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Review Article

Omics Insights: In Situ Tools Shed Light on Deep-Sea Microbial Activities

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Abstract

Extreme conditions, including darkness, elevated hydrostatic pressure, low temperature, and limited nutrient availability create unique deep-sea environments. Previous studies on deep-sea organisms/microorganisms have not only revealed the metabolic characteristics of various deep-sea life forms and their dynamic communities but also contributed to the development and utilization of deep-sea biological resources. Genomics studies of deep-sea organisms heavily rely on high-quality samples. However, environmental alterations during sample retrieval, from the deep sea to the research vessel, lead to microbial community changes and RNA degradation. Hence, various automatic devices are developed for *in situ* fixation of

samples and extraction of nucleic acids in the deep-sea environment, with subsequent processing conducted onboard. In recent years, *in situ* metagenomics, metatranscriptomics and metaproteomics (omics) have become powerful tools for exploring the metabolic activities and periodicity of the deep-sea inhabitants, which can provide insights for the survival mechanisms, element cycles, and dynamic changes of the 'microbial dark matter' in the deep ocean. This paper reviews the necessity of deep-sea *in situ* omics, outlines the development of ecological monitoring equipment for omics research in the deep sea, and discusses its applications in revealing novel genes and active substances in the deep sea, as well as understanding the distribution and ecological functions of deep-sea microorganisms. These *in situ* omics approaches are poised to revolutionize deep-sea research, enabling more accurate models of ecosystem dynamics.

Keywords: Time-course, microbial community, metagenomics, metatranscriptomics, water column

1. Overview of the deep-sea ecosystems

The deep sea represents an extreme environment characterized by towering pressure, extremely high or low temperature, pervasive darkness, limited nutrients, high salinity, and scarce resources. Deep-sea ecosystems are often regarded as the 'dark world', relying on chemosynthetic microorganisms as the foundation of the ecosystem[1,2]. Chemosynthesis is a process through which organisms generate energy by converting inorganic molecules into organic matter using chemical energy, rather than sunlight, as a source of energy. It plays a crucial role in sustaining life in the deep sea where sunlight cannot penetrate. As the ultimate frontier of Earth, this array of unique characteristics distinguishes deep-sea ecosystems from terrestrial and other ecosystems[3]. Over 50% of the ocean environment lies at depths exceeding 3,800 meters, where there is a wealth of undiscovered biodiversity[4]. In particular, the majority of microbial taxa, which represent the largest portion of Earth's biomass, remain uncharacterized. These unknown microorganisms may stand as new challenges to the current framework of biology[5].

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In addition, deep-sea ecosystems play a crucial role in global biogeochemical cycles and the evolution of life on our planet, although our understanding of this role remains far from comprehensive[2,6]. In the aphotic zones of the deep ocean, microorganisms rely on chemical energy to drive the synthesis of organic compounds, including carbon fixation, CO assimilation, and methane assimilation. Chemolithoautotrophic processes contribute substantially to the fixation of dissolved inorganic carbon (DIC) in the dark ocean[7]. In addition to classical chemoautotrophy, anaplerotic CO_2fixation plays a complementary role by replenishing intermediates for central metabolic pathways and biosynthesis, thereby supporting microbial growth under oligotrophic conditions. These microorganisms also mediate nitrogen and sulfur cycling through processes such as nitrification and sulfur oxidation[8], while symbiotic associations further contribute to carbon sequestration, collectively influencing global carbon fluxes. Abyssal trench systems serve as crucial nodes for the input of materials from the Earth's surface layer to its deep interior, with evidence of vast dissolved carbon reservoirs and abundant methane sequestration in these environments [9]. It is estimated that the rate of inorganic carbon fixation in the deep Atlantic Ocean ranges from 1.0 to 2.5 mmol/m^2 per day, accounting for 15% to 53% of the organic carbon input into the euphotic zone (0-200 m)[10]. In the mesopelagic northeast Atlantic, dark CO_2fixation has also been suggested to contribute to heterotrophic prokaryotic carbon demand at a magnitude comparable to that associated with sinking particulate organic carbon (POC) flux[11]. The organic carbon generated through these dark fixation processes may therefore complement surface-derived organic matter, contributing to microbial activity and the functioning of deep-sea ecosystems under oligotrophic conditions.

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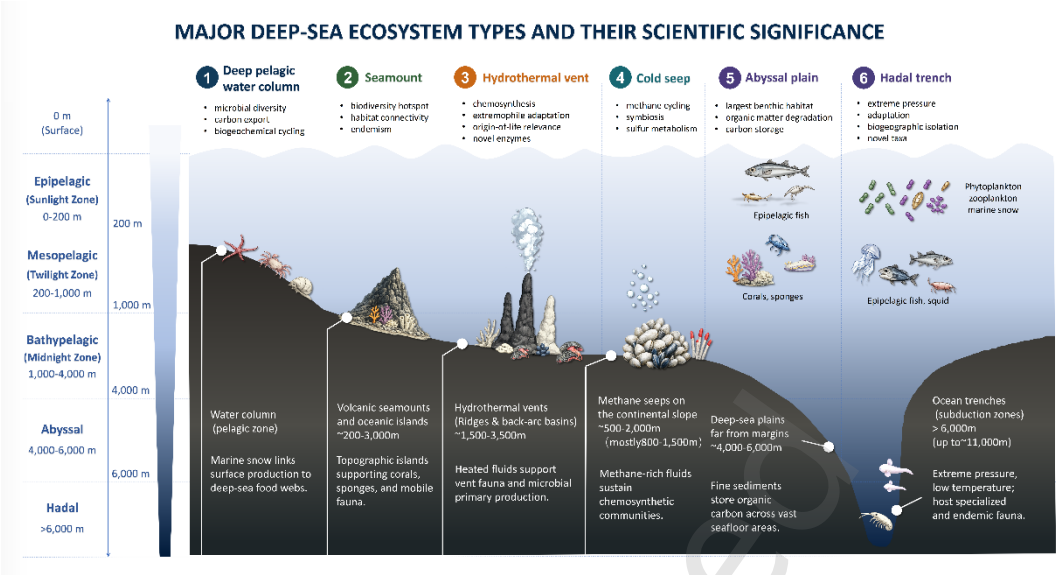


Figure 1. Major deep-sea ecosystem types and their scientific significance. Representative deep-sea ecosystems, including the deep pelagic water column, seamounts, hydrothermal vents, cold seeps, abyssal plains, and hadal trenches, are shown across ocean depth zones. These habitats differ in environmental conditions, dominant energy sources, biological communities, and ecological functions, providing diverse settings for studying deep-sea microbial activities and in situ omics.

As marine exploration advances, life in extreme conditions within the deep-sea environment is gradually being unveiled[12]. Extreme geochemical processes in the deep sea provide a variety of specialized habitat types, such as hydrothermal vents[13–15], cold seep[6], whale falls[16,17], seamounts[18,19], and trenches[20]. Together, these representative deep-sea ecosystem types and their scientific significance are summarized in **Figure 1**. These deep-sea organisms rely on the energy released by extreme environmental conditions for survival and prevalence[21]. In the deep-sea environment, there are widely distributed microorganisms that thrive under extreme conditions, including acidophiles (pH below 3), alkaliphiles (pH above 10), halophiles (above 2.5 mol/L), psychrophiles (able to withstand temperatures below 0°C), thermophiles (above 120°C), and barophiles (above 500 atmospheric pressure). By adapting the extreme characteristics of these environments, deep-sea life has evolved with high productivity and various specialized abilities[21]. For instance, hyperthermophilic archaea have evolved a combination of adaptive mechanisms, including the accumulation of compatible solutes, efficient expression and activity of pre-folded proteins, maintenance of membrane fluidity, and robust enzymatic catalysis[22]. These extremophilic organisms are featured with advantages in terms of unique metabolic functions, placing them at the forefront of scientific

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research and holding substantial industrial value[23]. The deep sea represents a rich source of bioactive natural products, and advances in new research technologies and molecular techniques that enable more comprehensive genetic screening are expected to accelerate the discovery of natural products from these microbial diversity hot spots[24]. For instance, microbial polysaccharides have become increasingly attractive products of interest across various industrial sectors. Hydrothermal deep-sea vent habitats may serve as a novel source of exopolysaccharides (EPS)[25]. Certain extremophilic bacteria originating from such environments have been demonstrated to biologically synthesize innovative EPS under laboratory conditions. These EPS exhibit unique characteristics in rheological properties, bioactivity, metal-binding capacity, and chemical composition, suggesting broad applications in the foreseeable future. Furthermore, deep-sea environments may also serve as a promising reservoir of much greater fungal diversity, some of which may produce biomedically useful metabolites[26]. Exploring and understanding the metabolism of life in extreme environments present unprecedented opportunities for deciphering the origin and evolution of life in the deep ocean under such challenging conditions[27,28].

2. Deep-sea *in situ* omics

Deep-sea *in situ* omics involves the high-throughput sequencing and omics analysis of nucleic acids and peptides that are extracted or fixed *in situ* within the deep sea and facilitated by the advancements in deep-sea automatic technology and next-generation sequencing technologies. As an emerging field, deep-sea *in situ* omics provides the potential to enable elucidation of the adaptation mechanisms, element cycles, and dynamic variations of deep-sea microorganisms[29]. The development of deep-sea *in situ* omics has been driven by the need to study microbial biodiversity, and to explore the origins of life, together with advances in technologies that enable in situ fixation and extraction, time-course sampling, and pressure-retaining workflows for more reliable, artifact-reduced results.

2.1 Rationale for deep sea *in situ* omics

While the immense practical and scientific value of deep-sea organisms and

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microorganisms is recognized, the unique extreme habitats render difficulties in the sampling, cultivation, and research of deep-sea life[23]. To better understand deep-sea active microorganisms, next-generation sequencing and omics analyses were employed to work on deep-sea samples, aside from the detection of microbial community structures based on analysis of 16S rRNA gene amplicons. Due to the relative scarcity of organic matter in the deep sea, the microorganisms there have evolved various transmembrane sensing systems to respond to environmental changes such as oxygen level and pressure[30]. The samples that are taken from deep-sea seawater to shipboard laboratories using water sampling bottles and gravity corer, are subjected to remarkable alterations in pressure, temperature, and gas composition. This, in turn, leads to the rapid proliferation of some microbial groups, and stress-induced alterations of gene transcriptional patterns[30,31]. Investigations of microbial communities in water layers of the Yongle blue hole showed that using *in situ* filtration and fixed samples could accurately decipher the prokaryotic communities dominated by sulfate reducing bacteria in the anoxic zone[32]. Exposure to oxygen might lead to prevalence of sulfur-oxidizing bacteria in the samples of the Yongle anoxic layers as demonstrated by the effects of sampling methods on the results[32]. Compared to DNA molecules, RNA is highly prone to degradation with a half-life of as short as 12 minutes[31]. Using samples without DNA and RNA preservation can produce artifacts in subsequent work, thus affecting the accurate measurement of functional genes as indicators of element cycles. The metabolic processes and *in situ* activities of deep-sea microorganisms remain elusive largely due to the lack of high-quality nucleic acid and protein samples[30].

In recent years, scientists have investigated the *in situ* gene expression of microorganisms at the RNA level using metatranscriptomics, providing more accurate profiling of the active genes of environmental microorganisms[33–35]. For example, the experiments using samples collected at a depth of >3,000 meters in the Mediterranean Sea showed (based on prokaryotic activity measurements and incubation experiments) that the oxygen consumption of prokaryotes under *in situ* conditions was consistently higher than that after decompression and incubation at atmospheric pressure[36]. When studying the *in situ* activity and physiological characteristics of deep-sea organisms using transcriptomics, it is crucial to either fix or extract

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RNA molecules *in situ* to prevent changes and degradation during the recovery process[37]. Research has shown that different sampling techniques influence the relative abundance of transcripts that are presumably indicative of the habitat[38]. In this context, the importance of *in situ* fixation is evident in reliably evaluating distinct microbial activities in response to deep-sea environments based on *in situ* metatranscriptomics to capture real-time changes[38].

In order to decipher the expression and functional mechanisms of genes in active deep-sea organisms, as well as to uncover unknown functional genes in these organisms, the development of key technologies for *in situ* biological sample processing and treatment is imperative. *In situ* RNA stabilization (stabilization before retrieval by remote control) instead of onboard RNA treatment (stabilization after retrieval) is desirable[38–40]. By enhancing sensitivity and mitigating biases inherent in PCR cloning, systematically assessing the effects of deep-sea sampling procedures under extreme physiochemical conditions, and acquiring insights into activities of microbiomes researchers can achieve more reliable results. [41] (**Figure 2**).

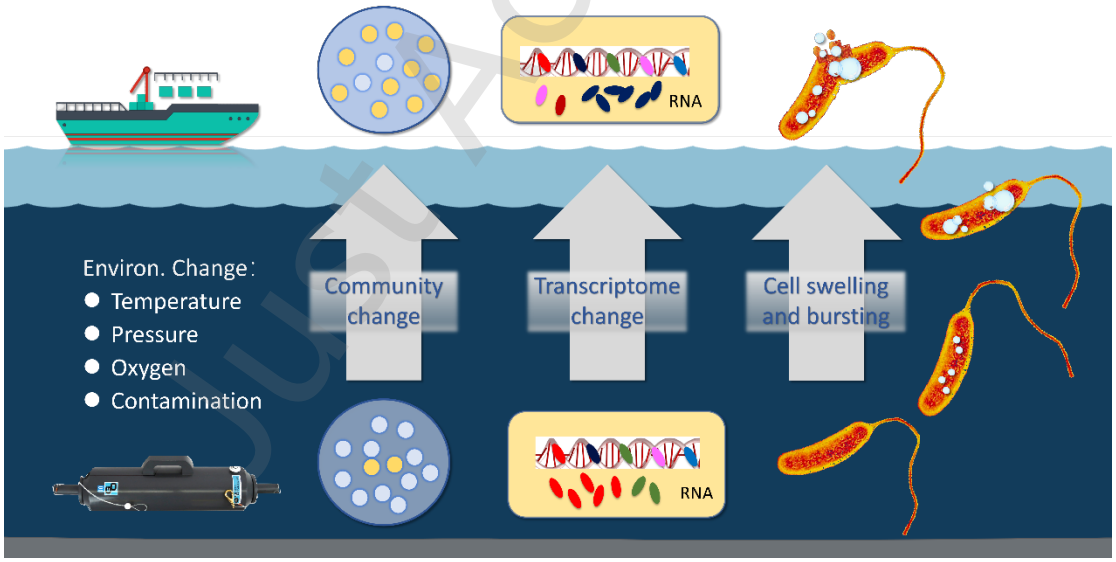


Figure 2. Environmental changes during deep-sea sample recovery (temperature, pressure, oxygen, contamination) can cause microbial community shifts, transcriptome alterations, and cell damage, leading to biased or unreliable results without in situ preservation.

While *in situ* fixation effectively minimizes artifacts associated with depressurization, temperature shifts, and oxygen exposure, it is important to recognize that the fixation process itself may introduce additional biases. Chemical preservatives such as RNAlater, Lugol's

solution, or lysis buffers can impose abrupt osmotic and chemical stresses on microbial cells. These perturbations may lead to selective cell lysis, membrane disruption, or differential preservation efficiency across taxa, particularly affecting fragile microorganisms such as certain archaeal lineages and protists. As a result, community composition and transcriptomic profiles may still be partially distorted despite *in situ* preservation.

Therefore, optimizing preservation chemistry remains an important consideration for deep-sea *in situ* omics. Future efforts may benefit from developing fixation strategies that better balance rapid biomolecule stabilization with minimal cellular disturbance, for example through pressure-adapted protocols, gradual chemical equilibration, or taxon-specific preservation approaches. A more detailed understanding of how different fixatives interact with deep-sea microbial physiology could further improve the accuracy and reliability of *in situ* omics analyses.

Additionally, using deep-sea environmental simulations for biological cultivation is an important method in the field of biogeochemical research worldwide[42,43]. However, the unique nature of the deep-sea environment makes it rather difficult to accurately simulate cultivation conditions in a laboratory setting. Shipboard microbial incubation of deep-sea samples may be affected by depressurization, air exposure, and temperature increase due to processing delays[44]. Furthermore, although simulation cultivation can address fundamental microbial metabolic characteristics, it cannot provide monitoring and assessment of the *in situ* ecological functions and dynamics of major ecosystem inhabitants. Some cultivable microorganisms, when subjected to changes in living conditions in a cultivation system, can exhibit a 'domestication effect' through continuous subculturing. The addition of organic carbon sources to the cultural medium may lead to gradual changes in the genomes of chemoautotrophic bacteria, resulting in lateral gene transfer and the development of facultative bacteria. Therefore, laboratory-cultured strains of bacteria often struggle to accurately reflect the real metabolic status in their natural habitats. Moreover, changes over time in the structure of prokaryotic communities are likely driven by different experimental conditions[36]. Therefore, *in situ* deep-sea microbial research holds greater ecological significance.

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2.2 Facility development for deep-sea *in situ* sampling and omics work

As we continue to expand exploration of the deep sea, some fundamental questions regarding ecosystems and life processes remain unanswered. These include: (1) the sources and primary contributors of organic matter in the deep sea; (2) the spatiotemporal dynamics and regulatory mechanisms of deep-sea ecosystems; and (3) the *in situ* activities and ecological functions of deep-sea organisms, among others. To address these essential scientific questions of deep-sea ecosystems, there is an urgent need for new research platforms and technologies[45]. In this section, we discuss representative deep-sea *in situ* platforms across four major methodological stages: autonomous sample collection, *in situ* preservation and fixation, time-course nucleic acid collection, and integrated molecular or observatory-based systems. These platforms are not equivalent in function. Rather, each stage reflects a different methodological response to the three questions outlined above. Early samplers mainly support the identification of organic matter sources and broad community patterns, preservation-enabled systems improve the fidelity of molecular measurements, time-course platforms address temporal dynamics, and integrated observatory systems increasingly link biological activity with environmental variability. To facilitate comparison among these platforms, their key characteristics are summarized in **Table 1**.

Table 1. Comparative summary of representative deep-sea in situ platforms

Platform	Max depth (m)	Sample volume / throughput	Temporal resolution	Pressure-retaining / preservation	Low-biomass fit	Main limitation / likely bias
RAS (early autonomous collection)[46–48]	5500	48 × 0.1–0.5 L; 75 mL min^{-1}	48 programmed events; 10 min–18 mo	No / none intrinsic	Low	Small per-sample volume; post-recovery molecular change
SUPR-V2 (large-volume preserved filtration)[49,50]	~4960 demonstrated	14 sample sets; 142 mm filters; ~2 L min^{-1}; up to 2 L whole water/filtrate per set	14 discrete sets per deployment	No / in situ preservative on filters	High	Filter clogging; mainly offline analyses after recovery

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ISMIFF (full-ocean-depth filtration/fixation)[51]	10,911	Up to 600 L per deployment	Single large-volume event per deployment; filtration up to ~20 h	No / in situ DNA buffer or RNAlater	High	Large, power-intensive; recovery still depressurized
MISNAC (time-course nucleic acid collection)[52]	3000	Up to 9 nucleic-acid captures per deployment; max filtration ~500 mL min^{-1}	Up to 9 programmed time points per deployment	No / in situ lysis + column capture of DNA/RNA	Moderate – High	3000 m depth ceiling; column efficiency may drop at low temperature
ESP (2nd gen) (integrated ecogenomic sensor)[53]	1000 demonstrated	132 pucks total; typical ~1 L per phase (configurable)	Phase-based missions; deployments up to ~30 d	No / onboard lysis + archived fixatives (incl. RNAlater)	Moderate	Targeted assays; modest per-phase volume; complex, bulky system
D-ESP (deep-sea molecular assays)[54,55]	4000	Up to 10 L raw-water intake before processing; targeted qPCR assays	Scheduled discrete assay cycles on observatory/lander timescales	No / preserved archives; qPCR on processed sample	Moderate	Sample decompressed before analysis; targeted rather than discovery-scale omics
Crawler Mobile Platform (observatory-linked ecology)[56]	Site-dependent	Imaging transects (20 m; standardized 120 m^2), not water filtration	Repeated surveys; monthly diversity and biomass indices	N/A / none	N/A	No direct molecular information; visibility and taxon-detection bias
In-Situ eDNA Sensor[57]	Not yet field validated	Dual-filter system; 47 mm filters; ~500 mL/min; ~5 L per sample	~2 h per analysis; near real-time detection	In situ lysis + qPCR analysis	Low–Moderate	Prototype stage, targeted qPCR only, primer dependent, not yet fully integrated or field deployed
Full ocean depth modular lander with pressure retaining sampler[58]	>10,700 m	135 mL PRS sample, paired with 2.5 L Niskin bottle	Single or discrete sampling events	Pressure-retaining recovery (~81%)	Moderate	Very small sample volume, single event sampling, not continuous, partial pressure loss, temperature increased during

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						recovery
Mesobot large volume eDNA sampler[59]	400 m	12 samples per deployment, ~40 to 60 L per sample	Discrete autonomous sampling along vertical transects	In situ filtration, filters frozen on dry ice after recovery	High for low biomass metazoan eDNA	Not pressure-retaining, tested only to mesopelagic depths, high extraction workload, and replicate variability.

Note: this table is intentionally selective rather than exhaustive. It keeps the comparison readable by focusing on representative systems with the clearest relevance to *in situ* omics and long-term ecosystem observation, while omitting highly specialized devices such as tissue homogenizers and broader network concepts such as Marine Observatory (MOB). Recent reviews also support the main critical point that emerges from the comparison: most current deep-sea *in situ* omics platforms have improved *in situ* filtration, fixation, or onboard molecular processing, but true pressure-retaining recovery remains uncommon, so depressurization artifacts are still a major unresolved source of bias in deep-sea biological sampling.

The earliest generation of deep-sea *in situ* technologies was primarily designed to achieve autonomous or repeated collection of seawater, suspended particles, larvae, and sediments, thereby establishing the basic sampling foundation for later preservation-enabled and integrated omics platforms. Representative systems included the Autonomous Microbial Sampler (AMS)[60], PhytoPlankton Sampler (PPS)[61], Remote Access Sampler (RAS)[47,48] and Water and Microplankton Sampler (WaMS)[62]. These early platforms were especially important because they allowed repeated or remote sampling across different depths, sites, or deployment periods, which helped address questions concerning the distribution of microorganisms, suspended organic matter, and particle-associated communities. However, their main limitation was that sample processing and molecular preservation were still largely dependent on post-recovery laboratory workflows.

These platforms mainly focused on sample acquisition, with or without subsequent

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preservation for laboratory analysis after recovery[63], and were mainly used for qualitative analysis of functional genes across temporal and spatial changes. The provision of RNA and protein preservation greatly enhances the dimensions of post-sampling analyses[63]. Thus, this first generation provided the logistical basis for deep-sea microbial monitoring, but it could not fully resolve *in situ* gene expression or metabolic activity because biomolecules may change during recovery, depressurization, warming, and oxygen exposure[31,37,39,64].

Building on early autonomous collection systems, the next major stage of technological development was represented by preservation-type samplers, which introduced *in situ* fixation and biomolecule preservation to improve the molecular fidelity of recovered samples. This stage directly addressed one of the central limitations of early samplers: although samples could be collected remotely or repeatedly, DNA, RNA, proteins, and cellular states could still be altered during recovery[31,37,39]. Preservation-type platforms therefore shifted the methodological focus from sample acquisition alone to artifact reduction and molecular stabilization.

Representative platforms in this category include the Autonomous Microbial Genosensor (AMG), Biological OsmoSampling System (BOSS), the Suspended-Particulate Rosette (SUPR)[65], and the Microbial Sampler-in situ Incubation Device (MS-SID). Among them, SUPR-V1 (Suspended-Particulate Rosette Version 1) is particularly representative because it combines large-volume particulate and microbial filtration with *in situ* preservation, making it especially suitable for low-biomass environments and for studies requiring sufficient material for downstream molecular analyses[65]. Its main strength is therefore not only the collection of suspended particles, but the recovery of enough preserved biomass for molecular analyses in environments where microbial abundance is low. However, because SUPR-type systems mainly preserve collected material for later laboratory processing, they still provide limited real-time information on microbial activity.

The upgraded SUPR-V2 (Suspended-Particulate Rosette Version 2) system further expanded the range of sample types and configurations while improving sample preservation through sealed sampling loops and *in situ* fixation[50]. This improvement increased sampling

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flexibility and reduced contamination or exchange with surrounding seawater after collection, making the platform more suitable for comparative studies of particle-associated communities and organic matter sources[50]. In parallel, more specialized preservation systems were also developed for targeted applications. Wurzbacher et al. have designed a novel *in situ* filtration and fixation sampler (IFFS) for analyzing the *in situ* expression of *actR* gene in aquatic environments. It is equipped with a pulse trigger system for *in situ* fixation of samples after filtering a specified volume of water (designed to filter volumes ranging from 100 ml to 900 ml)[66]. Compared with large-volume systems such as SUPR, IFFS is better suited to targeted gene-expression measurements where rapid fixation after filtration is more important than high sample throughput. Sanders et al. have designed an *in situ* Mussel And Snail Homogenizer (ISMASH) for *in situ* homogenization and sample preservation of biological tissues. This device can be deployed on the seafloor by ROV (remotely operated vehicle) manipulator arms after capturing hydrothermal vent snails. It is utilized to analyze the transcriptome and symbiotic mechanisms of co-occurring bacteria in vent mussels and snails[67]. ISMASH represents a different methodological direction because it targets host-associated tissues rather than free-living microbial cells or suspended particles. Its value lies in preserving tissue-associated transcriptomic signals before recovery, but its use is limited to accessible macrofauna and ROV-assisted deployments.

In situ Microbial Filtration and Fixation sampler (ISMIFF) system has been developed to meet the demands of full-ocean-depth microbial sampling and preservation. This platform represents a further methodological step because it combines autonomous microbial filtration, large-volume sampling, and *in situ* RNA fixation under full-ocean-depth conditions. It therefore directly addresses both low-biomass sampling and the preservation of molecular signals from hadal and abyssal environments. Capable of autonomously conducting microbial filtration and RNA fixation in deep-sea and abyssal environments, particularly in areas exceeding depths of 6,000 meters, the system has undergone rigorous pressure testing at 121 megapascals (equivalent to 1.2 times the pressure at a depth of 10,000 meters)[51]. It demonstrated the ability to perform *in situ* sampling at full ocean depths while dramatically increasing the volume of filtered water (up to 600 liters)[51]. Its major strength is the recovery

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of sufficient microbial biomass from extremely low-biomass water columns while minimizing RNA degradation and recovery-induced transcriptional artifacts[31,51,64]. This makes ISMIFF particularly relevant to questions concerning active microbial populations, methane cycling, and other ecological functions in deep and hadal water columns[51].

A recent ISMIFF-based study identified novel microbial lineages in the Yongle blue hole and revealed their roles in methane production and consumption in both oxic and anoxic zones[68]. However, as with other filtration-fixation systems, ISMIFF primarily captures microorganisms retained on the selected filter fraction and may underrepresent very small cells, extracellular nucleic acids, dissolved metabolites, or loosely attached particle-associated communities depending on the filtration configuration. Ongoing research efforts have prompted the creation of advanced *in situ* microbial sampling devices. These innovative deep-sea *in situ* microbial sampling techniques, involving microbial enrichment and nucleic acid fixation within the deep-sea environment, have fundamentally improved sampling efficiency and sample quality, which represent a major advance in deep-sea microbial research. Collectively, preservation-enabled platforms such as SUPR, IFFS, ISMASH, and ISMIFF mark a transition from simple sample recovery toward molecularly faithful sampling. They are especially important for addressing the first and third scientific questions raised above: identifying organic matter-associated microbial contributors and determining the *in situ* activities and ecological functions of deep-sea organisms.

Although deep-sea biological sampling and research have been conducted for many years, the ecological results of all typical deep-sea habitats can only reflect the static conditions at a certain deep-sea station and time point. This remains a major limitation because deep-sea microbial processes are shaped by episodic organic matter input, hydrothermal or seep fluid variability, particle flux, tidal forcing, and seasonal changes in surface-derived material[64,69,70]. Single-time-point sampling can therefore identify which organisms or genes are present, but it cannot adequately resolve when microbial populations become active, how long activity persists, or which environmental drivers regulate these changes. Obtaining an accurate description of microbial processes is crucial for gaining a deeper understanding of the dynamics and metabolic potentials of the microbial communities. Therefore, long-term, continuous *in*

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situ observations and sampling of deep-sea organisms are among the most reliable approaches for obtaining high-fidelity ecological and molecular data[64]. The Multiple *In Situ* Nucleic Acid Collection (MISNAC) apparatus has been developed to automatically conduct *in situ* microbial enrichment, cell lysis, and nucleic acid collection at different time intervals. MISNAC represents the transition from preservation at a single time point to programmed time-course molecular sampling. Its working principle is based on sequential microbial collection and nucleic acid capture during deployment, allowing microbial signals to be linked to defined incubation periods or environmental conditions.

The platform is not only suitable for studying changes in microbial communities over different time intervals but also for microbial enrichment and cultivation, investigating the *in situ* metabolic activities of microorganisms under the influence of various user configured conditions and substrates[52]. This makes MISNAC particularly relevant to the second and third scientific questions outlined above: how microbial communities change through time, and which organisms are metabolically active under specific *in situ* conditions. Genomics studies based on *in situ* acquired nucleic acids demonstrated that, compared to the conventional Niskin bottle sampling method, MISNAC can better replicate the structure of microbial communities and *in situ* gene activity in deep-sea environments by achieving time-course microbial nucleic acids[52]. This also enables the detection of varying patterns of major active indigenous groups over an extended time range[71]. However, MISNAC does not fully replace large-volume preservation platforms, because its strength lies in temporal resolution and controlled enrichment rather than maximum sample volume. Its results may also be influenced by substrate amendment, incubation chamber effects, or selective enrichment of organisms that respond rapidly to the experimental conditions[52]. Deciphering the genomes of different microbial ecotypes requires long reads generated from high-quality DNA and RNA using third-generation sequencing technologies. In this respect, employing deep-sea large-volume samplers such as SUPR, ISMIFF and MISNAC to extract up to 1 μg DNA or RNA can address this challenge. These platforms therefore provide complementary advantages: SUPR and ISMIFF increase biomass recovery from low-biomass water columns, whereas MISNAC adds temporal resolution and experimental control. Together, they support a shift from static

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descriptions of microbial diversity toward activity-resolved and time-resolved interpretation of deep-sea microbial processes. Over longer time scales, deep-sea *in situ* research, when combined with continuous environmental data from physical and chemical sensors, can reveal patterns related to microbial metabolic characteristics and community involvement, aided by computational algorithms[72–74].

The integration and autonomy of systems will be the developmental trend for advancing deep-sea *in-situ* laboratory technologies[54]. Integrated molecular platforms represent a further step beyond collection and preservation, because they begin to combine sampling, sample processing, molecular detection, and data transmission within autonomous or semi-autonomous systems. By 2007, the second-generation Environmental Sample Processor (ESP) had demonstrated field-deployable *in situ* molecular probe/array ("gene-chip") assays for detecting marine microorganisms[75]. Within the ESP, toxin surveillance has been implemented via immunoassay/ligand-binding chemistries conducted *in situ*, whereas quantitative toxin determinations by liquid chromatography–mass spectrometry (LC-MS/MS) are performed on archived samples following instrument recovery[76]. To minimize environment- and method-driven variability, the ESP autonomously filters seawater, performs on-instrument lysis, and chemically preserves (e.g., RNAlater) nucleic acids for downstream analyses[77]. The major strength of ESP-type systems is that they move molecular biology closer to the site of sampling, enabling repeated detection of selected organisms, genes, or biochemical targets with reduced dependence on shipboard processing. Their limitation is that many assays remain targeted rather than fully discovery-based, and some chemical or metabolite measurements still require analysis of archived material after recovery[77,75,76]. In parallel, deep-sea lab-on-chip platforms now perform *in situ* analyses under hyperbaric conditions (field-tested to ~4,800 m), improving sample integrity and enabling downstream genomic workflows[74]. These miniaturized analytical systems are valuable because they can measure selected chemical or biological variables directly under pressure, providing environmental context for omics data. However, their current throughput and molecular breadth remain limited compared with laboratory-based sequencing and multi-omics workflows. Thus, ESP and lab-on-chip platforms mainly address the second and third scientific questions by linking microbial activity and molecular detection

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to temporal environmental variability, but they are still less suited to broad, untargeted discovery than large-volume omics sampling followed by laboratory sequencing.

The Deep-Sea Environmental Sample Processor (D-ESP) was developed and deployed for a variety of molecular assays, including real-time quantitative PCR, within deep-sea environments. Compared with the broader ESP platform, D-ESP represents a more explicitly deep-sea-adapted molecular processing system, designed to perform targeted molecular detection under deployment conditions rather than relying only on recovered samples. This advanced system has been applied for *in situ* detection in methane seep areas at 800 m[55], integrated into the Monterey Accelerated Research System (MARS) seafloor observatory network. Its application to aerobic methanotroph detection illustrates how autonomous molecular assays can address the third scientific question outlined above, by linking specific microbial functional groups to active biogeochemical processes such as methane cycling. The successful deployment of the D-ESP marks a milestone towards the development of autonomous systems to facilitate extensive marine microbiological research. However, as with other targeted molecular platforms, D-ESP is most powerful when the organisms or functional genes of interest are already known. It is less suited to untargeted discovery than metagenomic or metatranscriptomic sequencing workflows, and its deployment[55] depth and assay range remain more constrained than those of simpler sampling and preservation devices.

The proposition of establishing an automated pipeline for multiparametric data acquisition, processing, and analysis in the deep sea has been made[78]. This pipeline comprises a series of consecutive steps that hold the potential for automation. Such pipelines are important because in situ omics data become more interpretable when paired with physical, chemical, imaging, and ecological measurements collected at comparable spatial and temporal scales.

Data from the Crawler Mobile Platform exemplify this approach[79]. This compact, mobile platform, equipped with caterpillar-like mobility, is tethered to a central seafloor node via an umbilical cable, facilitating power supply, remote user communication, and data transfer[80]. This system is integrated into the North-East Pacific Time-series Undersea Networked Experiments (NEPTUNE) cabled observatory operated by Ocean Networks

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Canada. Imaging is conducted, and diversity indices along with biomass (i.e., wet weight per m^2) are calculated on a monthly basis. Although the Crawler Mobile Platform is not an omics instrument itself, it illustrates how mobile observatory platforms provide the spatial and temporal ecological context needed to interpret *in situ* molecular data. Its main limitation is that imaging-derived biomass and diversity indices cannot by themselves resolve microbial gene expression, metabolic activity, or molecular function. Therefore, crawler and observatory systems mainly address the second scientific question, concerning spatiotemporal dynamics and regulatory mechanisms, and become most powerful when integrated with *in situ* molecular samplers such as D-ESP, MISNAC, or preservation-enabled filtration systems. Additionally, ocean observation systems are evolving to enable interactive experiments and remote testing of hypotheses from a molecular biology perspective[56]. This development represents the final methodological stage in deep-sea *in situ* omics: the integration of molecular sampling, environmental sensing, imaging, data transmission, and computational analysis within coordinated observatory networks.

Developing such infrastructures provides new avenues for interactive experiments. This requires appropriate spatial and temporal sampling designs across fixed and mobile platforms, including randomized measurements at different acquisition frequencies for imaging, environmental monitoring, and molecular sampling. Managing the acquired data quality involves evaluating the functional status of all platforms and sensors[81], as well as representing the data through interactive end-user interfaces to achieve result visualization[82]. Such network infrastructure should also possess the capability of artificial intelligence and automated data-management systems for sensor integration, anomaly detection, database organization, and hypothesis generation. A prime example is the proposal of a novel definition of Marine Observatory (MOB), which functions as an integrated infrastructure for observation, monitoring, and experimentation, targeting the collection of high-quality nucleic acids and high-resolution data within a confined geographical area. A MOB is composed of three major parts namely observation infrastructure, cyberinfrastructure, support capacity and one central control system[83]. This type of Marine Observatory can be integrated in larger networks and in Ocean Observing Systems from regional to global scale. All marine monitoring networks are

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increasingly shifting towards service- and end-user-oriented approaches, with their data management network infrastructure being upgraded to act as a "cognitive system" for data interpretation and response accordingly[83]. Such observatory-based systems are especially relevant to the second and third scientific questions raised at the beginning of this section, because they can link spatiotemporal ecosystem dynamics with *in situ* biological activity. However, their current value for omics depends on the successful coupling of observatory infrastructure with robust *in situ* molecular samplers, preservation systems, and downstream analytical workflows.

3. Applications of deep-sea laboratory technologies in microbiological research

3.1 Revealing novel genes and active substances in the deep sea

Deep-sea organisms inhabit extreme physical, chemical, and ecological environments, enduring dark, high-pressure, rapidly fluctuating temperature gradients, and high concentrations of toxic substances. These conditions have driven the evolution of unique biological structures and mechanisms that allow them to adapt in this distinctive environment[84]. New genes with specialized functions may evolve for the synthesis of secondary metabolites[85], particularly enzymes exhibiting remarkable temperature resistance, pH and salinity tolerance, and resistance to toxins[86]. These unique bioactive compounds in deep-sea organisms hold considerable application value, making the discovery of novel genes and active substances an important objective of deep-sea bioresource mining (**Figure 3**). In this context, deep-sea *in situ* omics not only improves sample preservation, but also increases the likelihood of recovering biologically meaningful genes and bioactive molecules that might otherwise be altered or overlooked during sample retrieval and shipboard processing.

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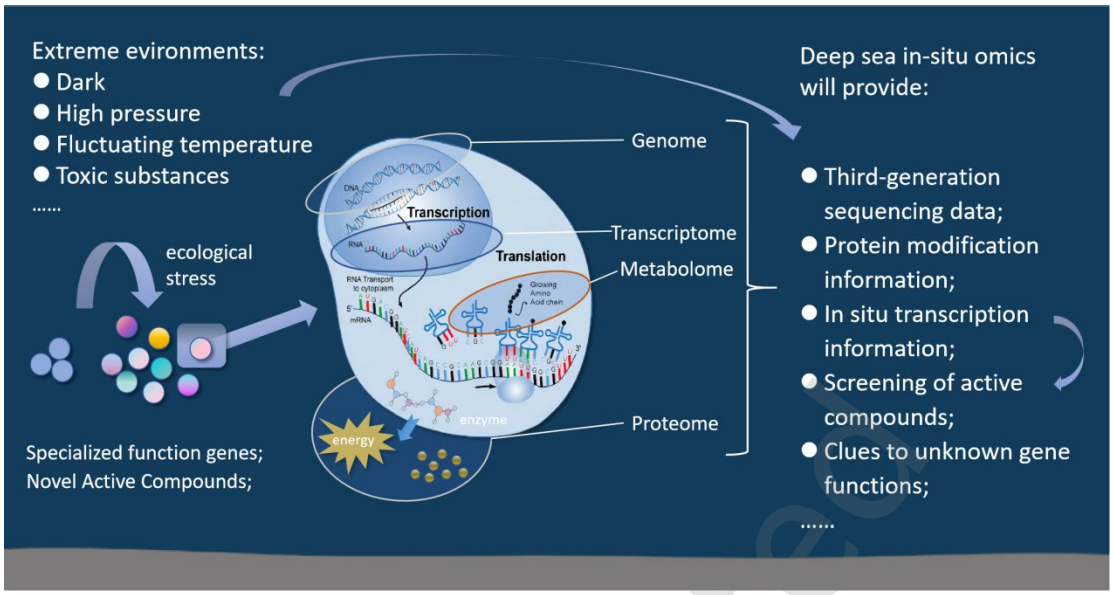


Figure 3. Deep-sea *in situ* omics framework. Extreme environmental conditions such as high pressure, low temperature, and toxic compounds influence microbial genomes, transcriptomes, proteomes, and metabolomes. In situ omics approaches capture these molecular responses, enabling the discovery of novel functional genes, bioactive compounds, protein modifications, and previously unknown gene functions.

This advantage is increasingly supported by recent studies showing that *in situ* approaches improve the identification of active populations and functionally relevant molecular targets in deep-sea ecosystems. Seafloor incubation experiments have shown that depressurization and sample-processing delays can substantially alter the apparent composition and activity of autotrophic microbial communities, indicating that post-retrieval analyses alone may misrepresent *in situ* functional states[30]. Consistent with this, long-term deep-sea *in situ* incubation combined with genomic and transcriptomic analyses has identified key bacterial populations involved in the degradation of animal- and plant-derived detritus in different oceanic regions, providing direct evidence that *in situ* approaches improve the detection of ecologically relevant active degraders[87]. In addition, multi-omics analyses have shown that *in situ* metatranscriptomic data can directly confirm metabolically active functional groups in the deep sea; for example, deep-sea sulfate-reducing bacteria were shown to perform dissimilatory sulfate reduction under deep-sea conditions[88].

Large-volume *in situ* sampling, microbial enrichment, and biomolecule preservation improve the recovery of nucleic acids from low-biomass deep-sea environments, thereby supporting the construction of high-quality long-read sequencing libraries and facilitating the

identification of gene clusters, enzyme-coding genes, and other biotechnologically relevant functional elements [89,90]. For example, deep-sea metagenome analysis expanded the known diversity of β-N-acetylgalactosamine-targeting glycosidases and uncovered phylogenetically distinct enzyme lineages, highlighting the value of deep-sea omics resources for identifying previously unrecognized catalytic potential[91]. In another recent study, a computational screening workflow applied to deep-sea hydrothermal vent metagenomes recovered 11 candidate β-galactosidases, 10 of which were subsequently validated in vitro, providing a direct example of how deep-sea omics datasets can support experimentally oriented enzyme discovery. In addition, earlier metagenomic mining of Arctic Mid-Ocean Ridge hydrothermal systems led to the characterization of several extremozymes, including the thermostable C11 protease globupain and the broad-substrate GH10 xylanase AMOR_GH10A, illustrating that deep-sea omics resources can yield functionally validated biocatalysts with potential biotechnological relevance[92]. When integrated with metagenomic and metatranscriptomic datasets, together with relevant ecological information, these resources provide an increasingly valuable basis for screening conserved novel genes and prioritizing candidate biocatalysts for deep-sea bioresource mining.

3.2 Understanding the distribution and ecological functions of deep-sea microorganisms

Deep-sea microbial communities exhibit striking diversity and are strongly shaped by environmental conditions and ecological interactions. To move beyond community descriptions and reveal the processes that microorganisms carry out, *in situ* omics approaches are indispensable. Combining metatranscriptomics, proteomics, and time-course sampling allows researchers to identify active indigenous species, separate them from background populations, and connect molecular activity to ecosystem processes. The following subsections describe how these strategies clarify the ecological roles of deep-sea microorganisms.

3.2.1 Ecological contributions revealed by *in situ* metatranscriptomics and proteomics

Deep-sea microorganism distribution is influenced by a myriad factors, especially the interactions of complex environmental elements that contribute to the significant dynamism

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and irregularity observed in the communities of deep-sea microorganisms[93]. Changes in factors such as substrate type (carbonate or sediment), microbial activity levels (ranging from low to active seep sites), sulfide concentration, and specific geographical regions (North versus South Hydrate Ridge) contribute to the variations in the composition and diversity of microbial communities in the deep-sea environments[94]. This variability, in turn, plays a role in the observed high diversity and complexity of marine microbial ecosystems. Constructing ecological networks based on deep-sea *in situ* omics data of active microorganisms by revealing their interactions such as competition and symbiotic relationships will lead towards the understanding of the ecological roles and dynamics of active microbial communities. Currently, the environmental samples from the deep ocean harbor a large part of inactive microorganisms from upper water layers. They mix with the active ones and hinder the identification of the ecological functions of active indigenous species.

Currently, due to the inability to culture most deep-sea microorganisms, analyzing metagenome-assembled genomes (MAGs) is the primary approach for deep-sea microbial studies[95–97]. By integrating metatranscriptomic data with MAGs derived from metagenomes, bioinformatic pipelines can help identify active microbial populations and infer their metabolic processes[98]. However, such integration is often challenging in deep-sea systems, where a considerable proportion of reads cannot yet be confidently assigned to reference genomes or functional databases, leaving many transcripts and proteins annotated only as hypothetical. Moreover, MAGs reconstructed from complex deep-sea communities are frequently incomplete or fragmented, which can limit the accurate linkage of genes, transcripts, and proteins to specific taxa or metabolic pathways. Despite these limitations, *in situ* sampling and preservation remain particularly important because they retain molecular signals closer to their native environmental state and therefore provide a more reliable basis for identifying active indigenous microorganisms, especially those occurring at low abundance. This is particularly important for comparing microbial activities across different deep-sea habitats and depths, where metabolic strategies can vary substantially[99]. Likewise, measurements of microbial activity under *in situ* pressure and gas conditions are critical for accurately quantifying nutrient cycling in deep-sea environments[100]. To fully realize the value of *in situ* multi-omics, future

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progress will increasingly depend on improved computational approaches, including long-read-assisted assembly, more accurate graph- and deep learning-based binning and refinement algorithms, and AI-assisted structural and functional prediction for poorly annotated proteins, thereby enabling more robust ecological interpretation of deep-sea microbial dark matter.

Marine microbial communities exhibit variations in metabolic activity and composition due to rhythms and seasonal changes. The application of the deep-sea *in situ* time-course samplers preserves sample integrity and quality, which enables metatranscriptomics study of rhythm and dynamics of different temporal scales in the deep sea. Quantitative or semi-quantitative analysis of microbial gene expression profiles is essential to determine their ecological contribution[71]. Furthermore, *in situ* cultivation of microorganisms in the deep ocean, using techniques such as isotopic labeling and fluorescence *in situ* hybridization, combined with *in situ* omics analysis, makes it possible to accurately determine the metabolic rates and changes of microorganisms in responses to the different cultivation substrates. This knowledge provides a basis for exploring microorganisms involved in organic matter degradation, methane oxidation, and other ecological processes[101–104], and allows specific environmental features to be replicated and measured in cultivation system[105].

To reveal the unique deep-sea biological processes, protein-level research is essential. Post-translation modifications on newly synthesized proteins, such as the protein phosphorylation, may provide evidence for novel mechanisms that may not be disclosed through metagenomics and metatranscriptomics. For example, proteomic comparison of a novel deep-sea sulfate-reducing bacterium (zrk46) under laboratory and *in situ* conditions revealed microscale processes of sulfate reduction specific to the *in situ* environment, likely linked to methane oxidation through direct intercellular electron transfer via cytochromes[88]. The proteins show various dynamic phenomena over a wide range of timescales, from picoseconds to milliseconds[106], and are definitely influenced by hydrostatic pressure and temperature in deep ocean. Traditional research methods cannot meet the requirements for *in situ* proteome extraction and storage in the extreme environments, which makes the subsequent laboratory work difficult to decipher the original proteome. Currently, *in situ* proteomics technology is still in its infancy stage, and further advancements are needed to overcome the

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limitations and barriers mentioned above.

3.2.2 Long-term, continuous *in situ* observations

Due to the inherently dynamic nature of microbial communities, the research in this field needs advancements toward the integration of multi-omics data with high spatiotemporal resolution and detailed environmental information. *In situ* omics, through systematic collection, integration, and modeling of high-throughput molecular data, facilitate more precise characterization of microbial communities in extreme environments where obtaining samples is typically a difficult task[107]. In the exploration of dynamic ecological processes in the deep sea, meaningful insights can only be obtained through *in situ* continuous monitoring and sampling, using multiple devices carried by mobile vehicles across a research region in different depths, sites, times and seasons (**Figure 4**). With collection of data and samples from more representative research regions of the vast oceans, we are able to construct models to interpret possible periodicity and formation of deep-sea ecosystem.

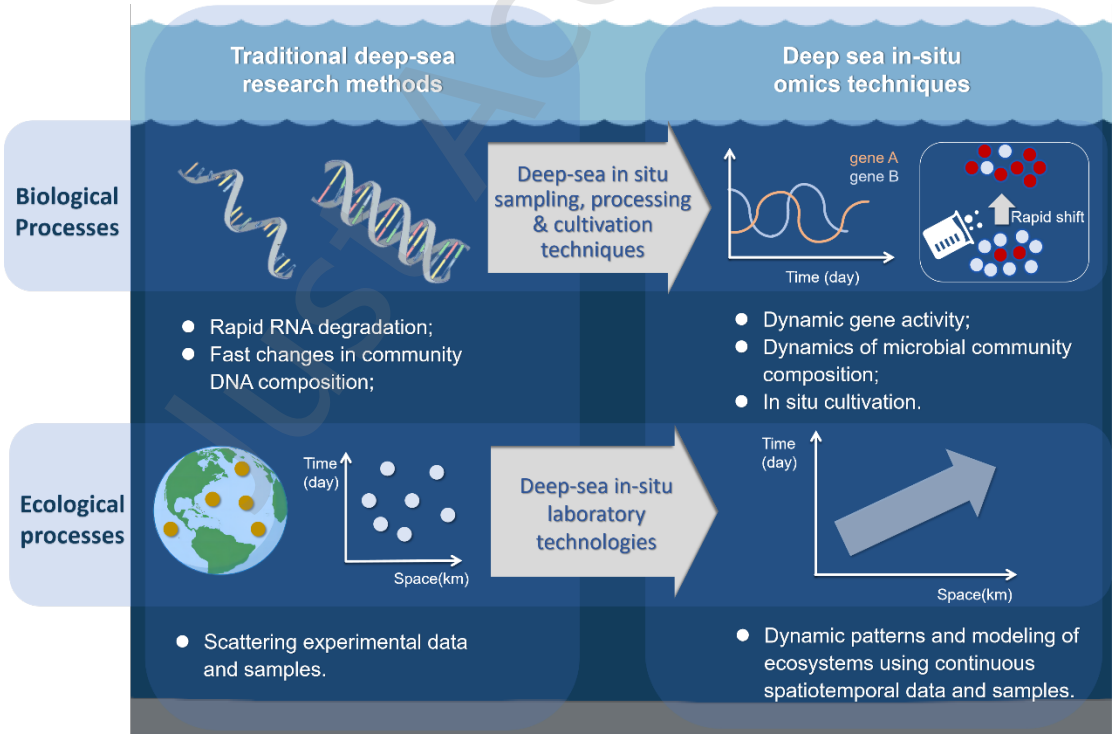


Figure 4. Deep-sea in-situ omics allows for the collection of continuous spatiotemporal data from the deep-sea environment and its inhabitants.

However, due to the current limitations of sampling equipment, conclusive findings

regarding the time scales of periodic changes in deep-sea microbial community structure and the functional gene expression patterns of dominant populations remain elusive. Research on the seasonal and diel gene expression rhythms of deep-sea microbial communities is still limited [108,109]. Given the heavy dependence of deep-sea organisms in oligotrophic environments on surface ocean photosynthesis[110], seasonal and diel rhythmicity is expected at specific depths. Sequencing the temporal transcriptomes of the deep-sea hydrothermal mussel *Bathymodiolus azoricus* revealed that tidal cycles predominate in the transcriptome and physiology of mussels at hydrothermal vents, while daily cycles prevail after laboratory acclimation. The presence of canonical circadian clock genes suggests that observed oscillations may be driven by environmental stimuli or endogenously regulated by biological clocks[111]. However, recent research has shown a dramatic decrease in diel periodicity with increasing depth, to the point where it becomes undetectable in the mesopelagic region. While some ESP platforms have made great progress in the autonomous collection and fixation of marine microorganisms[55,112,113], there are only a small number of studies on *in situ* sampling in the deep sea. Therefore, future research should concentrate on exploring diurnal rhythms in deep-sea microorganisms and obtaining more support for continuous environmental factors and *in situ* cultivation. This will drive a deeper understanding of the ecological adaptability and lifestyles of deep-sea organisms.

The study of deep-sea hydrothermal vent ecosystems began in the 1970s and unveiled novel metabolic characteristics of chemoautotrophic microorganisms and their central role in supporting the deep-sea biosphere[114]. Most microbial studies of chemosynthetic communities at hydrothermal vents and chimney structures were historically based on cultivation and gene amplicon sequencing techniques[115,116]. Ongoing research into other deep-sea ecological niches, such as cold seeps, mud volcanoes and brine pools, continues to provide unprecedented opportunities[117,118]. Studies of community structures in these niches allow reasonable inference of microbial mediated biogeochemical activities within these niches, including methane oxidation, carbon-nitrogen cycling, and metal ion deposition [99,100]. In past decades, investigations of the deep-sea dark biosphere near cold seeps and hydrothermal vents have depended on samples acquired by deep submergence vehicles (DSVs) and *in situ* experiments

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carried out with the aid of DSVs[119–123]. However, the results are not convincing because the fluid components that are sensitive to temperature and pressure will escape from the fluids and decompose into other substances[124]. The seepage from cold seeps and hydrothermal vents is heterogeneous on both time and space scales[69,70,125,126]. The dramatic exchange between vent fluids and surrounding seawater poses a challenge for sample collection. The acquired samples and *in situ* conducted experiments have been temporally and spatially random[127,128]. The formation of the cold seep and hydrothermal vent ecosystems, mechanisms of population dynamics, successional patterns, biotic interactions, and reactions to environmental disturbances require a large amount of *in situ,* long-term, and continuous observation data. Therefore, there is a growing demand for platforms that can be used for *in situ* and long-term observations[129].

Long-term and continuous observational data and time-course sampling can also provide insights into ecological dynamics that are not obtainable through conventional means. The dynamic ecological processes in the deep sea are also manifested in the fluctuations of energy and nutrients caused by geochemical and geophysical processes in the deep sea, which may reshape the microbial environment rapidly, leading to changes in community structure and ecological niches[130]. Certain deep-sea microbial communities play a pivotal role in driving the essential element cycles, such as carbon and nitrogen, which are indispensable for upholding ecosystem stability in oligotrophic settings[131,132]. These microorganisms exhibit niche separation, a phenomenon where functionally similar microbial entities occupy distinct ecological niches driven by specific environmental preferences. This niche separation effectively minimizes direct competition, facilitating the coexistence of these microorganisms[133,134]. For instance, nitrifiers illustrate niche separation by enhancing the efficiency of nitrogen cycling within the ecosystem. Although prior research has reported microbial community changes and metabolic activities, these studies have predominantly relied on metagenomic data, which may not entirely represent the actual ecological dynamics[135–137]. SAR202, the dominant bacteria degrading detrital organic carbon in the deep ocean showed lineage-specific dynamics in expression levels across different time intervals[33]. These subtle differences between the SAR202 lineages probably resulted from their substrate preference and

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availability across the day-night periodicity. Hence, the utilization of *in situ* omics techniques is essential to investigate the expression activity of these microorganisms in their natural environment. This knowledge is pivotal in achieving an in-depth understanding of the intricate dynamics within these distinctive environments.

More broadly, the significance of deep-sea *in situ* omics may extend beyond deep-sea ecology itself. Beyond its direct value for deep-sea ecology, *in situ* omics also has broader significance for understanding the origin of life and the potential for life in extraterrestrial environments. Deep-sea extreme habitats, particularly hydrothermal systems, have long been regarded as plausible analogs of early Earth environments and as useful natural settings for exploring the emergence of life under chemically and physically constrained conditions[138–142]. By enabling more faithful characterization of microbial activity, adaptive strategies, and ecological interactions under native conditions, deep-sea *in situ* technologies can therefore help reveal biological processes that are highly relevant to origin-of-life studies and to the assessment of habitability beyond Earth[143,144]. This relevance is further strengthened by the possibility that geologically active seafloors may also occur in extraterrestrial ocean worlds such as Europa and Enceladus[145], and by the broader role that deep-sea hydrothermal vent research has already played in shaping scientific discussion on life beyond Earth[27]. At the same time, the technological advances developed for deep-sea applications, including automated sampling, low-contamination and closed-system processing, pressure-tolerant engineering, long-term autonomous deployment, and *in situ* molecular assays, may also provide useful methodological references for future life-detection missions. In addition, insights into symbiotic associations in deep-sea ecosystems may further inform hypotheses on the ecological interactions that accompanied early life evolution [146,147].

4. Conclusion

With the continuous development of deep-sea sampling equipment, sequencing technologies, and computational tools, *in situ* omics has become an increasingly powerful approach for investigating the diversity, activity, and ecological functions of deep-sea microorganisms. By reducing artifacts associated with sample recovery and shipboard

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processing, *in situ* sampling and preservation provide a more reliable basis for reconstructing microbial metabolic processes, biogeochemical cycling, and ecosystem dynamics in the deep ocean.

Nevertheless, several limitations still need to be overcome. Many current platforms can perform *in situ* filtration, fixation, or molecular processing, but true pressure-retaining recovery remains uncommon, and depressurization, warming, oxygen exposure, and contamination can still influence biological signals. In addition, fixation chemistry itself may introduce osmotic or chemical stress, low microbial biomass can limit multi-omics analyses, and long-term time-series datasets remain scarce for most deep-sea habitats. *In situ* proteomics, metabolomics, and real-time molecular detection are also still at an early stage, while the integration of omics data with environmental sensors, imaging systems, and ecological models remains technically and computationally challenging.

Overcoming these limitations will require the development of more integrated deep-sea *in situ* laboratories that combine pressure-retaining or low-disturbance sampling, large-volume filtration, optimized preservation chemistry, autonomous time-course sampling, multi-omics processing, and real-time environmental monitoring. Such advances will help transform deep-sea research from static descriptions of community composition toward dynamic, activity-resolved models of deep-sea ecosystems[148,149]. In this sense, deep-sea *in situ* omics remains both a technically challenging and highly promising field, with the potential to reshape our understanding of microbial life and biogeochemical processes in the largest ecosystem on Earth.

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Author contribution statement

Yiling Du drafted the manuscript. Yinghui He, Alexandru S. Barcan, Yunning Wang, Li Tan, Federico Baltar, and Yong Wang reviewed and revised the manuscript. All the authors have approved the final manuscript.

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Data availability

Not applicable.

Declaration of competing interest

All the contributing authors report no conflicts of interest in this work.

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Use of AI Generated Contents and AI-Assisted Technologies Statement

None.

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