

# Microbial Adaptation to Climate Change Stressors Revealed by Environmental Metagenomics: Insights and Perspectives

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## Article Info

### Article history:

Received : 15.04.2025

Revised : 19.05.2025

Accepted : 24.06.2025

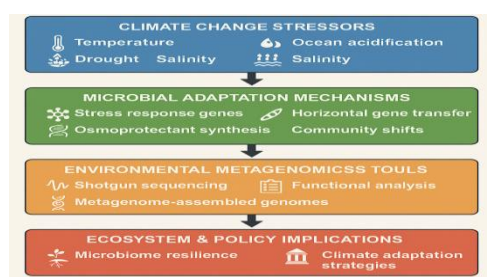
### Keywords:

Environmental Metagenomics,  
 Climate Change Stressors,  
 Microbial Adaptation  
 Mechanisms,  
 Horizontal Gene Transfer,  
 Metagenome-Assembled  
 Genomes (MAGs),  
 Microbiome Functional  
 Diversity,  
 Stress Response Pathways,  
 Ecosystem Resilience,  
 Biogeochemical Cycles,  
 Multi-Omics Integration

## ABSTRACT

The microorganisms play a central role in the regulation of the ecosystems on the Earth, controlling the cycles of nutrients, carbon storage and ecosystem stability. Nevertheless, increasing speed of climate change exposes microbial communities to numerous stressors, which include warming, changes in precipitation, drought, salt levels, and ocean acidity, as well as, the buildup of pollutants, which have significant impacts on microbial community structure and functionality. Knowing how microbes can respond to these stressors is crucial to understanding how the ecosystem will react and how climate change can be reduced. Environmental metagenomics has become an innovative instrument of investigating the genetic potential, evolutionary approaches, and functional resilience of microorganisms in a variety of environments, such as soils, freshwater, oceans, and harsh environments. The review is a synthesis of the recent developments in the field of metagenomic applications to reveal the microbial mechanism of adaption to stress by the expression of stress-response genes, horizontal gene transfer, mobile genetic elements, and metabolic restructuring as a response to climate-related perturbation. We put emphasis on case studies to show how microbial community structure changes, functional genes become enriched, and biogeochemical feedbacks change due to environmental change. These methodological frameworks are shotgun metagenomics, metagenome-assembled genomes (MAGs), and multi-omics methods, all critically reviewed in order to highlight their limitations and strengths. In addition, such issues as unfinished functional annotation, sampling bias, and computational limitation are also addressed in the context of extrapolating microbial understanding to predictive ecosystems. In the future, we highlight the need to combine environmental metagenomics with longitudinal surveillance, machine learning, and synthetic biology and improve our knowledge of microbial resilience, ecosystem stability, and climate adaptation mechanisms.

## 1. INTRODUCTION



**Fig. 1.** Conceptual framework of environmental metagenomics for microbial adaptation to climate change stressors.

*The Diagram shows climate change stressors (temperature, drought, salinity and ocean acidification) to microbial adaptation mechanisms (stress response genes, osmoprotectant synthesis, horizontal gene transfer and community shifts), to environmental metagenomics tools (shotgun sequencing, functional analysis, and metagenome-assembled genomes) and lastly their implications on the ecosystem and policy (microbiome resilience and climate adaptation strategies).*

Microorganisms are the pillars of life on the planet and are an essential factor in controlling the biogeochemical cycles on earth such as the carbon, nitrogen and sulfur cycles. Microbial communities

have a significant impact on climate feedback processes and ecosystem stability through carbon fixation processes, methane oxidation processes, nitrification and denitrification processes, as well as sulfur reduction processes. Any change in the activity of the microbes can thus trickle down to the extensive ecological and climatic effects. The increased rate of climate change is putting unprecedented stress on the microbial ecosystems. Global warming, frequent heat waves, drought, salinity, acidification of the oceans and build-up of anthropogenic pollution are some of the stressors that modify the survival mechanisms and metabolism of microbes, as well as their interactions and interactions within the community. Such perturbations do not only change the structure and diversity of microbial communities, but also cause changes in functional patterns that influence nutrient cycling, greenhouse gas emissions and general ecosystem resilience. A summary of the interaction between climate change stressors, microbial adaptation strategies, metagenomic strategies, and ecosystem scale consequences is shown in Figure 1.

Traditional microbiology, mostly culture-oriented has given valuable data on the physiology of individual microbes but is restricted in its extent. It is approximated that fewer than 1 percent of microorganisms are cultivable in laboratory circumstances leaving the rest of the microbial diversity untapped. Such culture bottleneck has been historically limiting on our knowledge of responses and adaptation of microbial populations to environmental stresses both at community and functional scales. Environmental metagenomics has overturned this situation by providing the capability to directly sequence the sum of total community DNA in an environmental sample without prior growth. The method offers the first access to the so-called microbial dark matter as it enables researchers to define taxonomic diversity, functional gene repertoires and adaptive strategies in vivo. Recent developments, such as shotgun sequencing, metagenome-assembled genomes (MAGs) and integrated multi-omics methods, have provided new opportunities to understand the mechanism of adaptation of microbes to climate change stressors. This review is three-fold: (i) critically review how environmental metagenomics has been used to demonstrate how microbial responses to climate stressors are functional, (ii) to synthesize the main findings connected to the functional responses including stress-response pathways, horizontal gene transfer, and metabolic reprogramming, and (iii) to discuss the current limitations and future directions of applying metagenomic applications to the predictive ecosystem model. Comprising the insights of the recent investigations, this review

creates a holistic view of the phenomenon of microbial resilience and their possible contribution to the elimination or enhancement of the effects of climate change.

## **2. Climate Change Stressors and Microbial Responses**

Microbial communities are very sensitive to environmental perturbations and are sensitive to changes in climate change and adapt in a diverse way. The metagenomic strategies have shown taxonomic change as well as functional change that helps the microbes to survive in the dynamic and often hostile environments. The temperature extremes, ocean acidification, salinity and drought, and the buildup of pollutants are considered the key stressors on the level of microbial ecosystems.

### **2.1 Temperature Extremes**

Temperature is a vital parameter that can have an impact on microbial physiology, metabolic activity and community stability. High temperatures activate the expression of molecular chaperones and heat shock proteins which preserve the cellular structure and enzyme with denaturation. Genome streamlining which entails the elimination of unnecessary genetic material has also been reported in thermophilic organisms, which enables them to utilize energy effectively in the face of heat stress conditions [1]. Thawing of permafrost due to the temperature change is another significant aspect of change because the permafrost contains previously frozen communities of microorganisms that are thousands of years old. The metagenomic study of thawed permafrost has revealed new genes related to stress resistance, antibiotic resistance and carbon metabolism. These microbial communities are not only able to adjust to the present warming but also lead to the emission of greenhouse gasses especially methane that cause a feedback mechanism that further enhances climate change [2].

### **2.2 Ocean Acidification**

An increase of atmospheric CO<sub>2</sub> leads to acidification of oceans with severe impacts on the marine microbial ecosystem. Acidification conditions affect calcifying organisms and change microbial communities of nitrogen fixation and carbon cycling. Studies of the microbiome show that in microbial communities with low PH, compositional restructuring occurs, in which taxa that can tolerate low PH environments are favored. Genes that encode proton pumps and membrane transporters are over-represented functionally indicating adaptations to ensure intracellular homeostasis of pH [3]. These changes may disrupt the marine food webs and affect the biogeochemical cycling of the oceans.

### 2.3 Salinity and Drought

Both the terrestrial and aquatic environment are becoming more and more prevalent to salinity changes and drought stress. Halophilic microorganisms respond by producing osmoprotectants like glycine betaine, trehalose and ectoine as well as specific ion transport systems that maintain intracellular osmotic equilibrium [4]. Microbiomes of soils in long-term drought conditions also have a low taxonomic diversity but are enriched with drought-tolerant taxa, such as Actinobacteria and Firmicutes. Arid soil metagenomic profiles suggest that there is an up-regulation of antioxidant enzyme encoding genes, sporulation genes and DNA repair protein encoding genes that result in resistance to desiccation stress [5], [8]. These adaptation characteristics guarantee the persistence of microbes and functional stability of the water-restricted environments.

### 2.4 Pollutants and Anthropogenic Stressors

The human practices associated with anthropogenic activities enliven the introduction of pollutants like oil and pesticides, as well as, heavy metals into the natural ecosystems. The response of microbial communities is through the activation of hydrocarbon catabolic pathways and also increased heavy metal resistance genes such as efflux pumps, metallothioneins, and redox enzymes [6], [9]. Notably, horizontal gene transfer (HGT) is a key factor in enhancing a faster response of the microorganism to pollutant stress. Plasmids, phages and other mobile genetic elements enable rapid transport of resistance genes to different taxa leading to community level resilience [7], [10]. The adaptive behaviours that were indicated by these metagenomic surveys are indicative of the wonderful ability of microbes to withstand anthropogenic stress and also bring up the issue of resistance trait diffusion.

### 3. Environmental Metagenomics: Methods and Approaches

Environmental metagenomics includes a collection of techniques that allow culture-neutral study of microbial communities, and which offer information about both taxonomic and functional potential in response to stressors of climate change. Recent development of sequencing technologies and bioinformatics has added to the toolkit of methodologies an untargeted, or randomized, approach to DNA sequencing, all the way to integrated multi-omics methods. All approaches make their own contribution to the study of the microbial adaptation mechanisms and the combined approach provides a detailed perspective of the microbial resilience in dynamic environments.

### 3.1 Shotgun Metagenomics

Shotgun metagenomics is a technique that sequences the whole set of DNA that has been extracted out of environmental samples, giving a resolution in depicting microbial taxonomic composition as well as functional gene repertoires. The shotgun sequencing in contrast to the targeted marker methods (e.g., 16S rRNA sequencing) defines the entire range of microbial diversity, such as rare taxa and novel genes. The approach has extensively been used to investigate microbial responses to climate-related changes, e.g. ocean acidification and soil warming. As an example, the metagenomic studies of acidified seawater by shotgun methods have identified enrichment of genes of proton transport and pH regulation, whereas warming of soils has reported changes in genes of carbon and nitrogen cycling. These results indicate that shotgun metagenomics can be used to expose the relationship between climate stressors with alterations in microbial functional capacity at an ecosystem level.

### 3.2 Metagenome-Assembled Genomes (MAGs)

The reconstruction of near-complete and partial genomes of microbes in mixed communities with metagenomic data is an essential development of metagenomic analysis: Metagenome-assembled genomes (MAGs) are a category of genome reconstructions that are constructed from mixed community data. MAGs enable researchers to attribute functional attributes to particular microbial taxa including hitherto uncultivable organisms by binning sequencing reads into genome bins. The MAG based studies have identified new stress adaptation genes, including CRISPR defense system, multidrug efflux pumps and oxidative stress enzymatic systems that are essential in helping microbes to survive subjected to climate stressors. Notably, MAGs offer evolutionary understanding of the approaches that microbes use to respond to environmental pressures resiliently, which aids in determining lineage-specific adaptations and genomic innovations to environmental change.

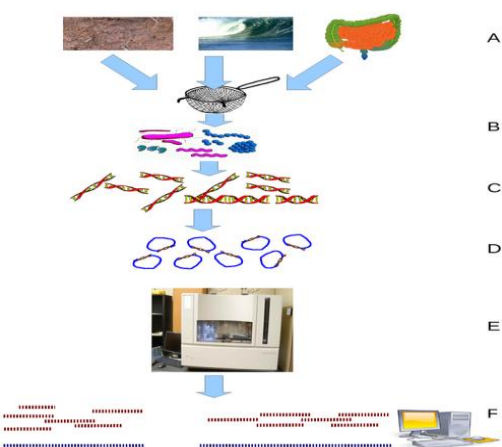
### 3.3 Comparative and Functional Metagenomics

The comparative and functional metagenomics methodologies complement sequence-based methodology because they are concerned with understanding gene functions in various conditions. Comparative metagenomics are cross-sample analyses, which enable the researcher to determine the difference in gene abundance between the control and stressed ecosystems. This has found application especially in the identification of enrichment of stress-response genes in drought prone soils or in aquatic systems that have been contaminated by pollutants.

Instead, functional metagenomics extends beyond that of identifying sequences by determining the traits of adaptive functions, which are then expressed in host systems and used to screen through environmental DNA fragments to screen for desired adaptive features, enzymes that confer saline tolerance or ability to degrade contaminants. The combination of these methods allows getting both correlational and experimental support of the adaptation of microbes to climate stressors.

### 3.4 Integrated Multi-Omics Approaches

Although metagenomics can offer a lot of valuable information on the potential of good genes, it fails to capture dynamic gene expression and metabolic activity. Integrated multi-omics, such as metatranscriptomics, metaproteomics, and metabolomics, can be used to supplement DNA-based technologies to show the active transcriptional and biochemical responses. As an example, metagenomics coupled with metatranscriptomics has implicated transcriptional activation of genes in nitrogen metabolism as a response to warming stress in agricultural soils as well as metaproteomics has established protein-scale responses to ocean acidification in ocean-dwelling microbial communities. These findings are further associated with the end-product pathways, e.g. osmolytes production or antioxidants production with metabolomics. Combined, multi-omics integration can offer a systems-wide view, where genetic capacity is measured and the functional response of microbial communities to climate change stressors can be obtained in real-time. Figure 2 summarizes the sequential steps that were followed in environmental metagenomic research, starting with sample collection and culminating into functional and multi-omics integration.



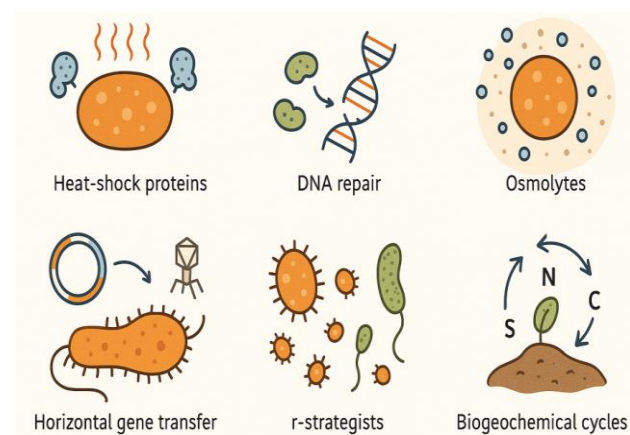
**Fig. 2.** Environmental metagenomics workflow.

*Flow diagram of metagenomic analysis: (A) soil, marine, and gut samples are collected; (B) microbial*

*community is isolated; (C) DNA is extracted; (D) clones or fragments are made; (E) sequencing; and (F) bioinformatic analysis is done. This figure represents the order of experimental and computational operations in metagenomic research, which is an additional display of the workflow to Figure 3 in the body.*

### 4. Key Insights into Microbial Adaptation Mechanisms

The metagenomic research has been a rich source of information on the various approaches that could be used by microorganisms to counteract climate change conditions. These adaptations occur on a variety of levels that include genetic and functional changes as well as ecological reorganization of microbial communities that have an effect on global biogeochemical cycles. Figure 3 depicts the significant microbial adaptation strategies that can be found through metagenomic studies.



**Fig. 3.** Microbial adaptation mechanisms revealed by metagenomics.

*An example of the major microbial adaptive responses to climate change stressors (including genetic and functional), horizontal gene transfer and mobile elements, ecological processes (r-strategists), and biogeochemical consequences (carbon, nitrogen, and sulfur cycling).*

#### 4.1 Genetic and Functional Adaptations

Genetic and functional changes can be considered as one of the main mechanisms of microbial adaptation to increase the stress tolerance. The prevalence of heat shock protein genes, DNA repair protein genes, and osmolyte biosynthesis pathways has been shown by environmental metagenomics. These genes can allow microbes to stabilize proteins, recover damage of their DNA due to stress and adjust intracellular osmotic pressure to changing temperature and salinity. Furthermore, there have been expansions in the number of gene families involved in managing



oxidative stress e.g. superoxide dismutases and catalases in communities subjected to large amounts of UV radiation and drought. Likewise, polluted environment has been observed to enrich xenobiotic degradation pathways, a fact that highlights microbial ability to process complex and in most instances, toxic compounds. These results contribute to the concept of functional versatility of microbial genomes as a driving force of resilience in shifting ecosystems.

#### **4.2 Horizontal Gene Transfer and Mobile Genetic Elements**

The other essential adaptation mechanism is horizontal gene transfer (HGT) which enables the quick obtaining and sharing of stress-response characteristics. Mobile genetic elements, such as plasmids, phages, and transposons, are used in the transfer of functional genes among the microbial taxa. Co-transfer of antibiotic resistance and heavy metal resistance genes has been reported in environments where the environments are subjected to anthropogenic stressors. This indicates that not only are individual populations of microbes able to adapt but they can also share genetic material on the level of a community, which increases the level of resilience. HGT is dynamic and this enables microbial ecosystems to react to environmental pressures faster than adaptation to such pressures based on mutation and vertical inheritance.

#### **4.3 Community Shifts and Ecological Strategies**

In the community scale, the metagenomics has shown changes in the microbial ecological strategies during the various climate stress regimes. r-strategists, fast growing species with high reproductive rates, were likely to dominate in those environments where the stressors change, or become unpredictable and thus enable the quick use of available resources. In contrast, slower-growing but more resource-efficient K-strategists continue to exist in the ecosystems that are under chronic or long-term stress. An example of high tolerance to and rapid reproduction is the dominance of Cyanobacteria in high-temperature freshwater lakes, which offers an ecological advantage due to its high tolerance to heat stress and rapid reproduction. These local level adaptations put more emphasis on the plasticity of the microbial ecosystems in preserving functional stability during changing climatic conditions.

#### **4.4 Biogeochemical Implications**

In addition to genetic and ecological adaptations, microbial reaction to climate change has far reaching implications to biogeochemical cycling. Thawing permafrost has been studied through metagenomics and shown to undergo changes in

the metabolism pathways of methane including an enrichment of the methanogenesis-related genes that enhance the emission of the greenhouse gases. This type of microbial activity is a very important positive feedback loop, because the released methane further increases global warming. Equally, ocean acidification was also demonstrated to affect microorganisms of nitrogen cycling, where changes in the nitrifiers and denitrifiers abundance altered ecosystem nitrogen fluxes. The results demonstrate that the microbial adaptation processes are not limited to the issue of survival, but they include active reorganization of the elemental cycles that govern the climate system on Earth.

### **5. CHALLENGES AND LIMITATIONS**

Regardless of the impressive advancements into the field of environmental metagenomics, there are a number of obstacles and constraints that prevent the exploitation of the technique to its fullest extent in the discovery of microbial resilience to climate change stressors. These constraints exist in biological, methodological and computational levels and the solution of the same is essential in order to develop the field. Incomplete functional annotation is one of the most challenging issues. Over 40 percent of genes found in metagenomic datasets are often described as being in the category of hypothetical proteins or have no known function to be placed into a recognized functional pathway. This knowledge gap limits our understanding of microbial adaptive responses in its entirety (particularly when addressing a new or extreme ecology when reference genomes are limited). Consequently, the ecological and evolutionary relevance of a significant portion of microbial genes has not been determined, rarely being incorporated in climatic response models.

The other weakness is the gap in data integration where the abundance of genomic information is not successfully connected to ecological and environmental data. Although metagenomics can give information about the diversity of microorganisms and their capacity to perform functions, these data must be related to ecosystem-scale processes, e.g. carbon circulation, greenhouse gas emission, or nutrient cycling, which also involve cross-disciplinary modeling. The absence of standardized models to combine microbial data and ecological or climate simulations is still a major hindrance to predictive knowledge. Interpretations are complicated by the sampling bias. Most of the metagenomic research is done on relatively easy to reach environments like agricultural soil, surface waters in the ocean, or a wastewater system whereas extreme environments, such as deep-sea vents, polar ice, deserts, and high-altitude ecosystems, are

underrepresented. This biased sampling leaves us with blank areas about the details of global microbial variety and adjustment. Additionally, having biases with time, when samples are taken at single time points, not seasons, or events across seasons, does not allow us to study dynamic responses of microbes to long-term stressors. Lastly, this discipline has got enormous computational needs in terms of large scale metagenomic analyses. The raw data produced through shotgun sequencing are terabytes that are processed with high-performance computing (HPC) and sophisticated machine learning pipelines in order to complete the process of assembly, annotation and comparative analysis. Numerous research organizations, especially in

resource-constrained environments do not have such computing facilities. Also, the lack of universally validating bioinformatics pipelines simply results in methodological variations that do not make cross-comparisons and meta-analysis challenging. Table 1, gives an overview of these key methodological and computational challenges. They collectively highlight the importance of better reference databases, standard protocols, the combination of multi-omics and ecological models, and fairness in access to computational resources. Addressing these drawbacks will be crucial to the progress of the predictive capabilities of environmental metagenomics on the subject of microbial resilience to a shifting climate.

**Table 1:** Challenges and Limitations in Environmental Metagenomics for Climate Change Studies

Challenge	Description	Potential Solutions
Incomplete Functional Annotation	>40% of genes remain uncharacterized, limiting understanding of adaptive pathways.	Expand reference genome databases; employ AI/ML-based functional prediction; increase experimental validation.
Data Integration Gaps	Difficulty linking metagenomic insights with ecosystem processes and climate models.	Develop standardized frameworks; integrate with ecological and biogeochemical datasets; adopt systems biology approaches.
Sampling Bias	Overrepresentation of accessible ecosystems, underrepresentation of extreme/remote habitats.	Expand sampling campaigns to understudied ecosystems; establish global microbial observatories; adopt long-term monitoring.
High Computational Demands	Large-scale metagenomic datasets require HPC and advanced pipelines; lack of standardization.	Enhance access to HPC resources; develop cloud-based platforms; create standardized bioinformatics pipelines.

## 6. Future Perspectives

The future of environmental metagenomics in climate change adaptation studies of microbes is in linking the innovative methodology with operational ecological and policy studies. Since the world is becoming exposed to environmental stresses, the world needs new approaches to transforming the metagenomic findings into foreseeable models and solutions that can be used to manage the ecosystems. The challenge of machine learning (ML) and artificial intelligence (AI) integration in metagenomic studies is one of the opportunities. The size and complexity of metagenomic data require more sophisticated methods of computation to find patterns and forecast adaptations of microbes as well as simulate community-wide reactions to proposed climate conditions. The hidden connections between genes, functions, and environmental variables can be established with the help of AI-based algorithms, and the predictive models can be constructed to predict the role of microorganisms

in carbon cycling, methane emissions, and nutrient turnover under different climate scenarios. Longitudinal metagenomic monitoring is another essential development that will be oriented at the creation of time-series data to represent the evolution of microbial adaptation through the seasons, years, and climatic conditions. By engaging in such monitoring, the approaches will go beyond the frozen snapshots to identify a dynamic change in community structure, functional gene activity and evolutionary approach. Developing the global networks of microbial observatories in terrestrial, marine, and extreme ecosystems will present the data that will be required to identify the early warning signs of the ecosystem instability and resilience limits. Synthetic biology has provided a revolutionary solution to utilizing the metagenomic understanding. Microbial strains with superior resilience can be engineered using genes known to play a central role in stress tolerance which are those involved in the production of osmolytes, DNA damage repair systems, and enzymes to

catalyze the breakdown of pollutants. The microbes that are resistant to climate are promising in the fields of agriculture (e.g., drought-resistant soil microbiomes), bioremediation (e.g., the degradation of pollutants in hostile conditions under the influence of microbes), and bioenergy production (e.g., optimization of methanotrophs to control methane sequestration). Nonetheless, the application of engineered microbes in the natural ecosystem will be required to be guided by ethical, ecological, and biosafety factors.

Lastly, the management of policy and ecosystems will be important in ensuring that the expertise of metagenomics will be utilized in the strategies of climate adaptation. Microbial adaptation can be used to inform the sustainable agricultural practice, fisheries management and to inform the carbon sequestration policies. An example of this is a better way of utilizing microbial-mediated soil carbon storage or control of microbial communities in aquaculture systems, which would be of direct benefit to mitigation and adaptation objectives. Inclusion of microbial information on governance systems of the environment will guarantee the microbial resilience as part of the climatic adaptation planning. Overall, the future of environmental metagenomics lies in the area of interdisciplinary cooperation, combining sequencing technologies, computational innovations, synthetic biology and policy frameworks to develop predictive and action knowledge. This integration will not only allow understanding the concept of microbial resilience better but also using it to protect the ecosystems and human society in a quickly changing climate.

## 7. CONCLUSION

The importance of environmental metagenomics has revolutionized the process of studying and learning about the microbial world, especially with climate change. This method, through being able to culture-independently sequence whole microbial communities, has revealed the genetic, functional, and ecological solutions that microbes employ to survive stresses like increased temperatures, alkalification of oceans, drought, salinity variations and polluted environments. The combined data show that microorganisms do not merely respond to the environmental change but are active participants the adaptive mechanisms of which directly impact the biogeochemical time scales, the ecosystem stability, and the climate feedback processes. The knowledge acquired so far demonstrates a broad range of adaptation mechanisms that differ in the activation of stress-response genes and horizontal gene transfer, transition in ecological strategies, shifts in the global carbon and nitrogen cycles. Such results emphasize the robustness and agility of microbial

life in a way that emphasizes its possible use as a buffer to some of the destabilizing impacts of climate change. Nevertheless, even with such advances, there are still great challenges. A significant percentage of genes in metagenomic data sets is functionally unsatisfied, there is a geographical and ecological bias in sampling, and integration of the microbial genomic data in predictive ecosystem and climate models is not yet well developed.

In the future, advancements in this area will be determined by the usage of multi-omics strategies (metagenomics, metatranscriptomics, metaproteomics, metabolomics) to include the genetic potential and the actual functional activity. It will also be vital to apply AI-driven analytics and machine learning to simplify the complexity of large-scale data and construct predictive models of the reaction of microbial communities to future climate conditions. The longitudinal tracking activities, across a variety of ecosystems and climatic conditions will be necessary to include the dynamic adjustment trends with time. Moreover, that acquired knowledge should be converted into implemented policies, whether of creating climate-hardy microbiomes, carbon-enhancing soils, or management in marine ecosystems, to utilize microbial activities in a sustainable way. To sum up, environmental metagenomics can not only give an understanding of how microbes adapt to their environment but also a model on how microscopic processes are connected to global climate resilience. Through targeted solutions to existing weaknesses and the incorporation of interdisciplinary innovation, the area has a massive potential to improve scientific knowledge and provide the practical means of ensuring the sustainability of different ecosystem within a fast-evolving world.

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