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## Major Research Program on Aquatic Microorganisms: Focus on Core Scientific Questions in Aquatic Microbiome Research (Postprint)

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### Abstract

The hydrosphere encompasses oceans, lakes, wetlands, rivers, glaciers, groundwater, and other aquatic systems, harboring more than half of Earth's microorganisms—including bacteria, archaea, fungi, algae, and viruses—and constituting the planet's largest microbiome. Existing research has demonstrated that aquatic microorganisms play a crucial driving role in the biogeochemical cycling of essential elements. However, the core scientific question of how microorganisms exert these driving effects within aquatic microbiomes remains poorly understood. Recently, the National Natural Science Foundation of China officially launched the Major Research Program “Mechanisms of Aquatic Microorganisms Driving Earth's Elemental Cycles” (abbreviated as the “Aquatic Microbiome Program”). This program aims to select representative aquatic environments to dissect microbial community assembly and its interactions with the environment, elucidate microbial material and energy metabolism mechanisms, and assess the ecological significance and contributions of microbial metabolic activities, thereby revealing the mechanisms by which aquatic microorganisms drive the biogeochemical cycling of elements such as carbon, nitrogen, and sulfur. This article briefly outlines the background and rationale for the program's establishment, as well as the major scientific questions and research concepts involved.

## Full Text

# Major Research Plan for Microbes in Hydrosphere: Focusing on Key Scientific Issues of Microbiome Research in Hydrosphere

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## Abstract

The hydrosphere encompasses oceans, lakes, rivers, glaciers, wetlands, groundwater, and other aquatic environments, harboring over half of Earth's microorganisms—including bacteria, archaea, fungi, algae, and viruses—and thus representing the largest environmental microbiome on the planet. Existing research demonstrates that hydrosphere microorganisms play a critical driving role in the biogeochemical cycling of essential elements. However, the core scientific question of hydrosphere microbiome research—how microorganisms exert this driving influence—remains poorly understood. Recently, the National Natural Science Foundation of China (NSFC) officially launched the Major Research Initiative titled “Mechanisms Underlying Elemental Cycling on Earth by Microorganisms in the Hydrosphere” (abbreviated as the “Microbes in Hydrosphere Initiative”). This initiative will select representative hydrosphere environments to analyze microbial community assembly and interactions with the environment, investigate microbial mechanisms of substance and energy transformation, and elucidate the ecological significance and contributions of microbial activities. The ultimate goal is to reveal the mechanisms by which hydrosphere microorganisms drive the biogeochemical cycling of carbon, nitrogen, sulfur, and other elements. This article briefly outlines the background and rationale for the initiative, as well as the major scientific questions and research plans involved.

**Keywords:** NSFC Major Research Initiative, microbes in hydrosphere, microbiome, elemental cycling

Microorganisms represent the earliest life forms to emerge on Earth. Among all existing life forms, they exhibit the most extensive distribution, largest biomass, and richest biodiversity. Biogeochemical studies have shown that microorganisms play a key driving role in Earth's elemental cycles, altering atmospheric composition through photosynthesis, chemosynthesis, and nitrogen fixation; promoting rock weathering and soil and mineral formation; transforming the speciation and composition of important elements such as carbon, nitrogen, and sulfur in oceans, lakes, and wetlands; and co-evolving with Earth's environment. Consequently, Earth's habitability is intimately linked to microbially driven geochemical element cycling.

Water is the fundamental substance for life to survive, reproduce, and evolve, and serves as an important medium for material and energy migration and transformation within the Earth system. Three-quarters of Earth's surface is covered by water bodies including oceans, lakes, rivers, and glaciers, while substantial groundwater exists in the lithosphere. Together with atmospheric moisture, these waters form a continuous but irregular sphere—the hydrosphere. It is estimated that with every two breaths humans take, one breath of oxygen originates from the ocean; half of the carbon dioxide produced by human activities is absorbed by the ocean; over half of atmospheric methane, an important greenhouse gas, is generated from wetlands, swamps, and rice paddies; the ocean stores vast amounts of solid methane; oceanic nitrogen fixation accounts for two-thirds of global natural nitrogen fixation; and 30% of atmospheric nitrous oxide originates from the ocean. Clearly, the hydrosphere plays a crucial role in global material cycling, and Earth's health largely depends on hydrosphere ecosystems. The enormous quantities and diverse metabolic capabilities of microorganisms maintain the healthy functioning of hydrosphere ecosystems and drive the cycling of Earth's important elements [1] [FIGURE:1].

Research on hydrosphere microorganisms over the past decade has greatly enriched our understanding of their key driving role in Earth's element cycles and enhanced our knowledge of Earth's ecosystems [2-9]. However, because traditional microbiology research requires pure cultures to deeply analyze physiological metabolism, genetic characteristics, and ecological functions, and approximately 99% of microorganisms in nature—including those in the hydrosphere—remain unculturable in the laboratory [10], our current understanding of microbial community formation, metabolic modes, environmental interactions, and ecological functions in different hydrosphere habitats remains far from complete. The mechanisms by which microorganisms participate in elemental biogeochemical cycling remain largely a mystery

Unraveling this mystery is the primary focus and main objective of hydrosphere microbiome research. The mechanism by which hydrosphere microorganisms drive Earth's element cycles represents the core scientific question of this field. Focusing on this question, the National Natural Science Foundation of China officially launched the Major Research Initiative “Mechanisms Underlying Elemental Cycling on Earth by Microorganisms in the Hydrosphere” (abbreviated as the “Microbes in Hydrosphere Initiative” ) in October 2016, following multiple rounds of expert consultation. This initiative will select representative natural hydrosphere habitats and employ interdisciplinary approaches spanning life sciences, Earth sciences, chemistry, and information science, including concepts and methods from hydrosphere microbiomics, to systematically reveal the mechanisms by which hydrosphere microorganisms drive elemental biogeochemical cycling. The initiative aims to refine theories on life-environment interactions and co-evolution, provide scientific foundations and solutions for protecting hydrosphere ecological services, addressing global climate change, and ensuring

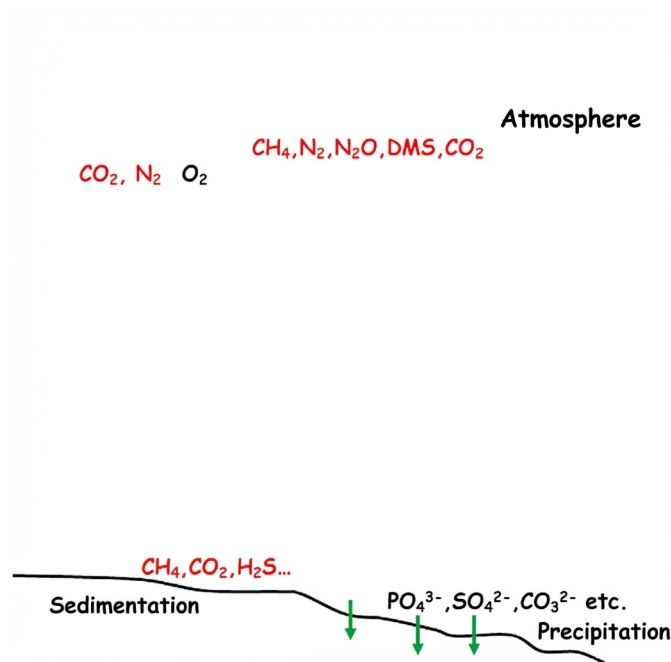


Figure 1: Figure 2

Earth's ecological security, and contribute to sustainable economic and social development. This article briefly reviews the background (current status both domestically and internationally), major scientific questions, and research plans of the "Microbes in Hydrosphere Initiative."

## 1 Current Status

Since the beginning of the 21st century, Western developed countries have shown unprecedented concern for hydrosphere environments, particularly the ocean, to address enormous challenges in resources, energy, and the environment [11]. In 2004, the United States released the "U.S. Ocean Action Plan," with two of its six major themes being "increasing scientific understanding of oceans, coasts, and the Great Lakes" and "enhancing the use and protection of ocean, coastal, and Great Lakes resources." In 2007, the U.S. introduced the "Ocean Research Priorities Plan and Implementation Strategy," prioritizing research areas such as marine biodiversity and ecosystems and the impacts of climate change on marine ecosystems. Japan issued its "Basic Plan on Ocean Policy" in 2008, proposing measures to advance marine surveys, strengthen marine science and technology research and development, promote marine resource utilization, and protect the marine environment. In 2010, the European Marine Board adopted the "Ostend Declaration," affirming that marine science and technology are indis-

pensable in addressing global economic, energy, and environmental crises. The United Kingdom released its “Marine Science Coordination Committee (MSCC) Delivery Plan 2025,” encompassing themes such as biodiversity and ecosystem function and marine biogeochemical cycling. In these marine strategies and plans, research on biodiversity and ecosystems has occupied an unprecedented prominent position.

Meanwhile, international communities have launched a series of major marine research programs. The Integrated Marine Biogeochemistry and Ecosystem Research (IMBER) program, jointly initiated in 2003 by the International Geosphere-Biosphere Programme (IGBP) and the Scientific Committee on Oceanic Research (SCOR), aims to investigate the sensitivity of marine biogeochemical cycles and ecosystems to global change. One of the three major scientific themes of the Integrated Ocean Drilling Program (IODP), led by the United States and Japan, is “deep biosphere and seafloor ocean.” Clearly, marine ecosystems and marine (micro)biological resources have become international research priorities in the near to medium term.

Over the past decade, the United States and the European Union have conducted multiple global ocean expeditions to survey marine microbial diversity. In 2004, Venter et al. [12] discovered microbial genes (unknown functional genes) in the Sargasso Sea whose number exceeded the total in public databases at that time. Between 2009 and 2012, the EU’s Tara Oceans expedition discovered 35,000 microbial species and over 40 million microbial genes, along with numerous marine viruses [13-17]. With the U.S. government’s official launch of the National Microbiome Initiative (NMI), research on microorganisms in various ecosystems, including hydrosphere environments, will enter a new stage of development globally.

China has a long history of research on microorganisms in terrestrial hydrosphere environments. Since the 1950s, studies have been conducted on microorganisms in lakes, hot springs, and wetlands. Starting in 2000, China launched application-oriented research on deep-sea microorganisms and their genetic resources. With China’s increasing comprehensive national strength and socioeconomic development needs, the country has attached unprecedented importance to marine rights and interests, elevating marine development strategy to an unprecedented level. The 18th National Congress of the Communist Party of China explicitly proposed to “enhance the capacity for marine resource development, develop the marine economy, protect the marine ecological environment, and resolutely safeguard national marine rights and interests, and build China into a maritime power.” The “National Medium- and Long-Term Program for Science and Technology Development (2006-2020)” also listed aerospace and marine technology as one of five strategic priority tasks for China’s medium- and long-term scientific and technological development, identified marine technology as one of eight key frontier technology fields, and prioritized marine ecology and environmental protection and efficient marine resource exploitation and utilization as priority themes, making marine science an important component

of basic research. The “National 12th Five-Year Plan for Marine Science and Technology Development” also proposed to “focus on expanding the depth and breadth of marine survey and research to significantly improve scientific understanding of the ocean.” The Ministry of Science and Technology, NSFC, and the China Ocean Mineral Resources Research and Development Association have all launched major marine and water-related research initiatives. Under the guidance of the maritime power strategy, the number of marine research institutions in China has recently experienced explosive growth, with rapid development of marine research vessels, submersibles, shipboard equipment, and shore-based research platforms [FIGURE:3]. China now possesses a group of research institutions and key laboratories engaged in hydrosphere research, forming an internationally competitive research team that has produced a large number of internationally influential research achievements [18-28].

## 2 Frontier Scientific Questions

The core scientific question to be addressed by the “Microbes in Hydrosphere Initiative” is: How do microorganisms drive element cycling in typical hydrosphere environments? This core question encompasses three levels of content.

### 2.1 Macro-Mechanisms and Ecological Effects of Microbial Participation in Biogeochemical Cycling of Carbon, Nitrogen, Sulfur, and Other Elements

This scientific question concerns the metabolic activity and flux contributions of hydrosphere microorganisms in the cycling of important Earth elements. Through correlative and systematic studies at different levels—genes, species, populations, and communities—the initiative will analyze the role of microorganisms in the cycling of important Earth elements and refine ecological theoretical models.

### 2.2 Mechanisms of Community Formation and Environmental Interaction of Hydrosphere Microorganisms Involved in Carbon, Nitrogen, Sulfur, and Other Element Cycles

This scientific question concerns the functional microorganisms and their community structures that drive the cycling of important elements in specific hydrosphere habitats, focusing on the effects of environmental factors and their changes on microbial community structure and the impacts of microbial activities on the environment. The initiative will focus on important carbon-sequestering microbial groups, microbial groups involved in greenhouse gas metabolism in wetlands (such as methanogenic and anaerobic methane-oxidizing archaea, soil ammonia-oxidizing archaea), microbial groups that form recalcitrant dissolved organic carbon in the ocean, and analyze microbial spatiotemporal distribution characteristics, diversity maintenance mechanisms, interspecies interactions, virus interactions, metabolic coupling,

and spatiotemporal responses.

### **2.3 Novel Pathways and Regulatory Mechanisms of Substance and Energy Conversion and Metabolism in Hydrosphere Microorganisms**

This scientific question concerns the pathways of substance and energy metabolism of microorganisms (including uncultured microorganisms) in specific hydrosphere habitats. The initiative will explore non-phototrophic carbon fixation pathways in marine archaea/bacteria, energy acquisition and storage mechanisms in life systems independent of photosynthesis, and other novel metabolic strategies.

## **3 Initiative Design**

Based on in-depth analysis of disciplinary development trends and China's research foundation, the "Microbes in Hydrosphere Initiative" will focus on the core scientific question of microbial driving mechanisms of important element cycles in typical habitats, selecting four types of representative hydrosphere environments (open ocean, coastal and estuarine areas, watersheds including rivers, lakes, and wetlands, and terrestrial special aquatic habitats) to investigate the biogeochemical cycling of three important elements (carbon, nitrogen, and sulfur). The initiative aims to elevate China to an internationally advanced level in hydrosphere microbiology research by the program's conclusion (2024), with China taking a leading role in some directions and achieving leapfrog development in the emerging interdisciplinary field of geomicrobiology. The initiative will develop scientific research in the following four directions.

### **3.1 Important Microbial Functional Groups in the Open Ocean and Their Mechanisms for Driving Carbon, Nitrogen, Sulfur, and Other Element Cycles**

The vast sea area far from continental shelves is called the open ocean, which constitutes the main body of the ocean and covers approximately 50% of Earth's surface, playing an important role in global material cycling. The deep ocean is mostly characterized by low temperature, high pressure, and perpetual darkness, but also contains various special environments such as hydrothermal vents and cold seeps, harboring numerous uncultured microorganisms. This direction will focus on the community formation of key functional microorganisms in oceanic water columns and extreme seafloor environments and their interactions with the environment, metabolic pathways related to carbon, nitrogen, sulfur, and other element cycles and their regulation, and the spatiotemporal distribution of key functional microorganisms and their driving and regulatory mechanisms in element cycling.

### **3.2 Mechanisms by Which Coastal and Estuarine Microorganisms Drive Carbon, Nitrogen, Sulfur, and Other Element Cycles**

Coastal and estuarine areas are among the most active hydrosphere environments for material transformation and energy flow, and also the most diverse in terms of microbe-mineral interactions. This direction will focus on microbial community formation and environmental interactions in coastal and estuarine habitats, mechanisms driving carbon, nitrogen, sulfur, and other element cycles, particularly the regulatory mechanisms for carbon sources or sinks, and novel pathways of microbial energy metabolism and carbon-nitrogen-sulfur element cycling in the euphotic zone.

### **3.3 Driving Mechanisms and Ecological Effects of Microorganisms in Watershed Water Bodies (Rivers, Lakes, Wetlands, etc.) on Carbon, Nitrogen, Sulfur, and Other Element Cycles**

Rivers, lakes, and wetlands exhibit high environmental heterogeneity and microbial community diversity, serving as major sites for carbon, nitrogen, sulfur, and other element transformations, with fixed organic carbon equivalent to nearly 40% of total oceanic carbon sequestration. This direction will focus on microbial community formation and environmental interactions in typical river, lake, and wetland habitats, metabolic mechanisms driving carbon, nitrogen, sulfur, and other element transformations and cycles, and ecological effects.

### **3.4 Characteristics of Microbial Carbon, Nitrogen, Sulfur, and Other Element Metabolism and Environmental Adaptation Mechanisms in Terrestrial Special Aquatic Habitats**

Hot springs, salt lakes, glaciers, acid mine drainage, karst groundwater, and others are all terrestrial special habitats. Microorganisms in these habitats possess unique environmental adaptation and growth metabolism mechanisms, containing clues for exploring the occurrence and evolution of microbial metabolic diversity. This direction will focus on microbial community formation and environmental interactions in terrestrial special aquatic habitats, special energy metabolism pathways of key functional microorganisms (groups), and molecular mechanisms driving organic matter synthesis and carbon-nitrogen-sulfur element cycling.

The successful implementation of the “Microbes in Hydrosphere Initiative” depends on deep interdisciplinary integration between life sciences and Earth sciences, as well as the development and application of new technologies and methods. The initiative encourages scientists to conduct substantive, high-level interdisciplinary collaboration, refine major scientific questions, and converge different disciplinary ideas and methods to achieve leading breakthroughs. Additionally, the initiative will vigorously promote the development and application of new methods and technologies for hydrosphere environmental monitoring, sample collection, microbial isolation and cultivation, and culture-independent

analysis, to comprehensively elucidate the important roles of microorganisms in the cycling of Earth's important elements at community, species, metabolic pathway, and gene levels.

Over the past two decades, with advances in genomics, bioinformatics, molecular ecology, geomicrobiology, biogeochemistry, biological oceanography, and other disciplines, as well as the development of novel sampling techniques, single-cell technologies, in situ detection technologies, and simulated cultivation techniques, researchers can finally extend their reach to the deep sea, extreme hydrosphere environments, and other most mysterious microbial habitats on Earth to explore the mysteries of microbially driven elemental biogeochemical cycling. The launch of the "Microbes in Hydrosphere Initiative" signifies that China has gathered sufficient confidence and made adequate preparations for leapfrog development in this significant hotspot area. The globally burgeoning microbiome research will also provide assistance and reference for implementing the "Microbes in Hydrosphere Initiative." It is foreseeable that with the impetus of this initiative, China will occupy a pivotal position in international hydrosphere microbiology research and make important contributions to maintaining Earth's ecosystem security and achieving sustainable socioeconomic development.

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## Figures



Figure 2: Figure 4



Figure 3: Figure 5

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