Molecular Analysis of Genetic Diversity in Environmental Microorganisms and Its Impact on Ecosystem Dynamics

Editors

Fatima Wisam Naji Wedaa
Collage of Science University Department of Forensic Science of Al Qadisiyah, Iraq

Nashwah jabbar Kadhim
Department of Forensic Science College of science university of Al Qadisiyah, Iraq

Doaa Talib Mohammed Shabib
Al_Qasim Green University College of Environmental Sciences Department of Environmental

Eman Hussen Kadhem Kazr
Al_Qasim Green University College of Environmental Sciences Department of Environmental

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Abstract

Molecular diversity analyses of environmental microorganisms, designed to enhance understanding of ecological processes, address core biological questions fundamental to both modern environmental science and its future application. Building on the premise that community genetic composition influences ecosystem function, these approaches provide essential insight into genetic diversity patterns and metabolic structure, both within individual ecosystems and in comparative studies across multiple environments. Such assessments form a key theme of the ecological investigations associated with this theme, focusing on the intrinsic biology of environmental microorganisms. By characterizing the genetic basis of microbial community resilience, adaptation, and functional richness in diverse ecosystems undergoing rapid environmental change, these studies provide an indispensable foundation for subsequent ecosystem- specific metagenomic and metaproteomic investigations and enable predictive analyses contributing to environmental conservation and restoration. Microbial ecosystems are modulated by numerous factors, including temperature, pH, salinity, nutrient availability, moisture content, C/N ratio, and mineral quality. Interactions between these and other environmental variables exert subtle influences on community composition and distribution. Community turnover in response to such strong natural gradients has been documented for surface environments.

At the same time, change in any of these parameters due to anthropogenic activities can upset community stability, provoke the blooming of specific microbes, or lead to the extinction of sensitive species. These events can lead in turn to an overall reduction in community genetic diversity or the alteration of

metabolic functions, both of which may jeopardize the resilience and stability of affected ecosystems, affect their ability to perform pivotal ecosystem functions, and increase the risk of ecosystem collapse. Factors driving observed changes in community structure and activity are therefore diverse and, in many cases, poorly understood.

Content

Chapter - 1

Introduction to Microbial Genetic Diversity

Molecular analysis of genetic diversity in environmental microorganisms and its impact on ecosystem dynamics

Microbial genetic diversity encompasses the variation of genetic characteristics of environmental microbial populations and the ecosystems they collectively form. As genetic variation enables populations to adapt, flourish, and survive, microbial diversity is an evolutionary and natural requirement for microorganisms' exogenous functionality within ecosystems. It occurs at four conceptual levels of genetic organization (gene, genome, population, species). Microbial diversity creation encompasses mutation, selection, recombination, and horizontal gene transfer, and encompasses the full impact of microorganisms on ecosystem processes, functionality, and service provision.

Microorganisms-bacteria, archaea, fungi, algae, and viruses-inhabit every conceivable water and terrestrial environment, and these environmental microorganisms perform critical functions within ever-changing ecosystems across all biological domains of life. Their accumulation of metabolic capability enables organic matter decomposition in biogeochemical cycles, while community structure-characterized through abundance, diversity, and composition-affects community functionality and the stability of the ecosystem. Within this context of environmental microorganisms, specific groups with characteristic morphology and physiology can additionally

function as ecosystem engineers that modify their environment and indirectly alter other species. Environmental temperature, pH, mineral composition, and moisture content also shape microbial community structure. [1, 2, 3]

Definition and Scope of Microbial Genetic Diversity

Microbial genetic diversity describes genetic variation among groups of sequenced genes. Most studies consider ribosomal RNA genes, allowing for genetic markers alongside phylogenetic species concepts derived from rRNA phylogenetic trees. Detecting diversity at a lower taxonomic level typically relies on variation of functional genes with direct implications for metabolism and ecology. Established concepts of genetic variation also apply to microbial systems, and analyses of the full genetic complement within a set of environmental conditions can reveal how populations respond and adapt to disturbances. Nevertheless, modern investigations frequently utilize markers covering only a fraction of the full genetic variation. This approach can be understandable given the vast genetic latency within environmental microorganisms, apparent from full omics/metabolic reconstruction of the respective natural sample. While vast genetic libraries exist in many natural communities, it remains that only a small fraction of the genetic potential is active under any given environmental condition. The resolution of the marker approach, practical for analysis at lower taxonomic levels, will generally improve as sequenced diversity approaches that of the environmental library within the region of interest.

Microorganisms are classified into two groups based on their habitat, namely environmental and human-associated. Environmental microorganisms can be further divided based on the type of energy and carbon source they utilize, as well as their ecological categories in biogeochemical cycles. Environmental microorganisms play a major role in elemental cycling, such as

carbon, nitrogen, sulfur, phosphorus, and iron, and they also influence the behavior and productivity of other organisms. Microbial community structure and composition are often influenced by environmental conditions, leading to changes in biosphere functions. Environmental microorganisms act as ecosystem engineers, modifying environmental parameters that can greatly influence other communities and species. Microorganisms are also particularly sensitive to many abiotic factors, such as temperature, pH, minerals, and moisture. [4, 5, 6]

Importance of Genetic Variation in Microbial Populations

Genetic variation within a population is crucial for its long-term survival, and populations with greater genetic diversity have an increased chance of surviving major environmental changes, such as climate change. Consequently, understanding the levels of genetic variation present in a microbial population, especially one occupying an extreme environment, is of great importance and has a fundamental influence on its ability to respond to changes.

Microorganisms are often subjected to various extremes in their habitats, including temperature, pH, salinity, substrate availability, and the presence of toxic compounds. Future climate changes, habitat disturbances, and anthropogenic pressures might significantly affect population structure, species richness, biogeography, and community assembly of microorganisms. How microbial genetic diversity is impacted by environmental change and resilience is therefore a topic of great interest. [7, 8, 9, 7, 8, 9]

Levels of Genetic Organization in Microorganisms

Microorganisms are composed of a few to several hundred genes expressed in a cell. Genetic variation at the level of individual genes is particularly significant for microbes that

multiply rapidly in unstable environments, but the organizations of different levels of genetic variation-gene, genome, local population, total population, and species-are also particularly relevant during species formation and elucidating phylogenetic/phylogenomic relationships.

The impact of genetic variation of microbes in natural habitats has been evaluated based on the analysis of natural sequences discovered before the advent of culture-independent techniques. Population genetic data provide insights into the evolutionary process affecting the distribution of genes contributing to the functional variation of species in natural habitats and emphasize the significance of genetic heterogeneity among natural populations in reducing genetic vulnerability in adaptation to environmental disturbances. High genetic variation within loci and extensive stratification among populations indicate that microbe populations are micromorphologically distinct, present a high degree of genetic diversity, and experience genetic drift and natural selection. The key biogeochemical pathway playing an important role in environmental change operates efficiently only in ecological contexts that are conducive to local species dominance. [11, 12, 13]

Evolutionary Basis of Microbial Diversity

Microbial organisms exhibit vast ecological and functional diversity and are the most abundant and diverse group in the biosphere. Despite their simple structure, their adaptability to extreme environments, economic importance, and ecosystem functions rely on their genetic and biochemical variation. Many authors define genetic diversity as the variation in genetic composition among individuals within populations, among populations within communities, or between communities in different environments. Microbial diversity is of paramount importance for ecosystem functioning. The primary process

shaping diversity on a very short time scale, along with mutation, is selection, while horizontal gene transfer, recombination, and genetic drift are more relevant on longer timescales. Genetic drift and mutation play a negligible role in the longer term, gene sequence variation between the closely related strains of the same species can be rather large.

Genetic diversity has been framed to improve our understanding of selection for ecological questions. It has also been explicitly linked to resistance and resilience to climate change. Genetic variation enables populations to adapt to changing environments. Genetic areas responsible for the variations defining two highly distinct ecotypes have been identified. The metabolic features conferring the advantage of a hot spring population are well documented. The metabolic differences coincident with variations in community compositional dynamics following salinization have also been revealed. [14, 15, 14, 15, 16]

Overview of Ecosystem-Level Impacts

Microbial communities represent a vast reservoir of genetic diversity, with implications for ecosystem functions, processes, and services. Biogeochemical cycles serve as solid examples of how variation in composition and abundance of microbial communities leads to fluctuations in the rate of ecosystem-level processes. Partnering with other microbes, plants, and animals, environmental microbes are also classified as ecosystem engineers. For a given ecosystem, microbial diversity and community structure remain constant over time, with changes occurring only when environmental conditions begin to drift outside the range suitable to the ecosystem.

Microbial organisms, being smaller than other organisms, are also more sensitive to changes in environmental conditions. In particular, temperature, pH, mineral concentration, and moisture are key determinants of microbial community structure. At any given moment, these conditions reflect the weather scenario (hot, cold, alkaline, etc.). Given sufficient time (short, medium, or long-term scale), and keeping other forces constant, natural events can generate a similar signal (temperature, salinity, etc.) for every ecosystem around the globe.

Chapter - 2

Environmental Microorganisms and Ecosystem Function

Environmental microorganisms can be classified into different groups based on their habitats, metabolic types, and ecological roles. These organisms influence biogeochemical cycles of various elements (C, N, S, P, etc.) through the activity of their community structures. Cellular composition and configurations can change substantially to accommodate the diverse demands of different environments. Temperature, pH, mineral composition, moisture, and other abiotic factors can regulate microbial growth directly or indirectly by affecting the activities and diversity of the microbial communities.

Research illuminates how microorganisms of varying ecologies may function as engineers of their ecosystems and affect ecosystem processes and services. Microbial communities are not only the primary producers and decomposers but can also have the largest share of the energy budget in many ecosystems. Variation in the composition and structure of the microbial community can affect the functioning of an ecosystem, altering key processes such as biodegradation, the nitrogen cycle, and the sustainability of important ecosystem services like freshwater provisioning. Consequently, some microbiologists consider microorganisms as ecosystem engineers with an essential influence on ecosystem processes and services. [17, 18, 19]

Classification of Environmental Microorganisms

Environmental microorganisms can be classified according to habitat characteristics, energy acquisition, and ecological role.

Apart from living in various habitats like soil, water (fresh or saline), and animals and plants, microorganisms are defined as micro-organisms that can obtain energy from a range of different processes. As primary producers and pioneer species, microorganisms are crucial for self-contained biosphere support systems and are the main actors in many biogeochemical cycles. They are also very active in the carbon cycle, playing pivotal roles in carbon storage and release, and in the nitrogen cycle, contributing to the production and consumption of greenhouse gases. In particular, methanogens are estimated to produce ~160 teragrams of methane per year, accounting for about half of the atmospheric methane. Other microorganisms are also closely involved in the sulfur, phosphorus, iron, selenium, arsenic, and molybdenum cycles.

The distribution of microorganisms in various habitats is primarily shaped by the stratifications of microhabitats. Subsurface microbial communities are significant because they dominate Earth's subsurface biosphere in terms of mass, and their metabolic activities and processes are of considerable interest. These thermally stratified structures are also thought to reduce H2 gas concentration and to play a decisive role in the stability of mud volcanoes. However, the inherent technical difficulties of accessing these extreme sub-seafloor depths have hampered the molecular characterization of microbial communities associated with deep subseafloor sediments. Therefore, biogeo-chemical investigations of natural subsurface laboratory systems are of high importance. [20, 21, 22]

Microbial Roles in Biogeochemical Cycles

Microorganisms play pivotal roles in biogeochemical cycles. Carbon is fixed from CO_2 during photosynthesis and converted into biomass by producers within bio- or phototrophic communities, then further decomposed by heterotrophs through aerobic or anaerobic respiration, fermentation, and

methanogenesis. Nitrogen and sulfur cycles are interconnected, with multiple nitrogen-fixing and nitrifying/denitrifying bacteria participating in nitrogen transformations (see below), while the oxidation of hydrogen sulfide to sulfur and thiosulfate and the subsequent reduction of thiosulfate to hydrogen sulfide involves phototrophic, sulfur-oxidizing, and sulfate-reducing microorganisms. In addition to the main biogenic elements (C, N, and S), dissolved organic matter, trace elements (e.g., iron and manganese), and sulfur species are also recycled by marine microorganisms. Furthermore, geochemical gradients within sediments act as ecological drivers, revealing the mechanisms underlying metabolic coupling and the transport of substance and energy associated with dissolved ions and groundwater, along with the involvement of microorganisms in nutrient regeneration and toxic element detoxification.

Microorganisms contribute to and modify the environment, affecting primary and secondary production and serving as the foundation of the environment. Changes in environmental factors and ecosystem shifts alter microbial communities, which in turn affect the functioning of animal and plant higher trophic levels. The stability of ecosystem structure is reflected in relevant functional redundancy, where microorganisms constitute a large number of species and genera serving similar ecological functions in biogeochemical cycles. Microbial diversity, influenced by habitat complexity, determines functional diversity at the community level and is the ultimate guarantee of environmental stability. Microorganisms can be regarded as ecosystem engineers that actively modulate nutrient cycling and induce significant changes in biogeochemical processes at both local and landscape scales. They also mediate the mutual retaliation between the abiotic and biotic environments. Factors such as temperature, pH, mineral content, moisture, and pollutants further influence microbial survival and physiology. [23, 24, 25]

Microbial Community Structure and Function

Microbial communities in the environment can be classified according to their habitat, nutritional and metabolic mode, and ecological role. From a functional point of view, microorganisms are the main drivers of geochemical cycles, as their metabolic characteristics make them more versatile than those of macro-organisms. They drive the carbon cycle through decomposition, fermentation, and methanogenesis, and the nitrogen cycle through nitrogen fixation, denitrification, and nitrification, among other processes. In nutrient-poor ecosystems, such as oceans, soil, and sediments, microorganisms can also determine key biogeochemical processes indirectly, by controlling the availability of energy and nutrients for other organisms.

The composition of microbial communities is often decoupled from ecosystem functioning and is more related to environmental filtering than to species interactions. Nevertheless, abundant and functionally essential microorganisms can modulate ecosystem processes and their stability. Microbial community structure and functional profile can be coupled to identify microbial ecosystem engineers, whose changes can have a strong impact on biogeochemical cycling and aquatic ecosystem stability. Moreover, although microorganisms are usually considered independent of abiotic forces, some climatic factors are critical for community assembly and ecosystem functioning. The abundance of thermophilic and halophilic microorganisms is determined in part by environmental temperature and salinity, and their respective habitat suites appear to be almost independent, probably due to harsh environmental windows. [26, 27, 28]

Microorganisms as Ecosystem Engineers

Microorganisms profoundly affect ecosystem functioning and stability and are termed “ecosystem engineers.” Their ability

to modify resources on different scales distinguishes them from other organisms. Microbes shape their environment by modifying nutrient concentrations, pH, and redox distribution, change substrate compositions through decomposition or photosynthesis, and form solid structures that provide habitats for other organisms.

Bacteria act as ecosystem engineers by precipitating minerals, such as magnetite, phosphorite, and calcium carbonate. The carbamoyl phosphate generated by the urease activity of bacteria increases the local concentration of ammonium and thus precipitates in ammonium-rich conditions. Sulphyric microorganisms play a significant role in the turnover of sulfur, carbon, and nitrogen. Analysis of the habitats occupied by sulfur- or iron-oxidizing bacteria detected hydrocarbons produced by anaerobic oxidation with sulfur oxide or metals as electron acceptors. Sulfate serves as an electron acceptor for the oxidation of organic matter. The product, hydrogen sulfate, can be transformed to hydrogen gas and subsequently used by purple and green sulfur bacteria in replenishing local sulfide-containing waters.

Microbial community assembling, diversity, and structure are three fundamental aspects in understanding microbial ecosystems. Disturbances such as nutrients, heavy metals, salinity, temperature, sheep grazing, and inundation may lead to an emphasized assemblage of rare OTUs. Temperature and nutrients affect community structure and function, the composition and structure of microbial communities successfully reflected the coastal ecosystem health of bacterial communities, and aquaculture nutrient enrichment influenced the composition of prokaryotic and eukaryotic communities. [29, 30, 31]

Microbial Interactions with Abiotic Factors

Environmental microorganisms, though often overlooked, are among the Earth's most diverse and abundant organisms.

Their sheers numbers are staggering: a plate of soil containing just a gram of material could house around 1 billion bacterial cells from up to 10 000 different taxa, while a pond with a volume equal to an average bucket could hold the equivalent of 3 million cells of over 10 000 different species per millilitre. Current estimates suggest that there could be close to 10 000 species of fungi in a single gram of soil and well over 100 species in a single grain of sand or silt. Such astonishing diversity often remains unappreciated because of the difficulty in isolating and growing it in the laboratory. The importance of such diversity becomes apparent in the roles these microorganisms play; they catalyse essential processes in nitrogen, carbon, sulphur, and phosphorus cycling. In this respect, they are the very basis of life. Indeed, nature has used microorganisms to govern its key biogeochemical cycles for hundreds of millions of years. Such microorganisms, through their involvement in a multitude of ecosystem processes and biogeochemical cycles, engineering of ecosystems, and response to abiotic and anthropogenic factors-temperature, moisture, pH, and pollution-affect a range of ecosystem services. Changes in biodiversity-both alterations to taxonomic community assemblages and shifts in the abundance of ecological groups-can impact the structure and functioning of an ecosystem.

Most biogeochemical cycles involve the activity of microbes and reflect their metabolic capabilities. The carbon and nitrogen cycles are the best studied, yet there are other equally important cycles for sulphur, phosphorus, and iron, among others. These cycles collectively determine the functioning of aquatic and terrestrial ecosystems. Understanding how microbial community structure in a given environment affects ecosystem functioning remains a key question in microbial ecology and environmental biotechnology. Microorganisms can be considered ecosystem engineers. They govern primary productivity in many

ecosystems, particularly in oceanic, freshwater, and saline habitats; contribute significantly to organic matter decomposition in soils and sediments; and influence geochemical conditions in the development and formation of many extreme environments. [32, 33, 34]

Chapter - 3

Molecular Markers for Genetic Diversity Analysis

Molecular markers are any characters or traits that can be measured at the molecular level, and they are the detectable variable phenotypes usually associated with specific genes or DNA sequences. Various types of markers have been used for genetic diversity assessment in microbes. Ribosomal RNA gene sequences remain the most widely used marker for community composition studies. However, the phylogenetic signal of a single ribosomal RNA gene is often insufficient to resolve relationships in closely related strains. Thus, more variable markers, mainly those of functional genes, are increasingly being used to obtain a deeper understanding of genetic diversity in environmental-related taxa. Genetic diversity at neutral regions, such as microsatellite or single-nucleotide polymorphism, can also give fine-scale resolution on population demographics, structure, and dynamics of certain microorganisms. Despite the multitude of potential markers available, their choice is rarely straightforward. Several basic considerations can ease the decision-making process, guiding investigators toward the most suitable molecular markers for specific research questions.

Ribosomal RNA genes, primarily used for community composition studies, are typically considered phylogenetically stable and universal. Their sequences have been analyzed in the majority of bodies of freshwater, seawater, and sediment, revealing a high diversity of microorganisms. Nevertheless, many crucial aspects of microbial ecology in these habitats

remain poorly understood. The small phylogenetic signal of the bacterial 16S ribosomal RNA gene in recent evolutionary time leads to limited resolution among closely related strains and has prompted an increased interest in congruent phylogenetic reconstructions obtained using, for example, structural domains, concatenated 16S-23S ribosomal RNA genes, and ribosomal RNA gene introns. Concatenated genes, by incorporating more sites to estimate a tree, form a logical solution to distinguish among lineages or to assess their temporal divergence. [35, 36, 37]

Types of Molecular Markers

Diversity analyses of environmental microorganisms exploit molecular markers of diverse types and characteristics, each with particular strengths and limitations that need to be congruent with the underlying questions. Sequence-based markers of ribosomal RNA genes are the most widely employed in microbial ecology, revealing taxonomic and phylogenetic diversity patterns, distributions, and community changes. Functional gene markers tackle metabolic capabilities and ecology. With the advent of high-throughput sequencing, microsatellites and single-nucleotide polymorphisms are being increasingly applied in diverse populations.

The selection of suitable markers is best made on a marker-by-marker basis and on the basis of the questions being addressed. Molecular markers should encompass (1) genes or domains with sequences conserved enough to enable amplification of DNA of all phylogenetic groups present in the environment under consideration; (2) variation sufficient to discriminate among close relatives; (3) a level of variability sensitive to the scale of interest; and (4) sequence databases large enough to allow for a reliable taxonomic classification. When questions are focused on ecological aspects, the incorporation of genes coding for key metabolic processes should be considered,

and the availability of primers for detecting particular lineages or groups of interest would be advantageous. Finally, recent methodological developments in microbial ecology allow for an unparalleled resolution of epigenetic variation and functional potential, enabling the tracking of diversity across samples and providing a functional perspective. [38, 39, 40]

Ribosomal RNA Gene-Based Markers

Ribosomal RNA (rRNA) genes are among the most broadly studied molecular markers in microbial ecology. The primary, prokaryotic 16S rRNA gene and the eukaryotic 18S, 26S, and 5.8S rRNA genes, encoding the ribosomal RNA subunits involved in protein synthesis, are ubiquitous in almost all kingdoms of life. Phylogenetic trees based on rRNA genes have helped to define microbial taxa, establish taxonomic relationships, and document evolutionary patterns.

Variations within the 16S and 18S rRNA genes have been employed to interrogate microbial community composition, connectivity, and dynamics. In metagenomic studies, the filtration and analysis of intact rRNA thereby reveal the für hermith discussion of active community members. The rRNA cluster invariably comprises the largest single pool of sequence data in environmental sequences, documenting diversity at all ecological scales in all habitats of microbial life. Diversity in the plastid 16S rRNA (chloroplast) library characterizes yield-associated assemblages in micro-eukaryotes, defining major groups within these phylogenetic networks.

Beyond taxonomic information, the abundances of specific rRNA sequences can provide insights into the activity of dominant lineages within microbial communities. Further supporting these functions, gene content matrices highlight the natural and sequential abundance of genes involved in heterotrophy and phototoxicity, their expression depth

representing the diverse potential for organic carbon metabolism in marine systems. [41, 42, 43]

Functional Gene Markers

Functional gene markers based on form-specific or function-oriented genes reveal information about environmental adaptation and metabolic properties of an ecological community. They provide a complementary perspective to ribosomal RNA gene-based markers by identifying whether particular substrates are actually utilized, metabolic pathways are functionally complete or incomplete, and specific ecological functions are present or absent. The most often studied functional genes are those involved in phosphorous metabolism, nitrogen cycling, carbon fixation, methane cycling, and sulphur cycling, as well as antibiotic resistance genes.

Microbial diversity can also be assessed using microsatellites and single nucleotide polymorphisms (SNPs). Microsatellites, or DNA sequences consisting of varying numbers of tandemly repeated short motifs, are ubiquitous in prokaryotic species and possess properties that make them suitable for population studies. The high mutation rate of microsatellite loci makes them valuable for assessing relatively recent population structure, gene flow, and ecological interaction. SNPs, which are single basepair variations between different strains or species, have abundant markers for a range of prokaryotic taxa. These molecular markers provide detailed population genetics and species niches in natural habitats over various spatial and temporal scales. [44, 45, 46]

Microsatellites and SNPs in Microbial Studies

There are several molecular markers that measure genetic differences among organisms. Examples include ribosomal RNA genes, which are found in all living organisms, and functional genes associated with individual metabolic pathways. Certain

markers, such as simple sequence repeats known as microsatellites, have many advantages. Other methods analyze single nucleotide polymorphisms. The choice of marker for a microbial study depends on several factors, such as the level and type of diversity to be measured. Resources with guidelines for marker selection can guide researchers through the process.

Microsatellites are tandemly repeated short sequences found in all living organisms and are abundant in bacteria. Next-generation sequencing technology has further facilitated the development of microsatellite markers in a variety of microbes. Several guidelines for microsatellite analysis have emerged, and their advantages make them increasingly popular in microbial studies. Simple sequence repeats can also be found in chloroplasts and mitochondria of higher plants and eukaryotes, creating the possibility of developing simple sequence repeat markers for hybrid and niche studies. [47, 48, 49]

Marker Selection Criteria

Assessing genetic variation in environmental microorganisms requires consensus on appropriate molecular tools to address specific knowledge gaps. Available molecular markers, grouped by type and speaker affinity, differ in their suitability for addressing ecological questions related to genetic diversity in natural and engineered microbial ecosystems.

Characteristics of the marker system, including expanse across biological kingdoms, functional relevance, community expression, ecological resolution, evolutionary time scale, genomic position (non-coding or coding), availability of non-target databases, multiplexing potential, affordability, and locus availability in reference genomes, can inform selection of markers for genetic diversity analysis. [50, 51, 52]

Chapter - 4

DNA Extraction and Sample Preparation

Sampling strategies should target habitat-specific conditions known to dictate microbial diversity in environmental ecosystems, such as temperature, moisture, pH, organic matter, and salinity. Many groups consist of low-abundance populations, and rarity introduces complications due to the stochasticity inherent in community assembly, which can result in significant seasonal shifts in operational taxonomic unit (OTU) composition. High-throughput sequencing is increasingly used to generate datasets aimed at elucidating the diversity and dynamics of environmental microorganisms around the world.

Microbial communities inhabiting environmental ecosystems can be highly diverse, and providing an unbiased representation of the community detected within natural samples can be extremely challenging. When considering the definition of the ecological niche proposed by Hutchinson, environmental microorganisms occupy a hyperdimensional e-Niche and community diversity varies temporally as well as spatially. Moreover, these environmental microbiomes are usually examined in a "snapshot" time-series basis, without replicates and focusing on populations with low-abundance or even rare representatives, with implications for detecting the complete signature of diversity associated with these ecosystems. Proper sampling strategies accounting for the environmental parameters regulating the community assembly and formation of its resident microbiome can promote a better representation of the

underlying biodiversity. Nevertheless, a thorough assessment of the sample size and sampling strategy design is still to be implemented, even in studies capturing these environmental parameters.

A myriad of different conditions present in microbiomes worldwide affect community structure and play important roles in shaping diversity and its ecological implications. Nevertheless, the development of suitable sampling strategies designed to obtain adequate snapshot time-series data of environmental microorganisms remains an area that should be further explored. [53, 54, 55]

Environmental Sampling Strategies

To assess the full extent of community diversity, microbial sampling strategies must be carefully designed because parameters such as the type, intensity, and spatial scale of the environmental sampling will influence the richness and evenness of diversity metrics. Environmental samples capture only a subset of the total community species pool, and sampling intensity determines the completeness of the discovered local species pool. Microbial communities are usually heterogeneous, and rare species occurring at very low abundance levels may not be sporadically detected in samples. Appropriate sample size calculations can guide the discovery of rare species by quantifying sample-sampling saturation and establishing how many individuals should be sampled.

Microbial diversity associated with complex matrices such as soil, sediment, and gut content can be explored by developing an efficient protocol for DNA extraction. The presence of minerals such as humic acids, phenolic compounds, and various polysaccharides hinders the efficiency of the extraction, and different combinations of commercial kits should be tested for optimum recovery and purification of high-quality nucleic acid

suitable for downstream molecular applications. Inhibitors isolated during extraction that may affect subsequent PCR amplification can also be removed with adsorptive materials. Quantifying DNA by spectrophotometry or fluorometry is useful to evaluate the quality of extracted products, and specific approaches can be adopted when handling extremely low DNA-concentration samples. PCR-based molecular techniques such as quantitative PCR (qPCR), digital PCR, and denaturating gradient gel electrophoresis (DGGE) continue to be powerful tools to assess environmental microbial diversity. [56, 57, 58]

DNA Extraction from Complex Matrices

Environmental matrices are often highly complex and can lead to reduced DNA yields and qualities after the extraction procedure. For example, it is difficult to extract DNA from samples such as sediments because the various processes (e.g. precipitation) can modify the consumption of the chemical compounds from microorganisms (opposing to the DNA extraction). Furthermore, such complex environmental conditions increase the risk of inhibition of downstream steps. Therefore, the DNA extraction methods must be carefully chosen for a given type of complex environmental matrix prior to the experiment.

The removal of inhibitors during the DNA extraction is extremely important in order to allow the successful amplification of any required DNA regions. Specialized protocols have been developed in order to allow for mitigation of inhibition. Inhibitor removal from DNA samples for further applications, such as for qPCR or NGS, should ideally be performed without affecting the microbial community composition or their relative abundance. For the preservation of extracted environmental DNA, it is always recommended to keep the samples at a temperature of −20 °C or lower in order to

prevent the degradation of stored samples, although storage at −80 °C is ideal. [59, 60, 61]

Quality Control and DNA Quantification

Various quality control (QC) steps should be considered for accurate representation of microbial diversity and populations during DNA extraction and molecular work. The quality and quantity of extracted DNA are crucial for successful downstream applications. Estimating DNA concentration is facilitated with spectrophotometric methods. However, these methods do not effectively detect co-extracted PCR inhibitors such as humic acids and polysaccharides. Quantitative PCR (qPCR) provides an efficient means of qualitatively determining DNA concentration and can identify residual inhibitors through use of quantitative standards. If quantifying target genes, qPCR can also mitigate problems caused by unequal target abundance of different community members. Methods targeting excess reagents-such as MAP, AMPure, and PgClean-Up that remove residual PCR primers-enhance 454 pyrosequencing quality and effectively remove tag-specific secondary PCR products without affecting original tags.

Removal of inhibitors is crucial to avoid failure or bias in subsequent analyses. Bilateral methods have demonstrated the ability to remove specific inhibitors from environmental DNA. Approaches employed are diverse, ranging from the use of activated charcoal and aeolian sand for organic inhibitor extraction to KiYb or KTE for more universal removal of contaminants. Subsequent analysis of restored samples often reveals them as more representative for community composition studies. To minimize degradation, it is generally recommended to store extracted DNA at −80°C and under optimal preservation conditions to ensure longer-term stability, or lyophilisation for shorter periods. Maintaining samples at −20°C for prolonged

periods induces significant changes in genetic composition. [62, 63, 64]

Inhibitor Removal Techniques

The discussion presents various methods designed to eliminate PCR inhibitors from environmental DNA extracts. Although such measures are not universally applicable, their inclusion is vital due to the potential effects of residual inhibitors on subsequent analyses, especially for complex environmental samples. The occurrence of molecules disrupting different reaction processes within PCR has been extensively documented, primarily focused on qPCR and assigning detection limits rather than examining community richness and abundance in detail.

Typically, different DNA extraction methods are effective in reducing inhibitor concentration in various complex samples. Nevertheless, some residual levels may remain, thereby affecting the downstream analyses of community composition and dynamics. A solution or combination of approaches that successfully impact inhibitor levels is usually recommended in the PCR step. During the preparation of that step, the addition of horse serum and bovine serum albumin has often been suggested for various samples. Enzymes such as Taq polymerase can also be included with similar aims. The presence of different metals has proven effective in countering the action of inhibitors derived from natural sources.

Different active charcoal products have been used as adsorbing agents in numerous contexts. The application of Nannochloropsis oceanica-detecting primers demonstrated the effectiveness of Norit Carbon 4B in eliminating any inhibitory potential from sediments native for that microalga. Pre-digested rumen fluid in the PCR step allows the detection of bacterial groups and species in ruminant faeces, but residual PCR inhibitors can nevertheless impact abundances. [65, 66, 67]

Preservation and Storage of Environmental DNA

Proper preservation and storage conditions of environmental DNA are crucial to minimizing degradation, maintaining sample quality, and allowing successful downstream analysis. Depending on environmental conditions, nucleic acid concentration, and the time interval between sampling and analysis, preservation and storage procedures often require optimization to enhance analysis potential. Additives can reduce the degradation rate of nucleic acids; for example, lowering temperature or adding glycerol or Tris buffer is considered successful. Polyethylene glycol (PEG), cetyltrimethylammonium bromide (CTAB), and cellular solutions have emerged as promising preservation solutions for environmental DNA preservatives. In an ecosystem survey, a final compound of 6% (w/v) PEG–3% (w/v) CTAB–3% (w/v) glycerol maintained amplified nucleic acids for >25 days at both 25°C and 4°C, while no visible bands were detected in the preserving buffer without PEG or CTAB.

Nucleic acid solutions can be successfully stored at -20°C for several months, while unnatural thermal cycles and longer timescales induce a growing drop in enrichment and amplification. Therefore, a temperature of -80°C is recommended for long-term storage. The addition of 25% (v/v) preservative solution G (at fourfold concentration) allows long-term (10-25 inches) storage of environmental DNA samples at various temperatures. When freeze-drying is used for long-term (over 2 years) sample storage, acids are effective at 4°C but unsuitable at 22°C. [68, 69, 70]

Chapter - 5

PCR-Based Techniques for Diversity Assessment

Common PCR applications in microbial ecology encompass two primary objectives: detecting specific target organisms by amplifying marker genes and quantifying microorganisms using marker genes or species-specific genes, often employing quantitative or digital PCR protocols.

Diversity screening and community composition profiling can also be achieved through denaturing gradient gel electrophoresis (DGGE) or temperature gradient gel electrophoresis (TGGE). DGGE separates nearly identical sequences based on melting temperature differences. Short sequences (~500 bp) from the V3 region of the 16S rRNA gene are commonly analysed via PCR DGGE. Although TGGE promises a simplified workflow, both methods sacrifice complete bacterial community profiles to address bias and low sequencing depth.

PCR bias and errors remain a concern for studies focused on diversity and community composition. Bias arises from preferential amplification of certain microflora, reduced amplifiability of rare microflora, and primer-template mismatches. Apart from using multiple primer sets and quantifying templates for each reaction, additional strategies may help reduce PCR errors and bias in diversity assessments. Incorporating multiple PCRs and analysing the most diverse PCR product or consensus sequences, including non-fluorescent controls and potential contaminants in subsequent analyses, and

amplifying and sequencing all PCR products can alleviate bias and enrich sequenced diversity. [71, 72, 73]

Conventional PCR in Microbial Ecology

Conventional PCR traces its origin to the serendipitous discovery of strand duplication by Kary Mullis and colleagues in the early 1980s. It exploits temperature-dependent structural transitions within haarpin-bearing nucleic acid chains to facilitate sequence-dependent strand annealing. Conventionally, unlabelled oligonucleotides act as forward and reverse primers that intuitively define the range of amplification. In its most rudimentary forms, it allows for direct visualization of products on agarose or polyacrylamide gels. Specialized versions have subsequently enabled semi-quantitative estimation of relative abundances, multi-product multiplexing, detection of low-abundance targets, and inclusion of even greater functionality as capillary electrophoresis with fluorescent labels. In PCR-SSCP, the discrete mobility of single-stranded products within a heterogenic population provides insight into divergence without dependence on sequence identity. Nucleotide substitutions and insertions/deletions (indels) can also be independently evaluated.

The rapid development of PCR and its applications have surpassed even Kary Mullis's most gifted junction predictions. As a method, it serves more like a toolbox than an inquisition. Its ability to enrich for even rare nucleic acid comparisons has seen it used to probe a greater range of questions than arguably any other molecular ecology technique. From microbiologist-aligned inquests such as quantifying the impact of trematode infestation on trematode numbers to more ecologically driven developments like comparing the relative abundance of acidophilus in different test subjects, PCR now plays a central role in most therapeutic biological DNA sequencing and a significant role in RNA-DNA or RNA-RNA investigations, whether using microinterest or Metatranscriptionomics. [74, 75, 76, 77]

Quantitative PCR (qPCR) Applications

Quantitative PCR (qPCR) allows for quantifying specific organisms or genes within a microbial community. With its proven precision and sensitivity, qPCR is frequently employed in gene-centered investigations, detecting and illustrating changes in the abundance of specific genes in response to environmental gradients. MGS data offer an opportunity to derive marker genes for quantification, improving the soundness of qPCR studies and enhancing microbial ecology knowledge. Abundance changes across diverse studies can be scaled into an ecological model. qPCR also enables detection in environmental samples when a marker is absent, protecting against bias and overreliance.

Primer design can skew functional gene abundances. Contamination during primer synthesis is generally prevented, as leading companies employ stringent measures. Control solutions are available for these rare instances; however, bacterial and eukaryotic controls are unfeasible if the target organism is absent, and no well-documented marine control exists for some groups. Environmental controls remain vital, as MGS often provide abundant taxa without dominant homologs and hence few scientific resources. Detection is also challenging when the gene copy number is below quantification limits because the gene often acts as a marker, even without functional overlap. Abundance alone offers limited insight; taxonomic and habitat diversity are rarely addressed, risking the loss of potential ecological signals. [78, 79, 80]

Digital PCR for Microbial Quantification

Digital PCR (dPCR) enables precise quantification of microbial abundance and community dynamics, addressing the limitations of conventional and quantitative PCR. Infection dynamics and biogeochemical processes often depend on specific organisms at low abundance, making reliable detection

and quantification essential for understanding ecosystem roles. Quantitative PCR provides abundance estimates with high sensitivity but lacks standardization for absolute quantification. Digital PCR overcomes biases associated with traditional PCR by generating abundance counts rather than relative proportions of pooled PCR products.

In digital PCR, an initial bulk PCR reaction is diluted to partition the template into many tubes or wells, enabling Poisson statistics to predict the number of positive amplifications. Digital PCR thus quantitatively estimates the abundance of target sequences by counting negative and positive reactions. This direct quantification has advantages over qPCR, including freedom from standard curves, reduced pollution risk, and a reliability range independent of bias. However, specific and well-optimized probe and primer sets are still required, and dPCR remains the least widely used method for microbial quantification. [81, 82, 83]

DGGE and TGGE Techniques

Traditional PCR detection, especially when combined with DGGE or TGGE approaches, forms an important basis for evaluating changes in environmental diversity in studies where complex diversity estimation is not necessary. DGGE exploits the differential migration velocities of PCR products containing a unique GC-rich clamping region. Attempting to identify species from DGGE profiles usually relies on separation of species in at least one run. The presence of bands is then used for semi-quantitative data, but such observations should be treated with care owing to potential DNA concentration bias in the profiles.

A slightly different but conceptually similar technique, temperature gradient gel electrophoresis (TGGE), is based on the same principle but relies on a temperature gradient to modify local electrophoretic mobility rather than a concentration

gradient in the gel. The reduced resolution is countered by the possibility of performing hybridization analyses using digoxigenin-labeled probes. DGGE and TGGE are generally easy to implement and lend themselves to either semi-quantitative or qualitative experimental designs, depending on whether different bands in the same profile are expected to contain different species or whether the same bands appear in different profiles. Nevertheless, several serious caveats still need to be considered for both techniques. [84, 85, 86]

PCR Bias and Error Management

Two prevalent sources of bias in microbial community analyses involve preferential PCR amplification of specific taxa during DNA sequencing library preparation and spurious sequence variations generated during amplification. Amplification bias is primarily introduced when using PCR primers that are mismatched with the target sequences, have low-quality bases, or are present in very low abundance within a particular sample relative to the rest of the primer pool. Such effects are usually well documented, with the most serious bias typically being introduced by overly degenerated primer pairs. However, there is little information on how mismatched PCR primers affect specific populations within a natural community.

Sampling depth, single versus multiple PCR reactions, amplicon length, primer pair combinations with different variable regions, and amplification product concentrations used for generating sequencing libraries all contribute to community composition dissimilarity within and between pyrosequencing datasets. Spurious sequence variations occurring during PCR cannot be completely eliminated, but pre-PCR and post-PCR strategies can reduce their number and impact. Pre-PCR considerations include the choice of DNA polymerase, the use of low copy numbers of PCR template, the design of primer pairs

that have an optimal match to the template, and the number of PCR cycles. Post-PCR strategies typically apply a cutoff or filtering procedure based on the abundances of individual sequence variants characterised by identical lengths and positions of potentially erroneous nucleotides. [87, 88, 89]

Chapter - 6

Next-Generation Sequencing Technologies

Next-generation sequencing technologies enable efficient generation of large volumes of DNA sequence data at reduced costs compared to traditional methods. Several high-throughput sequencing platforms are currently available, each with its distinctive features and capabilities. The most popular sequencing types are amplicon sequencing of conserved marker genes and shotgun metagenomic sequencing of complex environmental samples.

Amplicon sequencing focuses on specific genes marked by universal, highly conserved primers in a wide range of organisms. DNA fragments at desirable lengths containing the target amplicons can then be pooled and sequenced in a single run on respective sequencing platforms. This technique is highly suitable for assessing taxonomic or functional diversity of natural populations in a quick, cost-effective manner.

In shotgun sequencing, complex DNA templates are randomly fragmented and sequenced. A large number of short reads generated from environmental pools are assembled into concise contiguous sequences. Although requiring substantial computational resources, shotgun metagenomic sequencing provides in-depth functional and taxonomic descriptions of environmental samples.

Other technologies based on longer read lengths are also becoming available, including the IBM-developed 454 pyrosequencing technology with read lengths of 300–600 bp,

preparation of single molecule real-time (SMRT) sequencing ready DNA libraries for the Pacific Biosciences (PacBio) RS, and single molecule sequencing technology developed by Oxford Nanopore Technologies. These new and emerging technologies are attracting growing attention for their relatively were long read lengths and the capacity to generate sequence data with less template preparation. However, targeted amplification of mixed templates, typically performed in amplicon sequencing and DNA assembly of shotgun sequencing remains challenging.

The rapid development of DNA sequencing technologies enables researchers to generate large amounts of high-throughput sequence characterizing various environmental samples, Their importance in supporting microbial diversity has also been highlighted. Contemplating the growing pace of microbial community analysis in emerging areas of biological research using DNA-based methods, metagenomic studies represent only the beginning. Because of elevated environmental DNA sequencing costs, a major part of the volume of DNA sequence data at present is still generated from rRNA gene amplification and sequencing of different environmental samples. However, sample quality control, data quality control, error prevention, and accurate data preprocessing still remain crucial aspects of DNA sequencing. [90, 75, 90, 75, 90, 75, 91]

Overview of High-Throughput Sequencing

High-throughput sequencing platforms have transformed sequence analysis, enabling fast and cost-effective identification of diverse genetic materials. Amplicon sequencing, shotgun metagenomics, and long-read sequencing are the main approaches. Amplicon sequencing specifically amplifies target regions, concentrating coverage and minimizing sequencing costs. Shotgun metagenomic sequencing captures complete community DNA without predefined tag regions, which is

particularly beneficial for unknown communities. Long-read technologies hold promise for resolving difficult regions, including repetitive and complex genomic regions. Amplification-based approaches are central to next-generation sequencing, but PCR bias alters diversity representation, calling for thorough mitigation strategies.

Growing sample numbers lead to massive sequence data generation, necessitating sophisticated pipelines for quality control, preprocessing, and error removal. Extensive sequencing efforts are required to produce high-quality amplicon libraries, as sample biology and preparation are crucial for reproducibility and interpreting ecological roles. Within-study variability often overshadows between-study differences, yet amplicon sequencing efficiently reveals major community patterns across abiotic or biotic gradients while identifying environmental drivers of structure and dynamics. Nevertheless, patterns derived from heavily sampled laboratories may be spurious or temporally limited.

Amplicon Sequencing Approaches

Amplicon sequencing enables targeted sequencing of specific marker genes, or regions thereof, with dedicated primer sets. This high-throughput potential allows analysis of diverse marker resampling from genetic databases and nearly complete conservation of homology in universal primers (especially in Bacteria and Archaea). The nature of samples allows multiplexing during PCR and sequencing, with amplicon length optimised for sequencing on platforms with shorter read lengths.

How amplicon sequencing is used for estimating taxonomic diversity in microbial communities is well-known. Uniquely tagged primers enabling multiplexing facilitate analysis of samples with low bioavailability. Community compositions of components belonging to fewer abundant chambers in atrophied

and well-maintained anaerobic digesters serving separate biogas plants are assessed and reveal that operating combinations of bioaugmentation and routine cleaning reshape community assemblages. Amplicon sequencing, using these tag-enriched 16S rRNA gene libraries assists in examining how examining Systemic Verbenaceae Nexus of genetic metabolism changes co-addition of 4-hydroxybenzene and N-ethyl-p-phenylenediamine-hydrochloride salts alters microbial profiles and evaluates how such changes affect structure and metabolic function. Microbial assemblages in sediments from hot springs are profiled using a specific 16S rRNA gene primer-Pyrosequencing system targeting the full-length gene. [41, 92, 93]

Shotgun Metagenomic Sequencing

Shotgun metagenomic sequencing advances environmental bioinformatics by generating genetic material from bulk and niche-scale samples, indifferent to assemblage composition. Whole community DNA isolated from broad samples supplies packages that are conjectured to resemble biogeochemical contributors, and the pooled action of genes from metagenic data sets should dictate system metamorphosis. Nevertheless, community diversity continues to obscure lucid gene enveloping, rendering systemic meta-genomics similar to conventional organic surveys and offering scarcely more meaning than statistical descriptive braille.

In contrast, illumination- or pyrosequencing-based shotgun methods subsume all genetic bundles, empowering elucidation of metabolic processes, regulatory, signalling, or logistic methods, Eco-Efficiency Monitoring such as Nucleocytoplasmic Large Viruses bidirectional loci, and construction of temperature sheet profiles, animal risk lists, or zebrafish holo-residues, etc. Hybrid sequencing associates chemical composition, ocular reminiscents, and or design direct arrangement, but does not

correlate resources or predict dynamics within amid long contexts. Cathodic centre pipeline configurations boost RNA stability and concentration, reducing dye quenching, enhancing phrasing or adjusting highlight extension. Conserved Prokaryotic cores enable glimpse statement from hull-transmitted reverse assemblages, soil swab distribution-crystallisation of negative shrimps, and outer central virulence, dissipation, or biochemical signatures transubstatiating integration at DNA level.

Long-Read Sequencing Technologies

Four long-read sequencing technologies (MinION, PacBio, HiFi, and TGS) are customizeable by recent developments, including training new models on longer reads, denoising based on coverage or ABG models, and preparing specific datasets that discover variation not detected by shorter reads. PacBio HiFi reads enable increased assembly contiguity beyond Illumina reads. TGS can improve metagenomic predictions made using shorter read lengths and enable assembles with better continuity than Nanopore reads. Likewise MinION Predict can vastly improve genome reconstructions by mixing alternative bases according to error count. An ASGAP discovery trained on long reads can produce better de novo RNA-seq transcriptome predictions than methods observing shorter reads. Read length alone does not determine predictive performance, but partitioning reads still enables diluted training of other Nanopore models. The popularity of LRS continues to flare with efforts using these technologies for exploring fungal genomes and leveraging the longer read lengths in combination with short reads.

Poor-repeat modeling, unplaced sequences, and the accumulation of other unmapped sequences in Illumina assemblies adds complexity to Nanopore data use. Integration of MinION raw signal data allows detection of elemental or amino-

acid substitutions related to polyketide or peptide biosynthesis. Single-molecule multi-omics methods draw a more complete view of a cell through integrated genome assembly and Seq-Wise 5-hydroxymethylcytosine methylation mapping and direct RNA sequencing. Incorporation of Nanopore technology for full-length transcriptome or 5-methylcytosine RNA-modification detection improves knowledge of plant cell wall responses to S. girdneri. Full-length transcripts are combined with transcriptome stage-associated expression during grape berry ripening to identify expressing full-length lncRNA. Other research also suggest the introduction of LRS where RNA structural variation data may be important, as RNA structure incorporates a structural variable expressed over time in relation to prevails functions within warm-season grasses. [94, 95, 96]

Sequencing Data Quality Control

Essential quality control steps can help avoid or reduce the number of errors introduced during sequencing. The most problematic bases-with the highest chance of error-are usually located in the first and last few cycles of the read; therefore, truncating them is a desirable QC action justifies. Another important QC step is the removal of reads that fail the quality threshold, which is usually chosen according to the objectives of the study.

Most of the currently sequenced NGS data are short reads, generated mainly by the Illumina technology. Therefore, a lot of effort has been devoted to ensuring high-quality-filtered Illumina reads, as they can improve the valid results in the following biological analyses. Because error patterns vary by platforms, it is also critical to choose the right error-correction tools. Before biological interpretation of NGS generated reads, it is recommended that sequence data be checked for potential quality problems and filtered or correction be applied if necessary. And

because different tools use different algorithms, using multiple tools for the same analysis can also support obtaining more credible results maker-containing reads from metagenomics datasets. [97, 98, 75]

Chapter - 7

Metagenomics and Functional Diversity

Metagenomics enables investigation of the entire genetic repertoire present in environmental samples, including non-culturable and genetically divergent microorganisms. Study designs can be based on specific genes or functional novel inquiries. The use of highly variable regions of the ribosomal RNA for taxonomic diversity is common, while functional metagenomics allows the discovery of assigned and annotated metabolic pathways. Comparison across multiple metagenomes can relate gene content to ecosystem processes. For ecosystem function and species-specific metabolism, transcriptional analysis of messenger RNA using metatranscriptomics provides key evidence of activity at the time of sampling. Integration of metagenomics and metatranscriptomics, confirmed with metaproteomics, links gene presence with activity at the time of sampling.

Metagenomic and metatranscriptomic de novo assemblies can help to characterize novel and highly divergent species detected during an environmental survey or to evaluate the presence of yet unidentified metabolic pathways. Functional Metagenomics analyses can provide pioneering evidence of an unexplored ecological role, which is subsequently improved and confirmed by Metatranscriptomic and Metaproteome content. And also the pathways that characterize the select ecosystem can also be confirmed through the appropriate integrated approach. [99, 100, 101]

Concepts of Metagenomics

A broad definition of metagenomics involves sampling DNA from a natural environment and sequencing the organisms present in the sample. Commonly sequenced nucleic acids in environmental research include ribosomal RNA (rRNA) genes, functional genes, protein-encoding genes, and whole metagenomes. Based on the targeted nucleic acid, the sampling design can fall into one of the following four categories: community structure, community function, phylo/metagenomics, or time-series metagenomics.

Analysis of amplicon sequencing or marker gene data (e.g. 16S rRNA gene for bacteria) can provide estimates of community structure (composition, diversity, and abundance). Correspondingly, shotgun metagenomic sequencing or expression data from all or a subset of functional genes can characterize community function by providing information on metabolic pathways and diversity of metabolic functions. Phylogenomic data (deduced from the genome sequence of one or more members of the community) can be combined with shotgun metagenomic sequencing to study phylo-metagenomics. In such cases, phylogenetic distance matrices are plotted against the functional similarity matrix generated from the shotgun metagenomic data. Time-series metagenomics involves analyzing incoming DNA or RNA from the same habitat over time (e.g., water depth or season), and correlating community structure with environmental changes.

Functional annotation pipelines for functional-oriented metagenomes usually classify the set of identified genes into metabolic pathways. Generally available Metagenomic Rational Analysis Tool (MetaRAT) pipelines are used for functional gene annotation, while the Kyoto Encyclopedia of Genes and Genomes (KEGG) is the typical reference database for metabolic

pathway construction. Once a group of genes has been assigned to their respective pathways, comparative metagenomics can be performed to discover global and ecosystem-specific patterns in gene abundance. [102]

Functional Gene Annotation

The identification of enzyme-encoding sections within nucleotide sequences (genes) and their assignment of functional significance in overall metabolism (functional gene annotation) provides the most fundamental facet of modern metagenomic studies. Functional annotation typically occurs in a sequence of steps, with the first aiming to localize genes in the DNA sequences of interest followed by the second step of assigning functions to these identified genes. In principle, functional gene annotation can also be completed at the metabolic pathway level, but this idealized approach is seldom pursued probably because of its substantial methodological and computational costs. In a first approach, the open reading frames (orfs), i.e., segments of sequences void of stop codons, of all assembled nucleotide sequences-shotgun contigs or individual amplicons-are collated into a single group. Each individual orf is then compared with the database of non-redundant protein sequences maintained by NCBI (National Center for Biotechnology Information) using BLASTX alignments, and unique assignments of functional activity are provided according to the annotation of the best alignment.

Despite its high-throughput production, the sequence data generated via "omics" technologies also possess certain inherent limitations. A major challenge of such approaches lies in the accurate biological interpretation of the sheer quantities of data generated. Whereas metagenome data reflect present environmental conditions and sources, metatranscriptome data offer an insight into the activity of the community under

investigation, elucidating which metagenomic functions are actually expressed (inferred activity profiles; see below) thereby assisting in the design and implementation of optimal sampling strategies. Further investigation of non-coding (forward and reverse) RNA molecules allows function and activity profiles to be generated at the level of cellular components (membrane, cytoplasm, ribosome, protein complex) and metabolic pathway. [103, 104, 105]

Metabolic Pathway Reconstruction

The pathways of biological metabolism, governing chemical transformations in living organisms, underpin the essential processes of life. Environmental microorganisms mediate the geochemical cycles of elements such as carbon, nitrogen, phosphorus, and sulfur. Metagenomic approaches allow the identification of functional genes involved in both central huts and secondary metabolic pathways, showing distribution patterns through functional group detection and pathway reconstruction based on the gene complement of an environmental sample. Certain metabolic pathways can be predicted based on the detection of prevalent marker genes or by comparing the distribution of pathway genes with environmental factors. The distribution of an ecosystem's genetic repertoire can also be linked to specific functional roles or ecosystem processes by associating detected functional groups with biogeochemical or ecological variables. Indeed, functional predictions based on metabolic pathways provide insight into microbial ecology and biogeochemistry and, more broadly, into processes driving ecosystem dynamics.

Metagenome-enabled predictions of metabolic pathways provide insight into microbial ecology and biogeochemistry. Reconstruction of the gene content of the pathways central to the metabolism of carbon, nitrogen, sulfur, and other elements allows

identification of the main metabolic groups of the system and their relationship with the local environment. The distribution of functioning genes within selected metabolic pathways also sheds light on the activity and functional potential of the studied system. Such knowledge is essential for determining the role played by microorganisms in ecosystem processes. These predictions contribute significantly to our understanding of ecosystem dynamics and can help identify the set of environmental drivers regulating microbial community assembly in different ecosystems. [106, 107, 108]

Comparative Metagenomics

Metagenomic approaches based on high-throughput sequencing can exploit amplicon and shotgun sequencing for targeted and untargeted, functionally informative characterizations of DNA sequence diversity in complex environmental communities, respectively. When comprehensively profiled and interpreted, differences in the functional gene content and genetic potential constitute informative evidence of distinctive community membership, activity and physiology in response to environmental variation. Comparative metagenomics is the analysis of gene content comparisons across multiple metagenomes, often highlighting distinguishable metabolic pathways, genes and functions offered by ecosystem-specific microbial community members.

To facilitate interpretation, the relevant aspects of metagenomic functional gene annotation are outlined, followed by a description of the gene-content comparison process. Comparative metagenomics then underscoring the insights achieved by connecting differential gene potential with known associations in environmental ecology and biogeochemistry. Metagenomics departs from 16S rRNA amplicon sequencing in its application of shotgun sequencing to environmental DNA

without marker-gene amplification, with consequent data sets incorporating all constitutive gene content rather than solely phylogenetic information. The foundation of the comparative metagenomics approach is delineated functional-gene-by-gene, replicate-cohort-wise parsing of the extensive gene-function annotation and taxonomic-association resources provided by the Integrated Microbial Genomes with Environment (IMG/ER) database, enabling subsequent alignment of these aspects from different metagenomic studies.

Communities that occupy complementary niches may manifest discernible differences in the genes that they collectively carry or express and, by extension, in the processes that they deploy to fulfil ecosystem functions. Enzymes often found in microbial genomes involved in degradation have been shown to respond to changing conditions with differential expression. Such distinctions are therefore reflected in the gene content and activity of the bacterial community in the sewage treatment process, enabling expanded ranges of substrates detectable by the community and improved process stability. [109, 110, 111, 112]

Linking Genes to Ecosystem Functions

Linking Gene Content to Ecosystem Functions and Processes

A metagenomic study of the bacterial community present in the river water of the Kharkiv region revealed a set of predicted genes that function as environmental indicators. Genes related to the degradation of various recalcitrant substrates were also identified. Overall, the taxa in metagenomic samples are shown to reflect stable succession of functional gene groups in the constantly changing environment, a process that is likely driven by the high rate of mutation and horizontal gene transfer.

Microbial community DNA from speleotherapy caves in

southern China were examined by metagenomic sequencing. Analysis of functional genes indicated a high prevalence of sulfur-disproportionating bacteria, suggesting that geochemical alterations from brine flows and sulfur deposits have created a unique H2S-rich microecosystem. Environmental distribution of well-characterized H2S and CO2-dependent metagenomic-assembled genomes reflected distinct biogeochemical gradients. In sediments from the Selettine saline-alkaline lake, functional analysis of metagenomic data revealed natural genetic resources for bio-remediation and effective control of salinity and alkalinity utilized for ecological balance and health.

High-throughput shotgun metagenomic sequencing of DNA extracted from surface and subsurface sediments from a highly androgenic polluted freshwater ecosystem showed the present of 769,171 assembled genes. Distribution of functional and taxonomic categories indicated the subsurface sediment community had biodegradation potential, while the surface community was associated with anthropogenic stress.Research in Tianluhu Lake indicated both metagenomic and metatranscriptomic assemblies exceeded 10 million contigs. Analysis of the biosynthetic gene clusters, functional, and taxonomic composition profiles and community metabolic interactions and functional redundancy of the metagenomes detected-indicated the lake sediments as vindicated machinery for bioactive substance biosynthesis. [113, 114, 115]

Chapter - 8

Metatranscriptomics and Metaproteomics

Metatranscriptomics sheds light on environmental activity and enables analyses of functional responses to stimuli, contributing valuable insight into ecosystem dynamics.

Meta-RNA extraction faces substantial challenges due to low RNA concentration, high contamination levels, and the need for rapid processing to prevent degradation. Approaches employing TRIzol® and RNeasy® kits have emerged as a suitable alternatives, but extraction remains more complex than that for DNA. Applying a two-step protocol can significantly improve quality.

The metaproteomic workflow begins with sample lysis, followed by protein purification and separation. One- and two-dimensional separation techniques are commonly employed, and the protein composition is then analyzed by mass spectrometry, allowing for functional annotations. Integrating transcriptomic and metagenomic data makes it possible to profile functional activity at the transcriptomic, proteomic, and metabolic levels, facilitating a more nuanced investigation of ecosystem dynamics. Enrichment or depletion analyses based on metatranscriptomic data provides further insights into potential activity shifts or transient pulses within the environment.

Meta-RNA research thus far has elucidated the active members, key nutrient transformations, and responses of complex natural communities to disturbances. By exposing a microbial consortium capable of degrading pentachlorophenol to

phenol, for example, it has been possible to increase understanding of the functional shifts occurring within the consortium. [116, 117, 116, 117, 118]

Principles of Metatranscriptomics

Metatranscriptomics detects RNA from diverse members of an environmental community, facilitating the investigation of active, yet uncultured populations, and unveiling community dynamics in response to environmental changes. Natural environmental samples present challenges for RNA analysis due to low concentrations, high degradation rates, and the presence of contaminants. RNA sampling, extraction, purification, and sequencing require additional quality control steps compared to DNA analysis. RNA secondary structures also pose obstacles for high-throughput sequencing. Nevertheless, metatranscriptomics principles remain similar to those of metagenomics.

RNA extraction techniques account for cell lysis, cell type abundance and metabolic activity, and the composition of the environmental matrix. Strategies for preserving retained RNA prior to analysis include reducing temperature, acidity, and enzyme activity, and limiting microbial activity. Metatranscriptomics workflows simulate those used in metagenomic analyses. Total RNA is reverse transcribed into cDNA or directly sequenced using single-molecule real-time technology. Potential obstacles include contamination by host RNA from complex environments and overrepresentation of highly abundant taxa.

Functional gene predictions are assigned metabolic levels, and pathway reconstruction employs RNA-sequencing data and 16S rRNA reference databases. The results highlight gene content complexity at different hierarchical levels during dynamic events. Integrating metatranscriptomics processing ensures the conversion of metatranscriptomic data, enabling

functional activity examination during specific time periods of ecological significance. Functional activity contributions of community members are further evaluated using metagenomic, metatranscriptomic, and metaproteomic profiles.

RNA Extraction from Environmental Samples

Total RNA is a crucial information reservoir for studying metabolic activity in environmental bacterial communities. However, RNA extraction from various environmental compartments (e.g., solid, aqueous, and sediment) can be particularly demanding. The combination of high concentrations of polysaccharides, proteins, and humic acids in such samples can hinder the lysis step, and the presence of inhibitors of ribonucleases may also contaminate extracts. Moreover, RNA is much less stable than DNA and, therefore, requires greater attention in all extraction steps to avoid degradation. A variety of commercially available kits specifically designed for RNA isolation from soil and other environmental samples are now available.

Maintaining sample integrity is critical while extracting RNA from environmental sources. Expressions and concentrations should be chosen according to the extraction procedure, and the sampling site should also be taken into account. RNA concentrations must be checked using an RNA fluorometric quantification kit. Because RNA is more susceptible to degradation than is DNA, suitable precautions should be taken when processing the extracts and conducting downstream analyses. Accurate quantification of RNA can be done via agrose gel electrophoresis with a standard control to evaluate RNA integrity. [119, 120, 121]

Metaproteomic Workflows

Metaproteomics studies tackle protein, enzyme, and organismal functionalities in environmental metabolisms. The

typical workflow begins with sample selection, focusing on an environment representative of community composition, sampling conditions, and storage time. Next, data generation is achieved through mass spectrometry for either proteomes or peptidomes, complemented by metagenomics or metatranscriptomics for gene content and expression base composition. Finally, bioinformatics integrates and interprets these datasets, with an emerging focus on linking functional metaproteomic patterns to ecological variables such as sampling context or niche.

Metaproteomics currently hinges on high-throughput DNA sequencing and protein identification by mass spectrometry. Steps range from protein lauryl sulphate extraction, to high-resolution tandem mass spectrometry, and finally, sequence and function assignment using nucleic acid-derived databases. Complete DNA sequence databases yield better functional assignments than annotated databases. Integrated datasets increasingly rely on connections between distinct omics techniques, with metatranscriptomic data often linking potential activity with expression for targeted niches. Recent applications point towards predicting operational metaproteomic features from non-traditionally metaproteomic variable categories or functions. [122, 123, 122, 123, 124]

Functional Activity Profiling

Functional activity profiles elucidate the active members of microbial communities and highlight variations in activity on ecological and temporal scales. Metatranscriptomic surveys probe the community transcriptome, while metaproteomics captures the community proteome. Metatranscriptomic sequencing assesses active functional groups, such as methanotrophs, methanogens, nirK-harboring denitrifiers, and diazotrophs in peatlands affected by temperature and moisture.

Sample-specific issues arise in RNA extraction, including limited quantity or quality and co-purification of genomic DNA. To overcome these, enzymes, beads, or chaotropic agents tackle rigid cell walls and lysis stage processing requires care to minimize chemical-related transcript decay. Preservation techniques mitigate RNA degradation.

Metaproteomic workflows encompass protein extraction, separation, mass spectrometry, and protein-level annotation. Total extracted proteins classify communities into functional groups on ecosystem scales. Integrated analyses deliver activity profiles linking metatranscriptomics–metgenomics–metaproteomics; such profiles may even assign genes to their products. Combined datasets pinpoint active metabolic pathways, identify active contributors-e.g., Martin-Gonzalez *et al*. detect potential N2O sources in a subtropical freshwater wetland – and relate activity profiles and functions. Recovery patterns underscore the role of Paenibacillus during succession. High-throughput sequencing platforms produce extensive data and quality filtering through basespace, Illumina connectors, AAP306-F and AAP616-R primers and annu.wiz introduces stringent quality control. Model-based probe selection assesses taxonomic assignment confidence. When distinct datasets permit, vessel-, region- and habitat-type-based comparisons reveal functional similarities and contrasts.

Integration with Metagenomic Data

Comparative analysis of metagenomic data sets can reveal trends in functional capacity, gene composition, or taxonomic potential across ecosystems. Key aspects include identification of metabolic pathways important to specific biogeochemical cycles, detection of predicted marker genes in ecosystem community assemblies, or correlation of gene abundance with metabolic turnover or transformation rates. Integration of

metagenomic data with additional omics (i.e., metatranscriptomics and metaproteomics) can also provide insights on functional activity within microbial communities.

Environmental microorganisms are essential for key biogeochemical cycles, including carbon, nitrogen, sulfur, and phosphorus. Several studies have examined the structure of specific microbial communities in different environments, bridging functionality by quantifying the abundance of marker genes involved in critical metabolic pathways (e.g., denitrification, methanogenesis, anammox). Integration of community structure with pathway analysis revealed strong relationships between community diversity and ecosystem function in salt marshes and tidal freshwater systems. Complementary, metagenomic approaches to examine the functional potential of sediments have established links between marker gene abundance and apparent rates of change through experimental incubation, providing base data to predict longer-term responses to climatic change in these historically dynamic ecosystems. [125, 126, 127, 128]

Chapter - 9

Bioinformatics Tools for Diversity Analysis

Bioinformatics Tools for Diversity Analysis

Accurate analysis of genetic diversity within environmental ecosystems utilizes multiple bioinformatics tools tailored for sequence data. Various pipelines exist for processing sequencing data, simplifying steps including filtering, quality control, and clustering of operational taxonomic units (OTUs). Generating exact sequence variants (ASVs) has gained traction, deploying the DADA2 and Deblur methods for high-resolution data. Diversity patterns are often explored through OTU or ASV-based approaches, though a shift toward ASV usage is evident. Network-based analyses further elucidate community composition.

Sequencing datasets are interconnected across studies, with taxonomic classifiers assigning taxa to individual sequences. Multiple databases and classifier strategies exist for assigning functional roles to reads, yet selection impacts ecological interpretations. Metagenomic sequencing supports detailed functional analysis, while metatranscriptomic resources delineate functional activity profiles. Integrative analyses couple metagenomics with transcriptomic and proteomics data, fostering deeper understanding of community composition, activity, and diversity influences.

Bioinformatics Tools for Diversity Analysis

Accurate analysis of genetic diversity in environmental microorganisms relies on dedicated bioinformatics tools for

sequence data. Multiple pipelines streamline filtering, quality control, and operational taxonomic unit (OTU) clustering, while increasing awareness of false positives in diversity patterns. Generating exact sequence variants (ASVs) has gained traction with DADA2 and Deblur methods, enabling high-resolution analyses. Diversity patterns are often explored through OTU- or ASV-based approaches, with shifting focus toward ASV usage. Network-based strategies further elucidate community composition.

Sequencing datasets are interconnected across studies, with taxonomic classifiers assigning taxa to individual sequences. Numerous databases and classifiers facilitate functional roles for reads, but selection influences ecological interpretations. Metagenomic sequencing supports detailed functional analysis, and expanding metatranscriptomic resources delineate functional activity profiles. Integrative approaches couple metagenomics with transcriptomic and proteomic data, deepening understanding of community composition, activity, and diversity influences. [103, 129, 130]

Sequence Data Processing Pipelines

Regardless of the application or sequencing platform, the process of sequence data generation and processing consists of similar steps. The raw read output of high-throughput sequencing from various platforms shows different characteristics, such as length and numbers (e.g. from 50 to 300 bp with millions of reads per run). Therefore, there is a considerable difference between sequencing data from a few reactions and that obtained by high-throughput sequencing. The complexity of the high-throughput sequencing data processing is often underestimated and should not be performed by inexperienced or non-specialized researchers. The needed bioinformatics skills stretch the capacity of most microbiologists involved in metagenomics.

In an amplicon-based sequencing analysis, a specific region of the ribosomal RNA gene, for example, is amplified by the same PCR reaction and sequenced on the same chip. The advantage of this is that the sequence determines the diversity mainly of a single function, and interference effects from species or lineages with very high abundance are minimized. However, a shotgun metagenomic study of a natural microbial community can yield sequence information for the complete genomic fragments, from which functional and taxonomic information can be obtained. Therefore, although 16S rRNA gene amplicon-based sequencing approaches are widely used for examining bacterial and archaeal diversity across ecosystems, metagenomics sequencing approaches that can obtain a large number of sequences of the complete microbial community in a single run are highly valued and valuable for analyses of structure and function in microbial communities. [103, 129, 131, 132]

OTU and ASV-Based Analysis

Analyses of environmental microbe genetic diversity and community structure from DNA amplicon sequencing can either be operational taxonomic unit (OTU)- or amplicon sequence variant (ASV)-based. OTU clustering aims to group sequences into mixotrophic organisms-such as euglena, dinoflagellate, or algae-like protists-with sequences above pre-defined sequence similarity thresholds (typically ≥95%) representing different functional groups or species. ASVs resolve sequences at the single base level and can infer taxonomic information at higher taxonomic ranks. Taxonomy assignment can be performed using the Silva or Greengenes databases. Diversity indices, such as Chao I and Shannon-Wiener indices, are more commonly calculated on ASV tables. UniFrac distance metrics then compare community structure within a sample or between samples, while non-metric multidimensional scaling plots visualize community composition.

Environmental sequenced data cleaning effects can introduce bias into diversity measures and ecological interpretation. Read quality affects alpha diversity and ASV richness detection. 16S rRNA amplicon sequencing-related bias is corrected through computational strategies on both OTU- and ASV-based tables. Biotic or abiotic factors, such as alteration in carbon/nitrogen ratio, quantum of nitrogen, or addition of exogenous α-methyl-D-mannoside impact the structure, richness, and evenness of soil bacterial and archaeal communities. Pigment analysis, chlorophyll a concentration, or distribution of protist communities correlates with other features, such as heterotrophic community structure, and community composition or abundance links with environmental properties. [133, 134, 135, 136]

Taxonomic Classification Methods

Taxonomic information of community profiles can be gained from 16S rRNA-based amplicon data and metagenomic sequencing by assigning operational taxonomic units (OTUs) or amplicon sequence variants (ASVs) and by taxonomic classification of full or partial 16S rRNA gene sequences. Classification is based on the similarities of query sequences to those in reference databases. BLASTn is the most commonly used tool for taxonomic assignments, but several other algorithms and software packages are available, such as rRNA classifier in QIIME, the Ribosomal Database Project classifier, and the SINA web service. For long-read sequencing data, classifying complete rRNA sequences is advantageous because these sequences can be compared against full-length sequences in the SILVA database. Besides nucleotide similarity, other measures, including rank-abundance and composition similarities, can also help to determine volunteer sequences. However, more than sequence similarity is needed to classify ecological groups at high taxonomic levels. The phylogenetic

classification of unassigned organisms is thus crucial for moving toward a phylogenetic definition of ecological groupings.

Automatic taxonomic classification of microbial communities can be determined from metagenomic data. In addition to carry the taxonomic sign of organisms, shotgun metagenomic sequences also provide genotypic and functional information. Distribution databases that automatically annotate metagenomic sequences from assembled contigs are available, and popular tools such as MEGAN cluster these sequences according to their taxonomic affiliations. Moreover, sequence signatures of taxonomic groups from the training set are established, allowing the use of any unannotated shotgun metagenomic data to assign the sequences to the signature-based taxonomic system. [137, 138, 103]

Functional Annotation Databases

Functional gene annotations generated from metagenomic sequences provide important biological information about microbial communities. Several functional classification databases have been developed from meta-omics analysis, each having two-dimensional mesh layout or domain-based approach so that one can find key secondary biochemical reactions that are connected sequentially in a pathway increasing the prediction accuracy. The eggNOG and COG databases are based on conserved clusters of orthologous genes identified on the complete genomes of available prokaryotic and eukaryotic organisms.

eggNOG has been designed to characterize and predict the function of sequenced genomes; it has integrated the metabolic pathways that are kept aligned using secondary prediction, and the edges of the annotation network are treated as weights. Key secondary biochemical reactions that are connected in the pathway supported by substrate after finally one can convert into

another or physically collocated in the same ecological niche receive a high prediction accuracy with additional confidence supporting metagenomics functional interpretation. KEGG also gives importance to prediction reliability and provides facilitated tool to explore key environmental reactions presented in the db. The SEED project provides a highly accurate metabolic model that can detail for distinct environmental niches via sub-database running within the SEED. Assembled amino acid sequences from metagenomic shotgun project are functionally annotated within the TrEMBL framework in the UniProt resource and are assigned cluster of orthologous group. [139, 140, 141, 142]

Statistical Analysis of Microbial Communities

Microbial community analysis addresses how microbes respond to environmental change. These changes affect their community structure and functions and can be examined through various statistical analyses. Statistical analyses of microbial communities begin by preprocessing and quality-control filtering amplicon or shotgun metagenomic sequencing data. Next, operational taxonomic unit (OTU)- or amplicon sequence variant (ASV)-based approaches identify the community's taxonomic composition and abundances and explore associations between environmental factors and alpha diversity indices. The composition and structure of the microbial community can then be described using a beta-diversity matrix based on Bray–Curtis dissimilarity or weighted Unifrac distances. OTU or ASV tables can be further passed to taxonomic classifiers to comprehend the community composition at species or genus levels. Furthermore, sequences belonging to specific functional genes can be collated to generate a heat map or network showing their co-occurrence patterns. The microbial community can also be modeled as a network or tested for modularity. Microbial variables across treatments can be compared using nonparametric methods, and

divergent populations can be detected with linear discriminant analysis effect size (LEfSe).

Microbial communities serve as sensors of climate change, pollution, and other disturbances; their composition and functional potential are thus being increasingly investigated. As microbial communities can be both indicators and key players in environmental shifts, further developing complementary bioinformatic pipelines will enhance understanding of the response patterns. This will establish a comprehensive assessment of microbial communities and thereby contribute to ecosystem management. [143, 144, 33]

Chapter - 10

Horizontal Gene Transfer in Environmental Microbes

Genetic material in the majority of organisms is inherited from their parents through vertical gene transfer; inheritance through transfer of genetic materials from one individual to another in the same generation is known as horizontal gene transfer.

Horizontal gene transfer impacts microbial adaptation and ecology in intriguing ways including transfer of antibiotics resistance genes among pathogenic species, acquisition of a metabolic pathway that enabled them to invade nitrogen-deficient waters, and epiphytes learning from pathogenic cousins how to degrade plant polysaccharides. Adaptive gene transfer through plasmids among strains within a species or via bacteriophages among different species often connects distinct populations in a habitat. Essential genes in bacteria, e.g., ribosomal proteins, appear resistant to HGT. GMEs play important roles in the ecology of communities through pathogenicity and conjugation. Bacterial communities with high HGT potential could be more affected by environmental changes. Analysis of GMEs provides a useful tool for identifying hotspots of HGTs in environmental microbes.

The influence of lateral gene transfer on genetic variation dynamics and evolutionary change in prokaryotic communities is under growing investigation. Detection of GMEs can provide insights into microbe–microbe interactions in communities and help evaluate the impact of environmental stress on GMEs.

Methanogenic and sulfate-reducing archaeal populations exhibit reduced genome-wide HGT frequencies in a mercury-contaminated wetland, suggesting recruitment of new members from the surrounding environment is the favored response to this stress. [145, 146, 147, 148]

Mechanisms of Horizontal Gene Transfer

Horizontal gene transfer (HGT; also known as lateral gene transfer) refers to the acquisition of genetic information from another organism (producing recombinant sequences) without sexual reproduction via meiosis. HGT has facilitated the evolution of microbial populations, enabling rapid adaptation to selective pressures in changing environments and the acquisition of resistance to antibiotics and heavy metals. HGT also plays a central role in bacterial pathogenicity by facilitating the rapid spread of virulence-associated genes. The three principal HGT mechanisms in microorganisms are transformation, transduction, and conjugation. Transformation occurs when naked DNA is taken up from the surrounding environment by living cells. The host bacterium then integrates a portion of this DNA into its genome. Transduction involves the incorporation of bacterial DNA into a phage genome through the lytic cycle of a bacterial virus. The lysogenic cycle of the phage is initiated when the integrated phage DNA is excised from the host genome and transferred to another bacterium during the lytic phase. This DNA may also recombine with the new host genome. Conjugation entails the transfer of DNA (usually plasmid DNA) from one bacterial cell to another through direct cell–cell contact. Plasmids are small, self-replicating, extrachromosomal DNA molecules capable of carrying stabilizing genes, antibiotic-resistance genes, metabolic genes, and pathogenicity determinants. The ability to establish that connection depends on the presence of specialized genes, such as the fertility factor (fertility factor) found in Escherichia coli.

HGT represents a significant source of genetic variation at the population level. It caused increased genomic variation and genotypic diversity in natural microbial communities and driven the dispersal of AR genes into environmental bacteria. HGT appeared to be a major driver of γ-proteobacterial evolution in the contaminated Namib Desert groundwaters by contributing to habitat adaptation along with mutations and gene gain. In synthetic γ-proteobacterial communities HGT was preferred over point mutations to acquire novel traits. However HGT is about maintaining diversity as it increases diversity among species and of metabolic capabilities via acquisition of foreign genes. At the community level, HGT facilitates cooperation among species through the exchange of beneficial genes and the acquisition of antagonistic or infection genes. It shapes co-evolutionary dynamics, such as the emergence of clusters of co-occurring plasmids, in ecological settings as diverse as human gut microbiomes, ocean pelagos and deep-sea sediments. [149, 150, 151, 152]

Mobile Genetic Elements

Mobile genetic elements (MGEs) represent essential components of natural ecosystems associated with diverse microorganisms that shape evolution. By facilitating horizontal gene transfer (HGT) in prokaryotes, MGEs have significant effects on genetic diversity, population structure, adaptation, ecological connectivity, and adaptation to ecological niches. Prominent MGEs include insertion sequences, transposons, plasmids, and bacteriophages. Gene transfer agents (GTAs), viruses produced by some bacteria and archaea, mediate HGT while preserving some genetic material of the producing bacteria or archaea. Recent experimental results suggest that GTAs are modeled after bacterial virus particle production but are non-living agents of HGT that encode genes optimizing populations in fluctuating environments.

A growing body of metagenomic and phylogenomic data confirms the prominent ecological roles of MGEs in different ecological settings. Genetic studies in ancient microbial-community populations point to expansion of species and dispersal-limiting selection during favorable conditions, modulation of genetic isolation and exchange rates based on its importance for community functions, and long-term evolutionary drivers associated with local diversity gradients. Phylogenetic analyses of barrier-limited populations highlight the importance of nucleic-acid-composition thermodynamics for reproductive isolation and the variation of HGT dynamics across the tree of life and different ecological states within the same species. Together, these findings clarify the general impact of MGEs on genetic diversity and functioning of natural microbial communities. [148, 153, 154, 155]

Role of Plasmids and Phages

Plasmids, circular DNA molecules capable of autonomous replication, influence evolutionary adaptation and ecological dynamics. They facilitate horizontal gene transfer, conferring traits such as antibiotic resistance, virulence, and metabolic functions. Plasmids can initiate conjugation or undergo transformation and transduction via helper bacteriophages, extending the donor-recipient range for genetic material exchange. Phages, DNA or RNA-containing viruses, infect bacteria to reproduce within them. Recently, studies demonstrated packaged host DNA in phages, capable of infection and establishment in new hosts, suggesting phages as agents of horizontal gene transfer. These processes impact various environmental bacterial communities, encompassing human-associated and non-pathogenic bacteria, and facilitate resistance acquisition.

Bacteriophages constitute over 60% of total DNA in seawater. Studies reveal transduction-mediated transfer of antibiotic resistance genes among Vibrio cholerae and Escherichia coli. Bacterial phages harboring virulence genes have been discovered, implicated in pathogenicity, pathogenicity islands, and the acquisition of toxin genes by Staphylococcus spp. Such horizontal gene transfer confers traits pivotal for bacterial adaptation and ecology, including pathogenicity, antibiotic resistance, bioluminescence, and degradation of xenobiotic compounds. The horizontal transfer of carotenoid biosynthesis genes among Haloarchaea facilitates survival in hypersaline systems. This effect remains undetermined in riverine assemblages, but a genome-scale analysis detected abundant mobile gene families among aquatic firmicutes. [156, 157, 158, 159]

Impact on Microbial Adaptation

Environmental microorganisms living in various habitats experience harsh conditions that can threaten their existence, including temperature extremes, salinity, radiation, pressure, and presence of heavy metals. Such stressors often select for specialized populations that can survive and thrive in extreme conditions. Under these selective pressures, genetic changes can occur relatively quickly, resulting in unique adaptations, some with ecological implications. Genetic signatures of adaptation have been identified in microorganisms from a variety of extreme environments.

Genetic analysis of environmental microorganisms has revealed a range of previously unidentified (and generally uncultured) species adapted to extreme conditions. A functional signature enabling growth at high temperatures and other temperate adaptations has been detected in uncultured environmental DNA from various natural-temperature

environments. Adaptations to cold have been elucidated in metagenomic data from low-temperature environments (e.g. polar, deep-sea, high-altitude) and in psychrophile isolates. Several genes for cold adaptation have been identified, including those coding for cold-active enzymes used in biotechnology and biogeochemical cycling, as well as possible adaptations for thermal oscillation tolerance. [160, 161, 148, 162]

Ecological Consequences of Gene Transfer

The transmission of genetic information among microbes is a widespread phenomenon with crucial implications for community composition and ecosystem functions. Different mechanisms facilitate such gene transfer among prokaryotic cells, permitting variation in the genetic makeup of coexisting microbial communities over short ecological time scales. Moreover, the interaction of these multiple processes at the population and community levels yields highly complex and non-intuitive patterns of genetic polymorphism, cohesion or genetic differentiation among subpopulations, and functional adaptation. Local patterns of reproductive activity of the core organisms implicated in those processes (with an emphasis on HGT) can thus deliver primary knowledge on the evolution of natural populations and microbial communities in situ. Such information is highly valuable for understanding how natural ecosystems assembled in space and time and how their signatures respond to a given environmental variable at present or during past episodes.

The impact of HGT on adaptive responses to environmental change has been documented in different natural populations and communities. This enables studies examining the relative influence of HGT on patterns of genetic community structure and cohesion-considered together with a presence-absence-bias in community composition or potential reproductive barriers-and of interpopulation divergence on community and ecosystem

properties, as well as the ecological consequences and underlying environmental factors that modulate the extent and direction of such influence. In summary, an integrative approach characterizing different modes and aspects of genetic flow, as well as their interactions, constitutes an appropriate tool for unraveling the complex nature of ecological processes shaping natural microbial communities and populations in the environment. [149, 163, 164, 165]

Chapter - 11

Microbial Population Genetics in Natural Environments

The population structure of microorganisms in natural environments provides insight into their evolutionary processes. For any given habitat, the degree of separation between different functional groups will determine the extent of gene flow (migration) that occurs. Such gene flow is expected to be common between populations experiencing similar environmental conditions, but not between those in differing extremes. Studying gene flow can not only highlight potential barriers to genetic exchange, but may also elucidate the location of environmental gradients that are of bioecological significance, as observed with the halophilic radiation in the hypersaline cores of the Ceuta lagoons.

Population genetics provides a means to interpret the disruptive forces of selection, genetic drift and recombination. Mutation, selection and drift together shape population structure, and, over some temporal depth, should structure natural groups according to habitat. However, these forces alone cannot account for all of the observed relationships between phylogeny and population structure. Recombination can subvert the population structure inferred from phylogenetic trees, and its relative significance increases with population size and the frequency of homologous sequences. Thus, consideration of multiples species occupying the same niche is likely to yield a different perspective of the acting forces than studies focused on individual species.

Population Structure Analysis

Characterization of population structure and genetic connectivity within natural habitats offers insights into gene flow, migration, and barriers to genetic exchange. Analysis of factors shaping microbial population structure deepens understanding of the evolutionary dynamics of microorganisms. Population structure reflects associations among conspecific individuals and the potential for gene flow. Genetic connectivity within populations, determined by migration rates relative to effective population size, indicates spatial and temporal patterns of genetic exchange across environments of varying scale and duration. Both structure and connectivity reveal the potential influence of external factors that impede dispersal and exchange; for example, metagenomics studies spanning gradients ranging from hypersaline lakes to deep-sea sediments have identified physical obstacles to gene flow in microbial populations inhabiting extreme environments.

The magnitude and distribution of gene flow are critical components of evolutionary dynamics in natural populations of all species. The rate at which individuals enter or leave a population can shape local adaptation by maintaining free exchange among populations or, conversely, homogenizing the effects of divergent selection. In natural systems, differential dispersion of genotypes can create population structure, which, in the absence of gene flow, can drive independent diversification. The location of barriers to gene flow can also be identified using asymmetrical Horwitz tests, thereby elucidating which geographic features or environmental factors inhibit genetic exchange. Together, these approaches reveal the balance between dispersal and restriction that shapes population structuring and genetic connectivity. [166, 167, 166, 167, 168]

Gene Flow and Genetic Connectivity

Gene flow and genetic connectivity influence the evolutionary and genetic structure of populations, detected while analyzing gene trees, mapping data, or closely-related sequence variations. Migration enables gene movement among groups; barriers to gene flow, such as temperature, salinity, or land barriers, may also shape population structure. Microbial populations are assessed by neutral markers using traditional population genetic tests or recessive markers correlated with fitness to account for selection, drift, or recombination effects. Microbial groups adapted to similar environments but separated by geographic barriers exhibit expanded population genetic studies, including Arctic–Antarctic temperature dynamics, spatial–temporal genetic structure in freshwater lakes, and spatial–environmental congruence in marine communities.

Studies on terrestrial microbes in soils and sediments examine landscape fragmentation, pH effects, climate-dependent connectivity, reproductive strategies, and salinity-driven negative covariance. Increasing temperature and salinity expand genetic distance, leading to niche divergence and selection for temperate, saline, and acid–alkaline conditions. Patterns of positive linkage disequilibrium in a microbial community align with supernatural environmental conditions, while negative linkage patterns indicate potential environmental stability. Analyses of hypersaline–nonhypersaline community pairs support ecological niche differentiation through function suppression. [169, 170, 171, 172]

Selection and Genetic Drift

Selection and genetic drift are primary factors shaping genetic diversity within populations. These processes-acting on genetic variation arising from mutation and recombination-dictate diversity at all levels, but particularly at the genome and

population scale. Selection changes the frequency of alleles in populations and populations that are subdivided can evolve differently if gene flow is limited, for example by distance. Populations that develop in isolation can become genetically distinct as a result of drift and natural selection. Selection generates local adaptation and is particularly strong on loci involved in local environmental interactions, metabolically relevant functions, and loci associated with phenotypic traits that differentiate closely related species.

For microbial diversity, understanding selection acting on neutral loci, often distributed across the genome, provides important association signals that can highlight ecologically relevant functional diversity. Genetic structure formed by geographical distance, or other spatial factors, can predict the strength of selection acting on neutral loci within a population-a signal reflected across the population and species groups of multiple ecosystems. Yet selection, drift, gene flow, and recombination act simultaneously in natural populations. The intensity of each evolutionary force varies with ecosystem context, and balance may shift temporally as conditions change. The ability to uncover these dynamics enhances the understanding of basic evolutionary processes, as well as supporting applications to conservation, management, and ecosystem function.

Recombination in Microbial Populations

Recombination contributes significantly to microbial diversity. Microbial genes are horizontally transferred among individuals, distorting evolutionary isolation. Variation in community structure enables exchanged genes to be phylogenetically analysed by population genetics tools, determining the mechanism of transmission and evaluating the impact on ecological function. The presence of substantial

methanogen populations in anoxic sediments has elevated the flow of methane into coastal waters. Genes related to methane oxidation and sulphate-reducing bacteria ideally are studied for biological signatures of environmental factors.

The gene encoding β-glucosidase in a mixed culture is suitable for studying the distribution of β-glucosidase in the environment. Its low genetic variation indicates that mutations are rare and that the gene might have been transmitted in the past via HGT or point mutations, enabling the population to adapt to changes in environmental conditions. The microbial community structure of pristine and dieback coral-reef waters differs. HGT between sediment denitrifier communities enhances their reductive function in the nitrogen cycle, and low HGT rates allow the establishment of quasi-stable associations. [173, 174, 175, 173, 174, 175]

Evolutionary Dynamics in Ecosystems

Microbial populations continuously evolve in response to selection pressure and ecological constraint. Ecological and evolutionary changes occur rapidly during environmental perturbation, and the community can exhibit genetic variation before the onset of taxonomic shifts.

Characterizing the patterns of microbial genetic diversity within and among natural habitats is important for understanding how genetic diversity within functional groups influences community dynamics in these diverse regions. Evidence for local adaptation among populations across adjacent but ecologically contrasting environments, habitat-specific community assembly dynamics, the influence of spatial factors, and levels of genetic subdivision within natural populations are increasingly accessible. Parallel genomic changes adapted to environmental extremes further link adaptation and ecology, while the relative contribution of dispersal and selection to shaping population

diversity remains unresolved. Microbiology is a constantly evolving field, but recent advances in sequencing technologies and their decreasing costs have expanded our understanding of how microbial populations function and respond to natural and anthropogenic stressors. These developments are providing new insights into a broad range of questions, including the evolution of microbial communities in response to extreme environments and climatic change, the role of the microbial population in ecosystem interactions, and the biogeography of microbial diversity on geologic and climatic timescales. [177, 178, 179]

Chapter - 12

Microbial Diversity in Extreme Environments

The immense diversity of cells and cellular organisms is underpinned not only by ecological factors but also by biological and genetic differences, and it has led to complex structures and functions in the microorganisms present in natural environments. Simple unicellular organisms exist in habitats with extreme conditions, such as those found in deep oceans, in permafrost, or in areas with scorching heat. These organisms have evolved special proteins and other molecules adapted to extreme pH and pressure values. Enzymes produced by some of these organisms are important in the biotechnology and industrial sectors. For example, the enzymes and proteins from Thermus aquaticus and Pyrococcus furiosus are used as temperature-resistant catalysts for polymerase chain reaction (PCR).

Several groups of microorganisms, called extremophiles, possess specialized adaptations that allow them to live and grow under extreme conditions. Thermophiles inhabit temperatures ranging from 50 to 80 °C, while hyperthermophiles can survive at temperatures higher than 80 °C and even as high as 120 °C. Psychrophiles live at low temperatures, psychrotolerant organisms can grow at low temperatures but prefer higher ones, hypo-saline organisms can flourish at sodium concentrations ranging from 0.1 to 3.4 M, while halophiles require sodium concentrations above 3.4 M. Alkaliphiles flourish at pH values above 9, whereas acidophiles grow at pH levels below 3.9. Microorganisms found in deep sea, without light and high

pressure, as well as subsurface microorganisms, are also considered extremophiles. [180, 181, 182]

Genetic Adaptations to Extreme Conditions

Research on the genetic basis of growth and survival in extreme environments has elucidated the mechanisms behind microbial adaptation to stress. These investigations reveal a remarkably diverse range of genes and gene variants involved in adaptations to extreme conditions, and that shared gene variants between distantly related taxa are often responsible for similar adaptations. For instance, studies exploring microbial adaptation to hyperthermophily have identified the specific contribution of mutations within isolated genes, as well as those distributed across multiple genes within a pathway, in promoting temperature tolerance and stability. Other investigations have examined the relative importance of gene loss and gene gain for adaptation to thermophily and acidophily, and consequently enable the prediction of additional temperature or pH-dependent genes. These models can be applied to members of an environmental community to elucidate its evolutionary history and steps involved in adaptation to extreme conditions.

Distinct lineages of psychrophilic microbes have been uncovered at high relative abundances in extreme cold habitats, while less abundant and/or generalist Psychrobacter or Psychromonas taxa are known primarily from less saline Antarctic Ice and surface waters. Future studies integrating genome sequences with observation of populations in low-temperature habitats will clarify the relative importance of isolation-by-distance and response-to-temperature. A comparison of the deep biosphere of the Peru Margin with similarly cold, high pressure, nutrient-poor, deep-sea anoxic marine sediments has provided new insights into the microbial community composition and its metabolic potential at a

comparable depth. Genetic signatures of adaptation to hydrostatic pressure have been identified, together with the evidence of anthropogenic influences on deep-sea microbiomes over the last decades.

Thermophilic and Psychrophilic Microbes

Thermophilic microorganisms grow optimally above 45 °C, with taxa adapted to 60–90 °C (thermophiles) or >90 °C (hyperthermophiles). Their habitats include hot springs, geothermal regions, and deep-sea hydrothermal vents, where they contribute to biogeochemical cycles. Pyrolobus fumarii, thriving at 106 °C, is the most heat-tolerant species, supported by a high-Eb genome and unique protein properties. Microbes in cold habitats (psychrophiles) flourish at ≤15 °C, with optimum growth at ≤10 °C. Psychrophiles show metabolic and genetic adaptations to cold, including polyunsaturated fatty acid synthesis, cold-shock proteins, and seasonal community distribution shifts, with polysaccharides and glycosyl hydrolases serving as biomarkers.

Microbial life persists in Earth's most extreme and inhospitable conditions, including -30 to -1 °C, ≥100 °C, 5–35% salinity, -0.47 to -pH 7. The deep biosphere harbours diverse prokaryotic communities capable of sustaining themselves for millions of years without sunlight. These ancient, large-scale habitats, previously considered devoid of life, are now becoming hot topics of research. Animals and plants inhabiting extreme habitats provide numerous genetic and functional adaptations for biotechnological applications. Psychrophilic, thermophilic and acidophilic microorganisms, their evolutionary and ecological uniqueness, and potential biotechnological applications are the growing areas of attraction in modern biology. [183, 184, 3]

Halophiles and Acidophiles

In nature, microorganisms typically flourish in environments corresponding to neutrality, defined by a pH of around 7. Deviations towards higher or lower pH impose additional stress and often necessitate specialized adaptation. However, some microorganisms exhibit a marked preference for acidity or alkalinity and are optimally adapted to extreme pH conditions-either below pH 6.9, termed acidophiles, or above pH 8.9 (alkaliphiles)-and often grow only within a narrow pH range of 1-2 units. Acidophiles, including bacteria and archaea, are of particular biogeochemical interest for their roles in sulfur and metal cycling and metal mobilization in mining activities. Nevertheless, the majority of environmental diversity at extremely acidic pH is likely not cataloged in culture collections, as the number of isolated strains remains small relative to the level of environmental sampling.

Halophiles inhabit highly saline environments, where elevated osmolarity typically impairs growth of most life forms. In nature, salinities exceed seawater at the coasts of hypersaline lakes, salt evaporation ponds, certain soda lakes, and marine environments dominated by high evaporation rates (e.g., the Red Sea). Halophiles contain numerous structural and physiological modifications for adaptation, and several of these microorganisms accumulate-rather than excrete-osmotically active components. Nevertheless, the increasingly stringent requirements for isolation and the relatively small number of available strains compared with environmental diversity remain a concern. Deep-sea sediments and deep-subsurface environments constitute perhaps the least-explored extremes, yet the vast volume and biomass associated with these habitats confirm active microbial life even several kilometers below Earth's surface. [185, 186, 187]

Deep-Sea and Subsurface Microbial Life

Microbial life inhabits almost every environment on Earth, including deep-sea and subsurface ecosystems where sunlight and oxygen are often scarce. Large parts of the ocean floor remain dark and cold, encompassing extreme environments such as hydrothermal vents. The deep subsurface represents Earth's largest habitat, encompassing sediment, crust, and mantle regions where life resides at tremendous depths, temperatures, and pressures. Microbial abundance declines with depth, but their activities remain significant. Nonetheless, changes in microbial communities-and the functional genes they contain-indicate increasing vulnerability to climate change, highlighting the need for future research. Because microbial cells exist much longer than eukaryotic cells in similar environments, they serve as accident time capsules of changes in local and global environmental conditions. Microbial activities and communities reveal their adaptations to survive and thrive in the dark, cold deep sea and the deep subsurface.

Deep-sea hydrothermal vents powered by reduced chemical compounds represent a pristine microbial ecosystem where eukaryotes are not the major group forming the biomass. The energy derived from hydrothermal ventilation can support organic matter production, which in turn sustains unique communities of higher organisms, including fish and crabs. Microbes play a vital role in the biogeochemistry of the world's oceans and transport energy to wider ecosystems. The subseafloor biosphere is an extensive habitat, but microbial abundances are very low. Their distribution and activity are limited by nutrient availability. Sedimentation and the discharge of waste products strongly affect the microbial community composition picked from deep subseafloor sediments. Extreme chemolithoautotrophs are present in oceanic depleted sediments.

High-throughput sequencing has become the major focus of ecological research, with increasing interest in exploring metabolic potential and facilitating reconstruction of metabolic networks. Many general features of microbial metabolomic evolution in the deep ocean have been traced and further identified. [127, 188, 189]

Implications for Ecosystem Resilience

Contributions of Molecular Microbial Diversity to Ecosystem Dynamics and Resilience

Overall, contemporary analyses indicate that microbial genetic variation is a decisive factor shaping ecosystem functions and community stability in natural habitats. The presence of multiple ecotypes with different environmental tolerances and the different metabolic potentials captured by the entire community result in multifunctional ecosystems. Markers for distinct functions (i.e. genes), populations with different endo-/ exosymbiotic relationships, as well as genotype, are also modulated by disturbances and ultimately affect basal ecosystem functioning and stability. Genotypes possessing specific metabolic abilities can enable communities to rapidly respond to changing environmental conditions, offering one explanation for the persistence of microbial diversity during climate changes over Earth's history Scale, environment, and seasonal depth influence the distribution of diversity and its contributions to resilience. Eutrophication, land-use changes, organic pollution, overfishing, and global climate change severely impact surface ocean conditions. These changes affect community composition, oral biodiversity, and metabolic activity and cause functional ruination. However, certain metabolic activities remain undisrupted during these events, indicating differences in community response and adaptation according to season. When undergoing wide-ranging environmental remediation, microbial

biodiversity is known to react adaptively. Within the community, key genera/OTUs with specific metabolic functions become dominant, thereby effectively recuperating community function.

Rapid detection and prevention of wetland degradation are possible through the identification of functional microbial hubs; however, meanwhile, a wider aspect of extracellular microbial activity is constantly being neglected. Microbial AT-rich regions detectably respond to Cu and Pb concentrations at trace levels, enhancing the intention of AT-rich regions as indicator probes for environmental monitoring. Genetic diversity provides a DNA-based indicator and signature for assessment of the ecosystem associated with the share of Archaea and its extreme microhabitats. The necessary dataset is now available; the solutions proposed are ecosystem-oriented and yet embrace a much wider application potential that includes terrestrial, freshwater, and a range of biogeochemical and applied aspects. [190, 191, 192]

Chapter - 13

Microbial Interactions and Community Dynamics

Microorganisms constantly interact with one another and larger organisms, forming networks of relationships that influence their community composition, population levels, activities, and overall metabolism. The various types of interactions include symbiosis (e.g., parasitism and mutualism), competition, and antagonism, and signals resulting from these interactions can result in significant changes in community function. For example, autoinducers are used in quorum sensing, a density-dependent mechanism that is widespread among bacteria and facilitates regulation of community responses. Biofilm formation, communication via excretions and transports, integration with eukaryotic hosts, heterotrophy, and oligotrophy represent key aspects of microbial interactions affecting functions and reservoirs in microbial communities.

Microbial interactions can be studied using network analysis to infer the likely types and strengths of associations between members. This approach has recently been applied in metagenome studies on diverse biomes, including lakes, oceans, beaches, ice cores, and even biological soil crusts. Analyses yielded expected relationships, such as positive correlations of copiotrophs with inorganic pollutants and negative correlations of abundant populations with community efficiency in biodegradation. Microbial succession in community composition shifts during hydrobiological periods, and environmental factors drive the variations rather than intrinsic interactions. [193, 194, 195]

Symbiosis and Mutualism

Symbiosis and mutualism are pervasive yet often underappreciated aspects of biological systems. Microbes engage in a variety of interactions, some of which are directly visible, while others remain cryptic. Quorum sensing mechanisms permit exchanges of information and chemical signals, and analytical frameworks like meta-DNA, RNA, and protein sequencing allow indirect investigation of these important processes in natural communities.

Cooperation, mutualism, or symbiosis-interactions favoring at least one participant without harming the other-occur broadly among various taxa. These concepts are often conflated, even though so-called mutualists may incur costs. In the common spider Pholcus phalangioides, parasites, Sphingobacterium, and filamentous bacteria serve as food sources throughout development but do not contribute to digestion in adulthood. Mutualistic interactions have distinctive features not seen in commensalism or antagonism. Cross-feeding between heterotrophic bacteria enriches for cooperation, enhancing productivity. Importantly, microbes also grow in adversarial settings that promote antagonism leading to virulence. Such studies may reflect cooperation or help test invader hypotheses.

Microbial interactions typically involve small-scale biochemical exchanges, yet meta-transcriptomic sequencing can also reveal potential signaling networks. Each participant secretes specific molecules, which can be grouped according to their density. In one acid mine drainage biofilm community, quorum-sensing signals conjugated at or near a threshold likely influenced pathway usage, metabolic fluxes, and community structure. Networks reconstructed from the corresponding metabolic repertoire define these bacterial communities, and discrete signaling systems enable contextual activity

coordination. [196, 197, 198]

Competition and Antagonism

Microorganisms inhabit complex communities that are shaped by interactions among individuals, members of different communities coexisting in the same habitat, and with environmental factors (called abiotic components), such as nutrients, temperature, pH, moisture, and salinity. Microbe–microbe interactions include symbiosis (benefiting both partners), mutualism (benefiting one partner without harming the other), competition (detrimental for both), and antagonism (benefiting one partner and harming the other). Symbiosis and mutualism can also serve as enablers for other processes that shape microbial communities, such as communication through quorum sensing, signaling networks, and biofilm formation.

Competition, in contrast, is widely accepted as a detrimental interaction among individuals belonging to the same or closely related species, which leads to resource depletion and has a critical role in shaping community assemblage and functionality. Antagonism is a kind of relationship that different groups of organisms develop with microbial species that produce secondary metabolites with antibiotic activity. The inhibition of growth or activities of the antagonized partner may be a consequence of the metabolic products found in culture supernatants or even require physical contact of the antagonizing microorganism, especially in the case of bacterial predators, such as the protozoan group of organisms. Co-cultivation of different bacterial genera has shown that a more exhaustive evaluation of microbial biodiversity in the ecosystem is possible by the application of antagonistic microorganisms associated with culture-dependent techniques. [199, 200, 201, 202]

Quorum Sensing and Communication

Microbial abundance cannot explain community-level behavior. Populations communicate and coordinate activity through small signaling molecules, a process termed quorum sensing (QS). These systems allow cells to assess their population density, acting as a switch to initiate communal behavior only when cells reach a certain abundance. Classes of these signaling molecules vary across taxa but broadly consist of a secreted signal and a cognate receptor that activates transcription of target genes in a cell-density-dependent manner. Many bacteria, especially among Bioinformatics Tools for Diversity Analysis Gram-negs, are known to use such signaling systems. Some secrete acylated homoserine lactones (AHLs) and activate transcription through LuxR-type receptors. QS circuits modulate a range of processes, including virulence, horizontal gene transfer, sporulation, and light production. Fungi, cyanobacteria, and yeast also utilize similar QS circuits.

Signaling networks can integrate several QS systems, including both inducer and response diversity. Gram-positives communicate through peptide-based QS and rely on oligopeptides that enter the cell and induce target gene expression through two-component systems. In many cases, biogenesis is long-range, whereas the receptors can extend shorter distances. These systems influence the production of various secondary metabolites, biofilms, natural competency, virulence, sporulation, and cell–cell interactions. Supporting the diversity of chemical/biochemical signaling systems, other eco-labeling molecules enable establishment and development in plant, fungal, and algal host associations. Such interactions depend on the FP of the community during abroad handshake. Various communication networks underpin the maintenance of bacterial community structure and sharing of metabolic resources. Using

network inference from expression profiles, co-occurrence patterns and prediction accuracy incorporate combined gene regulation stability into community-wide signal transduction.

Biofilm Formation

Cells of many microorganisms are capable of adhering to surfaces and to each other, forming multicellular assemblages with considerable physiological integration, known as biofilms. The biofilm mode of life is widespread and ecologically important, playing a major role in microbial dynamics in natural ecosystems (e.g. rivers, lakes, oceans, and soil) and man-made environments (e.g. drinking water distribution systems, industrial cooling waters, ship hulls). Biofilms are crucial in biogeochemical cycles and are involved in processes such as solar energy capture, carbon fixation, anaerobic and aerobic decomposition of organic matter, metal oxidation and reduction, production of ammonia from organic nitrogen and its recycling, denitrification, sulphate reduction, iron reduction, methane formation, and methane oxidation. These processes are often catalysed by specialist biofilm communities, where the spatial organization of microorganisms in complex three-dimensional structures provides distinct metabolic microenvironments.

Biofilms formed on submerged surfaces in aquatic environments can be viewed as miniature, simplified benthic systems. Microbial cells colonising such surfaces require organic nutrients for growth. Such nutrients may be derived from assimilable organic carbon compounds excreted by the initial colonisers and/or from organic matter in the surrounding water column. In lakes, these compounds may be of riverine or allochthonous origin. There is also substantial evidence supporting the importance of catchment-area hydrocarbons in contributing to the biofilm community structure. [203, 204, 205, 203, 204, 205, 206]

Network Analysis of Microbial Communities

Microbial community structure reflects the processes that generate ecological diversity. Patterns of interactions among species can change over time and are driven by internal community assembly processes and external environmental perturbations. A network approach enables identification of the main functional groups of a microbial community and the interactions between them. A core set of interactions can be characterized as positive, neutral, or negative for the stability of the community and the maintenance of ecosystem functions. These interactions can also reveal whether microorganisms play a keystone role in a community or ecosystem. Different kinds of organisms can be identified as hub, module, or connector species on the basis of their unique features of interconnectivity and richness within the structure. Quorum-sensing systems enable communication and coordination of responses among groups of bacteria, fungi, and plants, supporting multispecies interactions within a robust and resilient biotope. Such analyses of species interactions can help to establish virtual ecological networks, predict ecosystem assembly, and infer ecological roles and functions for poorly characterized groups.

Network analysis of microbial community structure is emerging as a promising tool to analyze complex interaction maps and gain deeper understanding of linkages between species and interactions with metabolic potential. Microbial communities are subject to changes in composition and dynamics as a result of both internal assembly processes and external perturbations, including flooding, nutrient enrichment, and heavy-metal pollution. During their growth, Polysccharide-producing bacteria from the genus Sordariomycetes play an important role in the construction of exopolymeric substances, which can stimulate the growth of other microorganisms by

supplying nutrients, thereby replacing the role of aerobic bacteria and acting as ecological connectors in such a culture system. Analysis of networks and functional interactions can shed light on the relationships among species in different environments and their implications for community functions. [207, 27, 208]

Chapter - 14

Environmental Change and Microbial Genetic Diversity

Environmental changes affect biology and ecology of organisms with different spatial and temporal scales. These changes can also alter the microbial community composition and diversity, disturbing functional groups and changing the functional structure, species richness, and genetic diversity indices of microbial communities. Microbial diversity can respond rapidly to ecological fluctuations, e.g., to climate change, pollution, and human disturbances, and may retain significant signatures of these events.

The impact of climate change on microbial communities has received increasing attention due to the crucial role of microorganisms in modulating ecosystem functions. However, early studies focused mainly on temperature change, while further work has begun to include additional factors. In natural ecosystems, microorganisms interact closely with each other and with other organisms. Quorum-sensing-signal molecules serve as local indicators of state change, leading to community-level macroevolution driven by semicoherence via an epigenetic metabolic memory effect. [209, 27, 210]

Climate Change Impacts on Microbial Communities

Climate change is a well-known phenomenon affecting global weather patterns. Consequently, rising temperatures-combined with other ongoing environmental changes-are impacting terrestrial ecosystems, including soils and aquatic

environments (freshwater and marine). Organisms inhabiting such environments are sensitive to temperature variations, which influence their biodiversity, community composition, functional diversity, and adaptability. Microbes play substantial roles in regulating ecosystem functioning through their contribution to biogeochemical cycling. Recent studies reveal how climate change drives shifts in microbial biodiversity and community structure, with subsequent effects on microbial activity, biogeochemical processes, and feedback to climate change.

Apart from temperature increase, other signs of climate change (e.g., pollution, ocean acidification, and deoxygenation) exert pressure on microbial populations and communities. These organisms function as early-warning indicators of environmental hot spots within ecosystems. In particular, shifts in microbial community composition reflect the cumulative impact of several influencing factors. Despite present-day community potential, microbes can adapt capably to changing environmental conditions. Climate change response mechanisms mainly rely on microbial metabolic diversity and adaptation. Response strategies to climate change and pollution therefore differ among microbial communities in different ecosystems. [211, 212, 213]

Pollution and Anthropogenic Stressors

Climate change, along with pollution and other anthropogenic stressors, profoundly influences the composition and activities of microbial communities in natural ecosystems, ultimately affecting their functioning. As the climate changes, microbial communities experience changes in species richness, evenness, network structure, community membership, and functional profiles. Life cycles of microbes shift due to temperature, such as the accelerated metabolism of plant pathogens leading to premature leaf fall. The resuscitation of dormant spores under warmer/N-enriched conditions enhances

pathogenic behavior. Human-induced global increases in both temperature and nitrogen deposition are predicted to shift bacterial community composition in grassland soils in opposite directions. For soil microorganisms, long-term (22 years) warming modifies taxonomic and functional diversity, with temperature gain affecting specific functional traits. In estuarine and coastal regions, microbial communities respond neither to short-lived climatic forcings nor to seasonal processes, but they experience marked modifications as a consequence of longer-term environmental changes and global human-induced stressors. The response of the bacterial community to urban pollution is more linked to the concentration of heavy metals than to organic matter and nutrients. Microbes are also affected by changes in land use and ecosystem disturbance, such as water pollution and invasion by alien species.

Climate change plays a crucial role in influencing microbial community composition and functional capacity, serving as a relevant predictor of changes related to both nitrogen and phosphorus pollution. Pollutants elicit a rise in distributional density of anthropogenic-concerned opportunistic species, while the functional redundancy decreases. A plastic response of the microbial community to agricultural water pollution is evident, but specific microbiotechnological adaptations (e.g. bioplastic accumulation) are encountered only in select seasons of routine farming. In disturbance-prone environments, the native bacterial community exhibits a resilient behavior. Under long-term fertilization, the microbial community functions and structural patterns in the top-layer soil horizons are remarkably aligned with soils^39ChatGPT 3.5.

Land Use Change and Habitat Disturbance

Climate change, pollution, land use change, and habitat degradation affect ecosystem functioning and stability, including

microbial communities. These changes may shape these communities directly or indirectly by influencing environmental factors like pH, temperature, salinity, moisture, and contaminant concentrations. Microorganisms are highly adaptive to changing environments, and the abundance of specialist populations often reflects the conditions present. However, identifying potential changes in community composition remains challenging. Adaptation of microbial populations to altered climates may be associated with a reduced diversity in community structure. Extreme diversities of microbial communities have been linked to ecosystem health, while diversity loss indicates declining stability. Many microorganisms-particularly in soils-resist contamination, and their presence is an essential characteristic of healthy habitats.

Land use often involves the conversion of forested or natural ecosystems to agricultural or intensive food production systems. Despite the changes in community structure and detection of indicator groups, present levels of diversity remain consistently high. Pollution and contamination vary over time and across the landscape, with the drainage of polluted rivers conveying contamination pulses to downstream areas. These affected locations tend to exhibit shorter-term temporal responses-deviating from land-use conversion frontiers-enhanced by known association and correlation functions among co-occurrences of community members. Other disturbances, such as flooding, also trigger shaped responses, further highlighting the complexity of interactions mediating these biological responses in changing ecosystems. [214, 215, 214, 215, 216]

Adaptive Responses to Environmental Stress

Environmental stress is a crucial and well-studied topic in the field of microbiology, routinely assessed and closely monitored. The lack of predictive capability for microbial responses to

climate change, which acts on several fronts (e.g. heat waves, water scarcity, soil salinity), is a major gap in the knowledge required for the future management and regulation of ecosystems and ecosystem services. Both biogeochemical cycles and higher trophic levels depend heavily on microorganisms. Microbial metabolism represents a major source of greenhouse gases and a key avenue of recovery from pollution stresses. There is also a growing awareness that microbial diversity is both essential for maintaining ecosystem resilience, security and health and indicative of an ecosystem's health status.

Natural ecosystems host diverse groups of microbes that functionally interact with each other and with other groups of organisms, including plants and animals. They also contribute to the bioavailability of essential nutrients and improve care at the human-animal-plant interface. Different microbial species possess different genetic and physiological functionalities that make them appropriate for specific environmental conditions, thus allowing their survival and long-term establishment in extreme environments. In environments exposed to rapid and violent shifts, the species that can withstand them may be different or only a subset of the total present. What remains almost enigmatic is how environmental changes induced by humans and through natural causes affect microbial communities at selected sites around the world.

Microbial Indicators of Ecosystem Health

The impacts of climate change on human life are evident; hence, the emphasis on using enzymes and metabolic products produced by microorganisms at high temperatures (thermophiles) or low temperatures (psychrophiles) in the biotechnological process is increasing. Microbial communities are influenced by natural environmental disturbances (earthquake, landslide, or flood) and human-induced

disturbances (industrialization and urbanization). With the rising population, the number of factories and sewage along river banks and lakes is progressively increasing, which is causing microbes to adapt to degrading conditions. Archaeology is becoming increasingly important for the reconstruction of ancient human and environmental ecosystems. In-depth culturing and screening of unculturable microorganisms at high salt concentrations serve as an important resource for potentially valuable natural products. The analysis of extremophiles sheds light on the exploration of the deep sea and the underground world. The concerns associated with chronic and delayed crises such as climate change, noise pollution, and biodiversity decline are expected to take the place of immediate pollution problems and feature a larger global division of labor. With the growing understanding of environmental change and its effects on biological groups, the genetic mechanisms of microbial response, adaptation, and resilience to external influences are being elucidated, thereby providing references for setting key indicators for ecosystem health.

The community structure, diversity, species richness, function, activity, and activity dynamics of microorganisms are affected by temperature, pH, salinity, moisture, organic matter, nutrients (nitrogen, phosphorus, potassium, iron), heavy metals, and hydrocarbons, as well as their interactions. Under long-term and extreme disturbance conditions, potential indicator species appear at the gene-content level, and the contribution of functions related to adaptation may increase as stress intensifies. With respect to extreme conditions, the response ability and strategy of microorganisms can serve as indicators of extreme degradation, whereas the response ability and strategy of soil microorganisms to moderate disturbances can explain changes in ecosystem function. [212, 217, 212, 218]

Chapter - 15

Applications in Environmental Biotechnology and Conservation

Microbial diversity affects ecosystem functions and services, and this knowledge base can therefore serve a variety of applications in environmental biotechnology. Various microorganisms can alleviate the adverse effects of pollution through bioremediation, where applications of specific biogeochemical functional groups or metabolic capabilities can restore the original community composition and metabolic function. The potential exploitation of microbial diversity for bioaugmentation and inoculation can facilitate the accelerated removal of contaminations. The knowledge of distribution patterns also provides opportunities for biotechnological applications. Microbiomes associated with living organisms can be engineered to yield improvements in human health, agriculture, and biotechnology. Understanding how environmental change alters microbial functions and what mild perturbations are sensed as stress can inform ecosystem conservation.

Microbial diversity pattern alterations are useful as indicators of ecosystem health and climate change impacts. Disturbance-adapted species richness and diversity patterns can be used as early warning signals. Such changes can provide clues toward restoration and help design biobanks for ecosystem recovery. Ecosystem repair or assistance is now the most common principle in restoration ecology and thus contains knowledge-based microbial diversity information of special relevance for

restoration. Microbial priority effects are increasingly receiving attention, and their contribution to ecosystem recovery enhances the significance of predicting and managing microbial diversity in restoration processes. Microbial ecology is on the brink of opening a novel field beyond conservation: microbial base effects and population supply capabilities provide the underpinning ideas for ecosystem engineering and actual ecosystem repair. [144, 219, 220, 221]

Bioremediation and Bioaugmentation

Integrating microorganisms in the bioremediation of polluted ecosystems can speed up the degradation of the contaminants. Two major types of bioremediation, biostimulation and bioaugmentation, can be identified. Biostimulation is the supply of nutrients to enhance the growth of the indigenous microorganisms, and bioaugmentation is the addition of natural or engineered microbial communities, which have the intrinsic capability to metabolize these compounds, to the contaminated sites. In polluted areas for which a thorough biochemical knowledge of the contamination is available, the natural populations of indigenous microorganisms can be isolated, characterized, and employed to initiate the degradation processes. For this, significant genomic and ecological information should be screened in advance. These approaches are frequently used in oil-contaminated, heavy metal-polluted, and organophosphate-polluted ecosystems.

Using naturally selected microorganisms or engineered microorganisms or enzymes in bioremediation can reduce the time required for the bioremediation process and transform otherwise hazardous compounds into less toxic compounds. Bioaugmentation also helps in restoring the microbial diversity of the polluted site. Many pollutants can also be bioremediated indirectly. Several microbial model organisms (e.g.,

Pseudomonas, Shewanella) have been employed in developing biotechnology-based products and bioengineering techniques to mitigate various types of environmental pollution. Methods developed in synthetic biology can synthesize engineered microbial consortia for bioremediation. The use of synthetic ecosystems allows researchers to clarify the key functional microorganisms and metabolic pathways in pollutant degradation. [222, 223, 224]

Microbial Resources for Biotechnology

Microorganisms and their metabolic capabilities can be harnessed directly or incorporated into synthetically designed systems for biotechnological applications. Several biotechnological processes and products currently utilize microorganisms. Metabolites and biomass from specific strains or natural microbial communities are currently used. Microbial strains encoding specific enzymatic and metabolic capabilities are applied for bioconversion, bioremediation, or bioconversion and act as biocontrol agents. Members of natural microbial communities can also be applied either without any supplementation or as inoculum for processes such as aquaculture, fermentation industry, bioremediation, or recovery of metal values.

Information generated through genetic diversity-based investigations can be linked with the corresponding biosynthetic, catabolic, and metabolic pathways associated with target bioproducts for applications in synthetic biology. Recent advances in sequencing technology and subsequent developments in gene detection, annotation, or reconstruction pipelines enable rapid identification of genes with biosynthetic, catabolic, or metabolic capabilities present across diverse environmental niches. Further, specific gene orthologs can also be expressed or integrated in robust host systems to reestablish

the natural functions. In addition to natural products, microbes have been utilized to convert feedstock to biofuels as well as biochemical products. Genetic resources for these applications have been derived from both culture-dependent and culture-independent sequenced databases.

Increasing socioeconomic and political pressures have triggered the exploration of unchartered natural resources for potential Biotechnology Applications (BTA). Despite having enormous industrial potential, microorganisms inhabiting extreme terrestrial habitats are still unexplored due to limited microbial genetic resources in biotechnological repositories and their inability to grow in standard laboratory conditions. Additionally, adaptation to extreme habitats has been hypothesized to influence the distribution of certain specific gene families. Consequently, insights into the genetic potential of these microorganisms can direct the development of biotechnological applications. Recent advances in sequencing technology and computing power have enabled discovery of information even from poorly represented ecological niches. Hence, elucidating genomic information from severely undersampled extreme-conditions such as hyperacidic subterranean habitats offers an opportunity to discover in nature encoded biotechnological resource.

Conservation of Microbial Diversity

Microorganisms play major roles in all ecosystems, including their evolutionary history and metabolic functions. If these roles are disturbed, ecosystem processes will be altered, and thus also the services they provide to life on Earth. New technologies have made the analysis of microbial diversity in natural environments more accessible, and the assessment of diversity and functions has many potential applications, including conservation. To use microorganisms for biotechnological applications or restoration,

knowledge about their community structure and genetic capabilities is essential. The conservation of natural ecosystems can also aid in the preservation of microbial diversity and its potential applications.

Microorganisms can directly influence the resilience of ecosystems and indirectly indicate ecosystem health. Microbial genetic variation has been suggested as an early warning system for detecting environmental disturbances. Changes in microbial diversity may indicate shifts before changes in higher trophic groups. Environmental microorganisms are among the first to respond to changes in environmental conditions. Microbial communities not only respond to these disturbances, but they can also be used for the mitigation of damage: bioremediation is a good example. Pilot studies involving bioremediation for arsenic or oil degradation by microbial inoculants have shown promising results in test plots but need to be validated in real-life situations. [225, 226, 227, 225, 226, 227]

Ecosystem Restoration Strategies

Microbial diversity plays a critical role in ecosystem structure and function, including human well-being. Nevertheless, diverse environmental stressors and climate change have altered microbial communities and ecosystem health. To conserve microbial diversity and the services it provides, conservation approaches should aim to minimize environmental disturbance. At the same time, the ecological principles governing functional microbial diversity should be integrated into ecosystem restoration strategies. The microbial community composition, and hence its ability to carry out important functions, is also intrinsically linked to the health of the ecosystem. Restoration of these ecosystems should incorporate the specific requirements of microbial communities for biological recovery.

Water quality can be improved through bioremediation

processes such as bioaugmentation, biostimulation and heterogeneous bioremediation, which enhance the functional diversity of microbes. Oil-degrading consortia enriched with native indigenous microorganisms from oil-contaminated environments have been effective for oil spill clean-up operations. Moreover, other microbial resources can also be employed for novel environmentally friendly green technologies. Continued effort is therefore needed to explore, isolate and characterize novel microbial species, particularly through the adoption of recent advancements in sequencing technology and systems biology, thereby potentially generating a microbial list of future microbial factories capable of biotechnological innovations. [229, 230, 231]

Policy and Management Implications

The rapid advances in deep sequencing technology, coupled with increasing recognition of the critical roles of microorganisms in modulating ecosystem processes, have spurred an exponential rise in the application of microbial ecology in diverse areas. For instance, functional metagenomics allows the discovery of novel enzymes, microorganisms serve as bioindicators of environmental stress and health, and bioremediation studies engage microbial communities for environmental management. As society increasingly looks to microorganisms for sustainable solutions to overcome environmental challenges, the loss of microbial diversity through pollution, habitat disturbance, and climate change poses major concerns. Yet many microorganisms remain uncultured and poorly characterised; their environmental distribution, evolutionary patterns, and ecological functions are only beginning to be understood. Given that more than half the biosphere's genetic diversity resides in uncultured microorganisms, an improved understanding of microbial

diversity is essential to predict ecosystem response to environmental changes or perturbations.

The need to conserve and restore microbial biodiversity is widely recognised. While such initiatives were initially focused on promoting the conservation of macroorganisms, ecosystems clearly require the services provided by diverse microbial communities for their continued survival. Protecting ecosystems will, therefore, indirectly safeguard microorganisms and the services they provide. The advance of molecular ecology is also allowing the microbially driven processes sought in restoration to be better understood, thereby enabling the incorporation of microbial principles in restoration planning. [232, 233, 234, 235]

Chapter - 16

Future Perspectives and Emerging Technologies

Recent years have witnessed the rapid emergence of novel techniques and technologies in microbial ecology. Single-cell genomics has enabled uncultured microorganisms from previously intractable environments to be physiologically and metabolically better understood, while advances in sequencing technology and bioinformatics have prompted a rapid increase in high-throughput metagenomics and -omics studies. Coupled RNA and protein expression abundance data can now allow for a better understanding of microbial activity networks. Metatranscriptomics and metaproteomics can provide definitive indicators of microbial roles in ecosystems and their adaptive responses to environmental change rather than relying solely on inferred functional signatures from metagenomic studies. Multi-omics integration provides holistic insight into the functional mechanisms of community assembly and interactions in diverse environmental and engineered settings. The roles of artificial intelligence and machine learning technologies have also been expanding across all scientific domains, including microbial ecology. However, not all applications of deep learning in microbiome research have advanced knowledge development; uncritical training and testing datasets with mixed literature anomalies and artefacts tend to generate misinterpretations of underlying patterns and concreted beliefs. Furthermore, the pursuit of knowledge surrounding synthetic ecology continues to mature and develop. Debates over pathogen development persist new ethical concerns, including the potential risk of Ebola- or

other infectious pathogen-based bioweapons. Finally, although the vast majority of microbes in ecosystem samples remain uncultured, the application of study methodologies is gradually entering Europe.

In addition to actively using the available toolbox of next-generation sequencing technologies for microbiota analysis, future efforts also focus on expanding it further, especially using a metatranscriptomic and metaproteomic approach in concert with metagenomics. The application of AI is being developed and incorporated, especially in building machine-learning classifiers driven by balanced and validated datasets and integrated solutions for specific questions. Such methods will involve robotic or deep-learning-assisted identification of candidate key OTUs, signalling pathways, and other available parameters in physical and chemical data preceding model establishment. Comprehensive discussions on SCG approaches for specific study questions are also informing future applications. Synthesised and metagenomic data inform artificial-life development discussions and exploration of biotechnology applications, such as novel antimicrobial therapy developments that would enhance antipharmaceutical properties without altering normal human flora.

Single-Cell Genomics

The sequencing of single cells opens a new avenue for exploring the yet-to-be-cultured fraction of environmental microbes. The earlier strategy of directly sequencing genomic DNA from single cells has been improved, permitting the sequencing of large and complex genomes. Using several of the latest approaches makes it possible to obtain 15% of genomic information of any cell in a suitable environment (those that match with large populations with environmental samples). These developments have established single-cell genomics as a

primary tool for access to microbial dark matter and for the biological interpretation of environmental metagenomic data by permitting the filling of the genomic information gaps in the environmental metagenomic sequences.

More recent applications show that it is also possible to obtain active single-cell genomes through metatranscriptomic sequencing using PCR amplification employed to produce a metaparasequence of the amplified transcript of a single cell. The combination of approaches, metagenomics, metatranscriptomics, and single-cell genomics, now enables population-level data acquisition for the uncultured majority of active microbes in the environment. The approaches lay the foundation for systematic investigations of the largely unknown fraction of active and/or abundant but previously recalcitrant microorganisms.

Multi-Omics Integration

Integrates transcriptomic, proteomic, and metabolomic data into metagenomic frameworks, facilitating a holistic grasp of ecosystem functioning. Transcriptomic and metaproteomic data elucidate functional activity corroborating metagenomic insights, while combining omics-layered information fuels a comprehensive understanding.

Metagenomic frameworks decode ecosystem gene abundance and content, triggering bursts of exploratory interest. Nevertheless, functional content represents only latent potential; expression dynamics, governing protein activity manifesting in biochemical events, require additional focus. Functional metatranscriptomic inquiries endeavor to clarify transient expression shifts-augmented by metaproteomic data chronicling biochemical activity progresses. Integrating such insights into metagenomic backgrounds intensifies comprehension of environmental functioning. These emergent spheres exploit three-dimensional gene architecture: metagenomic templates,

metatranscriptomic spatiotemporal expression matrices, and metaproteomic biochemical activity maps.

Transcriptional specialization remains temperamental: while certain categories warrant exhaustive RNA detailing, others receive scant representation in metatranscriptomic sketches; however, a backdrop of activity-driven processes and transcript parallels centered on targeted elements presents a coherent window. Augmenting this three-dimensional stratified approach unlocks even greater understanding of ecosystem function. Increasingly lucid description of nuclear skeletons convulsing through data-processing flows transition this lens into an operational embedding.

AI and Machine Learning in Microbial Ecology

Artificial Intelligence (AI), Machine Learning (ML), and potentially Deep Learning (DL), are rapidly revolutionising many fields, including Microbial Ecology. Increasingly more studies employ AI to examine microbial community structures, examine the distributions of labile substrates, predict metabolic capabilities, randomise sequencing data, improve biosynthetic gene identification, and predict the impact of biogeochemistry and community dynamics on ecosystems. Artificial Neural Networks (ANN) and Support Vector Machines (SVM) are the most widely used existing supervised models. In particular, ANNs have offered advances in toxin prediction and have the capacity to model global-scale empirical relationships in Microbial Ecology.

In general, supervised ML methods require training datasets to predict existing properties or hidden relationships among multiple microbial features. Often, an environmental variable of interest acts as a predictor. When ecological field data is deemed insufficient to capture environmental features, randomisation is employed either by resampling or through hydrodynamic

modeling. These missing features are then interpolated and estimated based upon the rest of the variables using common regression models such as ANN, SVM, Random Forest, Canonical Correspondence Analysis, or Classical Regression. These models multiplex local field data to provide supportive knowledge for possible co-occurrence clusters and potential relationships, indicating a trajectory for research and hypotheses testing. However, for predicting the distribution of labile substrates, only two artificial intelligence-based studies are available.

Synthetic Ecology Approaches

The recent advances in technologies for studying microbial communities at the molecular level have revealed high novelty and complexity in their composition and functions. These findings open an era toward the synthetic use of natural microbial diversity for applications in different areas of environmental biotechnology. Systems based on microbes are generally more efficient for environmental remediation but suffer in their robustness during production or application because of the inability to adequately modulate the composition of their microbial communities. Synthetic ecology aims to overcome this problem by building optimized communities for well-defined applications. For this approach, fundamental concepts and considerations derived from microbial ecology are critical, such as the choice of ecological strategy (r/K selection), the selection of key function(s) for engineering and the establishment of appropriate bioreactors or devices with adequate operational conditions.

In addition, for successful application, further knowledge on the genetic and metabolic diversity of natural populations is necessary to provide the appropriate building blocks and a sufficient supply strategy, as well as an analysis of the

environmental impact of the application to environmental health. Microbial information capable of supporting the development of synthetic ecology is abundant in the current scientific literature, encompassing aspects related to metabolic and functional community profiles, responses and adaptations to anthropogenic and global change, as well as interaction and network patterns. The proper integration of such diverse information should allow the construction of synthetic ecology systems with increased robustness and versatility for diverse applications.

Future Challenges and Research Directions

Various biotechnological, ecological, and climatic challenges created by human activities require mitigative efforts focused on restoration and management of environmental ecosystems. To ensure ecological health and sustainability, innovative concepts, technologies, and applications derived from the foundational knowledge of microbial diversity in natural ecosystems are being actively employed. A biotechnology-related future research direction involves the search for innovative microbial strains capable of effectively degrading diverse pollutants. Biodiversity-related studies emphasize assessment of ecological health and biological responses to climate change, pollution, land use change, and other anthropogenic stresses. Efforts are ongoing to identify microbial indicators of environmental quality, adaptive strategies employed by microbial communities, community responses to ecosystem stressors, and response mechanisms of key metabolic pathways. These studies may help evaluate present and future pollution level changes, community responses, and potential gene expression and functional alterations in response to external perturbations.

The development of emerging technologies has made possible the exploration of scarcely abundant and uncultured taxa, metabolic behavior and functions of microorganisms

engaged in specialized ecological processes, and microbial interaction patterns in natural communities. Integration of new technologies for simultaneous interrogation of different informational layers and artificial intelligence-assisted analysis will further contribute to improved understanding of microbial roles in ecosystem dynamics and enable competent elucidation of the relationships between genetic diversity and ecosystem functioning. New and unanticipated biological connections are constantly appearing in nature, and an understanding of these discoveries and the underlying principles will guide microbial ecology research in the future.

Conclusion

Evidence-based molecular analyses show that genetic variation is an essential precondition sustaining their population dynamics and ecosystem functions. While the frameworks of biogeography and ecology have formed the historical basis for understanding patterns of diversity in natural ecosystems, the creation of explicit concepts of genetic diversity and of analytical tools directed at the genetic scale have revolutionized the study of the biology and ecology of natural ecosystems in recent years. This research activity has brought an unprecedented understanding of the genetic ecological and biogeographical principles determining how environmental change influences diversity at the genetic level in natural populations, and in turn, how such diversity underpins the functioning of ecosystems. More than that, it has provided a platform for seeing such change-now operating at increasingly short timescales-as fundamental in re-shaping and, more strikingly, collapsing the conditions governing ecological support for life.

The rapid advances in molecular techniques-the ability to access the actual DNA, RNA and protein data of microorganisms and utilize these data to investigate populations in their natural habitats-have provided strongly new perspectives on microbial ecosystems with exciting possibilities. These so-called meta-omics techniques provide the capacity to study composition and diversity, metabolic activity, and functional potential of whole communities at high spatial and temporal resolutions; a similar facility can also be applied to assess aspects of the associated environment-such as chemical analyses of soils or waters coincident with biological assessment of sediment cores for example-affording the perspectives on functional ecology.

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