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Original Research

Viruses are a key regulator of the microbial carbon cycle in the deep-sea biosphere

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ABSTRACT

The marine biosphere profoundly influences atmospheric chemistry and climate through its carbon cycle. Viruses, the most abundant and diverse entities in marine ecosystems, significantly shape global carbon dynamics by infecting microbes and altering their metabolism. Both DNA and RNA viruses drive these processes in surface oceans, yet their roles in the deep sea—a sunlight-independent ecosystem that stores vast carbon reserves—remain largely unexplored. Here we show that viruses regulate the microbial carbon cycle in the deep-sea biosphere, based on viromic analysis of 66 global sediment samples spanning 1900 to 24,000 years. We identified 324,772 DNA viruses and 61,066 RNA viruses, revealing high diversity and long-term persistence. These viruses co-participate in host carbon metabolism via synergistic genes that encode carbohydrate-active enzymes, with DNA viruses primarily aiding synthesis and RNA viruses supporting decomposition. Integrated virome and microbiome data indicate that viral genes form novel metabolic branches, compensating for host deficiencies and enhancing pathway efficiency in processes like fructose-mannose and pyruvate metabolism. Our findings position deep-sea viruses as key regulators of marine microbial carbon cycling, with implications for global biogeochemical models and climate resilience. This work offers the first holistic perspective on DNA and RNA viruses in deep-sea carbon dynamics, illuminating their ecological significance across geological timescales.

1. Introduction

The biogeochemical element cycle, which involves the exchange of elements among the Earth's biosphere, lithosphere, hydrosphere, and atmosphere, enables elements such as carbon and nitrogen to be recycled and reused throughout terrestrial and marine ecosystems [1,2]. The profound influence of organisms on the global carbon cycle has been recognized for decades [3]. In addition to plants, some microorganisms, including bacteria and archaea, have a significant impact on the carbon cycle, although the mechanisms remain unclear [4]. In atmospheric chemistry, the climate system, and long-term planetary habitability, the marine biosphere plays a central role in the carbon cycle [5,6]. Marine organisms extract dissolved CO_2 and associated carbonate minerals from the ocean, generating organic molecules that can be exchanged in the atmosphere [7,8]. The marine biological carbon pump reduces atmospheric CO_2 by lowering the carbon content at the surface while increasing total carbon storage in the ocean [9]. Viruses are the most abundant and diverse entities in marine ecosystems [10,11], and, as “regulators” of marine biological communities, have a great impact on the cycling of biogeochemical elements such as phosphorus, and especially carbon and nitrogen, within marine ecosystems [11–13]. Many microbes participate in the complex biogeochemical transformation of carbon and nitrogen compounds, whose ecological functions are potentially regulated by viruses in different ways [14]. The role of marine viruses in biogeochemical cycling, including carbon and nitrogen dynamics, has been well characterized [15,16]. However, the influence of deep-sea viruses on the carbon cycle remains largely unknown.

The deep sea possesses a distinctive sunlight-independent ecosystem that differs fundamentally from the sunlight-

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dependent terrestrial ecosystem [17]. As the most abundant biological entities in the deep ocean, viruses play important roles in the biogeochemical cycles of deep-sea ecosystems [10,11]. However, the mechanisms by which viruses mediate these processes remain unclear. In marine ecosystems, viruses shape biological communities by regulating host abundance and diversity through cell lysis, lysogeny, and/or gene transfer [18]. In cold seeps, they influence element cycling, such as carbon, sulfur, and nitrogen, by inducing the mortality and/or metabolic augmentation of hosts [19], thereby playing a key role in deep-sea ecosystems. Studies have shown that the decomposition of benthic viruses releases approximately 37–50 megatons of carbon per year globally, highlighting their substantial impact on deep-sea ecosystems [20]. Accumulating evidence suggests that viral activity plays a globally significant role in regenerating organic carbon and nutrients in marine ecosystems [21]. Although viruses have been found to exert profound effects on the global carbon cycle, their specific mechanisms in deep-sea ecosystems remain poorly understood. Our previous studies have demonstrated that deep-sea viruses actively participate in host microorganisms' biosynthesis and energy metabolism by encoding compensate metabolic genes [10,11]. Based on these studies, it is hypothesized that deep-sea viruses may harbor carbon metabolic genes that can intervene in microbial carbon metabolic pathways, thereby potentially modulating global carbon cycling.

In this study, to elucidate the roles of viruses in deep-sea ecosystems, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) viruses, as two distinct viral types [22], were purified from 66 deep-sea sediment samples, and the virome of global deep-sea sediments was characterized. The results demonstrate the potential essential roles of DNA and RNA viruses in the microbial carbon cycle of deep-sea ecosystems.

2. Materials and methods

2.1. Sediment samples

To evaluate the role of DNA and RNA viruses in the carbon cycle of deep-sea ecosystems, sediment samples were collected globally from four typical deep-sea ecosystems: cold seeps (8 samples), hydrothermal vents (43 samples), seamounts (3 samples), and ocean basins (12 samples). These samples were obtained from the Pacific, Atlantic, and Indian Oceans during China's Dayang No. 1 survey cruises conducted between 2010 and 2018. Sealed sampling boxes released from the ship or a *remotely* operated vehicle (ROV), were used to collect the sediments, thereby ensuring minimal contamination. Upon retrieval, the surface layer of each sediment sample was removed to eliminate potential exogenous contamination, and the samples were stored at −20 °C until analysis. The sampling depths ranged from 1100 to 5878 m, with an average depth of 3000 m, and the sediment layer depth was 15–20 cm.

The geological ages of the deep-sea sediments were determined using radiocarbon dating analysis conducted at the Beta Analytic Testing Laboratory (Miami, USA), as described previously [23]. The foraminifera were collected from the sediment and subjected to radiocarbon dating using accelerator mass spectrometry for carbon-14 (^{14}C) analysis [23]. Sediment samples lacking foraminifera were analyzed using organic carbon. The samples were treated with hydrochloric acid, which was heated to release CO_2, and catalyzed into graphite for ^{14}C dating. The traditional ages were calibrated using the MARINE20 database and corrected for the global ocean carbon pool effect [23].

2.2. Metagenomic analysis of deep-sea viruses

Viral particles were purified from each deep-sea sediment sample using Milli-Q water, as described previously [23–26]. For DNA viruses, viral genomic DNA was extracted from the purified viral particles and subjected to metagenomic analysis [25]. For RNA viruses, viral genomic RNA was extracted, transcribed into complementary DNA (cDNA), and analyzed via metagenomics [23,26]. The purified viral particles and extracted viral genomes were analyzed by polymerase chain reaction using the bacterial 16S rRNA gene-specific primers (515F, 5′-GTGCCAGCMGCCGCGG-3'; 907R, 5′-CCGTCAATTCM TTTRAGTTT-3′) (M = A/C; R = A/G) to exclude microbial contamination. Metagenomic data from 66 deep-sea sediment samples in which both DNA and RNA viruses were simultaneously characterized [23,25,26] were subjected to the identification of viral contigs. Viral contigs were screened from contigs using VirSorter2 [27], DeepVirFinder [28], VIBRANT [29], and CAT [30]. Contigs (≥1.0 kb) were screened using the following algorithms: (1) VirSorter2 (categories 1 and 2); (2) DeepVirFinder (score 0.9 and $p < 0.05$); (3) both VirSorter2 (categories 1–6) and DeepVirFinder (score 0.7 and $p < 0.05$); or (4) all the contigs screened by VIBRANT. The contigs that were not selected using the VirSorter, VirFinder, and VIBRANT algorithms were further screened using the CAT algorithm. Contigs with less than 40 % of genomes classified as bacteria, archaea, or eukaryotes were considered viral contigs. All the viral contigs identified using Virsorter2, DeepVirFinder, VIBRANT, and CAT algorithms were pooled together, and the duplicate sequences were removed. The remaining viral contigs were clustered into viral operational taxonomic units (vOTUs) using CD-HIT v4.8.1 with the parameters -c 0.95, -aS 0.8, -d 400, -T 20, -M 20,000, and -n 5) [31]. The completeness of the vOTUs was evaluated based on CheckV v7.0 analysis [32].

2.3. Taxonomy identification of vOTUs

The viral taxonomy of each vOTU in the 66 deep-sea viromes was identified as described previously [23,25,26]. In brief, the open reading frames (ORFs) of the vOTUs were predicted using Prodigal [33]. The predicted amino acid sequences of the ORFs were then searched against viral genomes in the viral RefSeq database (release 201) to determine the viral taxonomy of the vOTUs using blastp [34] and vConTACT2 [35]. A vOTU was classified as a known virus if more than 50 % of its ORFs matched a known viral genome and the bit score of blastp to the known virus exceeded 50 [36].

2.4. Isolation of deep-sea microbes for metagenomic analysis

To characterize the metagenomes of the deep-sea microbes, mixed microorganisms were isolated from each of eight deep-sea sediment samples (DP003, DP004, DP005, DP006, DP007, DP008, DP009, and DP010) from cold seeps and subjected to metagenomic sequencing analysis. The isolation of the deep-sea mixed microbes from sediments was performed according to previously established methods used in our laboratory [37]. Briefly, 20 g of sediment was mixed with 10 mL of SM buffer (25 mM Tris-HCl, 200 mM NaCl, 20 mM $MgCl_2$, pH 7.5) [38] and shaken for 25 min. After the mixture had been centrifuged at 100×*g* for 5 min, the supernatant was collected. This process was repeated five times. The supernatants were pooled together and centrifuged at 300×*g* (4 °C) for 5 min, followed by centrifugation at 5000×*g* (4 °C) for 30 min. The resulting pellet was resuspended in an SM buffer. This differential centrifugation process was repeated until the supernatant became clear [37]. To exclude contamination of exogenous DNA, the isolated mixed microbes were treated with

DNase at 37 °C for 1 h. The mixture was then centrifuged at 5000×*g* (4 °C) for 30 min to collect the bacterial pellet. The isolated microbes were observed under a transmission electron microscope (Hitachi, Japan) to assess their morphology and purity [37].

2.5. Metagenomic analysis of deep-sea microbes

Bacterial genomic DNA was extracted from the mixed bacteria isolated from each of eight deep-sea sediment samples (DP003–DP010) using the FastDNA SPIN Kit (MP Biomedicals, USA) according to the manufacturer's instructions. The extracted bacterial DNA was then subjected to sequencing at Mingke Biotechnology Co., Ltd. (Hangzhou, China). In brief, the extracted DNA was analyzed using 1 % agarose gel electrophoresis, followed by quantification with the QuantiFluor-ST fluorescence quantitative system (Promega, CA, USA). Next, 300 bp fragments of DNA were generated by ultrasonication. A paired-end library was constructed using the TruSeq DNA Sample Prep Kit according to the standard protocol (Illumina Inc., San Diego, CA, USA), followed by paired-end sequencing (2 × 101 bp) on an Illumina HiSeq (2),000 system (Illumina Inc.). The TruSeq PE Cluster Kit v3-cBot-HS and the TruSeq SBS Kit v3-HS were used according to the manufacturer's protocol (Illumina Inc.). The raw data were trimmed to remove duplicate and adapter reads. Subsequently, clean data were used for de novo contig assembly using Megahit software (https://github.com/voutcn/megahit). Basic bioinformatic analysis was carried out as described in our previous studies [10,23,25].

2.6. Annotation of gene function and metabolic pathways

Amino acid sequences of viral and microbial proteins were aligned against the Kyoto Encyclopedia of Genes and Genomes (KEGG; release 59.0) [39] and Evolutionary Genealogy of Genes: Non-Supervised Orthologous Groups (eggNOG) (v4.5) databases [40] using blastp (parameter: WU-BLAST 2.0 and *E*-value $\leq 1 \times 10^{-5}$). Each viral or microbial protein was searched against the eggNOG ortholog group (OG) to identify the cluster of orthologous groups (COG) by the highest-scoring hit(s) containing at least one high-scoring pair with a bitscore of over 60. KEGG annotation was performed using the KEGG Orthology-Based Annotation System 2.0 software with default parameters in single-best-hit mode. The viral or microbial proteins in the KEGG OG were mapped to the KEGG pathway database. Additionally, the amino acid sequences of viral or microbial proteins were used to identify carbohydrate-active enzymes (CAZymes) on the Database for Carbon and Amino Acid N-Glycosylation Sites (dbCAN) web server [41] based on CAZyme family–specific hidden Markov models.

2.7. Gene abundance quantification analysis of metagenomic data

To calculate the relative abundance of genes in each deep-sea sediment sample, we mapped clean reads from metagenomes to the viral contigs using the CoverM package (https://github.com/wwood/CoverM) with contig mode. We used reads per kilobase of transcript per million mapped reads (RPKM; equation (1)) and transcripts per million (TPM; equation (2)) to quantify gene abundance in metagenomic data:

$$RPKM = \frac{R}{L \times T} \quad (1)$$

where *R* refers to the number of reads that map to a specific gene, *L* refers to the length of the gene in kilobases, and *T* is the total number of reads in millions that align to all genes in the sample. This formula normalizes the read count by both the gene length and the total number of mapped reads in the sample, accounting for biases such as gene length and sequencing depth [42].

On the other hand, TPM is another normalization method that adjusts for sequencing depth and gene length:

$$TPM = \frac{10^6 \times (R/L)}{\sum (Ri/Li)} \quad (2)$$

where *Ri* indicates the number of reads that map to a gene of all genes, and *Li* is the length of the corresponding gene in kilobase [43].

RPKM was used for comparing gene abundance within single deep-sea samples, correcting for gene length differences within the samples, while TPM was more suitable for cross-sample comparisons by normalizing both gene length and total sample abundance.

3. Results

3.1. Global virome of deep-sea DNA and RNA viruses

To investigate the role of deep-sea viruses in the marine carbon cycle, virome datasets containing both DNA and RNA viruses were characterized from 66 deep-sea sediment samples collected globally (Fig. 1a). These samples represented four distinct deep-sea ecosystems—cold seep, hydrothermal vent, seamount, and ocean basin—and spanned the Pacific, Atlantic, and Indian Oceans. The sampling depths ranged from 1100 m to 5878 m, covering typical deep-sea ecosystems. Radiocarbon dating analysis revealed that the sediments were between 1900 and 24,000 years old. Metagenomic analysis of the 66 deep-sea sediments generated 347,017 DNA viral contigs and 64,636 RNA viral contigs. The sizes of the viral contigs ranged from 1000 base pairs (bp) to several kilo bp (kb), suggesting that the purified viral particles had excluded contamination by extracellular vesicles since these nanoparticles usually contain small RNA [44]. These contigs were clustered into 324,772 DNA vOTUs and 61,066 RNA viral vOTUs (Supplementary Fig. S1 and Table S1), indicating the presence of diverse DNA viruses and RNA viruses in the deep-sea sediments.

To determine the taxonomy of these deep-sea viruses, 411,653 clustered vOTUs were compared against known viral genomes in the National Center for Biotechnology Information RefSeq database. The results showed that 11.3 % (46,306) of the vOTUs matched known viral sequences (Fig. 1b), with DNA viruses comprising 97.7 % of the matches and RNA viruses making up 2.3 % (Fig. 1b).

Taxonomic classification of the known deep-sea viruses revealed that the DNA viruses were assigned to 56 viral families, while the RNA viruses were grouped into 18 viral families (Fig. 1c and Supplementary Table S2). Among the DNA viruses, the most abundant families were Circoviridae (24.0 %), Siphoviridae (20.8 %), Microviridae (11.3 %), and Myoviridae (10.7 %) (Fig. 1c). Among the RNA viruses, the most predominant family was Retroviridae (36.9 %), followed by Metaviridae (20.9 %) and Totiviridae (12.9 %) (Fig. 1c).

These findings highlight the rich diversity of viruses in the deep-sea environment and underscore their potential role in the marine carbon cycle.

3.2. Roles of deep-sea viruses in metabolism

To explore the roles of deep-sea DNA and RNA viruses in metabolism, we performed COG annotation of the DNA and RNA

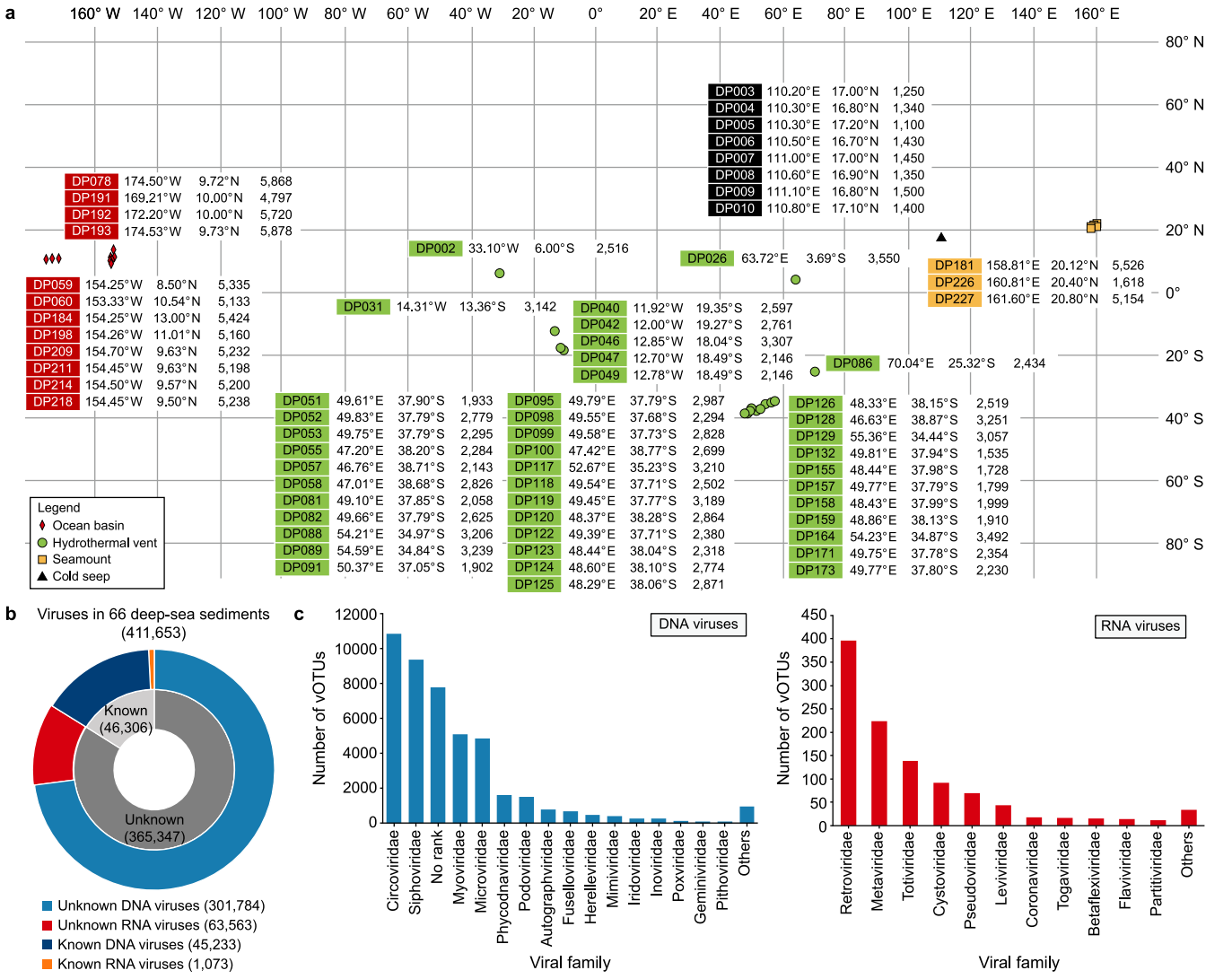

Fig. 1. Global virome of deep-sea DNA and RNA viruses. **a**, Distribution of deep-sea sediment samples. Detailed information about the samples is listed. The first column displays the sample name, the second and third columns show the longitude and latitude of the sampling stations, respectively, and the fourth column indicates the sampling depth (in meters). **b**, Proportion of the known and unknown viral operational taxonomic units (vOTUs). The number in parentheses represents the number of vOTUs. **c**, Abundance of the assigned families of deep-sea viruses. For the DNA viruses, "Others" includes 40 viral families with fewer than 100 vOTUs. For RNA viruses, "Others" represents 7 viral families with fewer than 10 vOTUs.

viromes by comparing the predicted ORFs of the deep-sea viruses with the eggNOG database. The results showed that the viral ORFs were broadly distributed across all COG functional categories, although the majority were annotated as "function unknown" (Fig. 2a). The viral genes were enriched in categories related to "transcription," "replication, recombination, and repair," "cell wall/membrane/envelope biogenesis," and "general function prediction only"—all of which are critical for viral reproduction and survival (Fig. 2a). However, "transcription" was the most abundant category in the known functions of the deep-sea DNA viruses, while "plication, recombination, and repair" predominated in the deep-sea RNA viruses (Fig. 2a).

To further investigate the metabolic potential of deep-sea viruses, we compared the viral genes from both the DNA and RNA viromes against the KEGG pathways. Of the 21,593 annotated viral genes encoded by the DNA vOTUs, 65.3 % (14,339 genes) were classified as "metabolism" (Fig. 2b). For the RNA vOTUs, 72.8 % (12,969 genes) of the 17,813 annotated viral genes were also mapped to "metabolism" (Fig. 2b). The total number of functional genes in the RNA viruses was comparable to that in the DNA viruses, suggesting that most of the viral genes whose functions could be predicted were involved in metabolic processes. Further analysis revealed that the viral genes were associated with "carbohydrate metabolism," "amino acid metabolism," and "nucleotide acid metabolism," with carbohydrate metabolism being the most abundant category in both the DNA and RNA viruses (Fig. 2c). These findings suggest that deep-sea viruses may play an important role in marine carbon metabolism.

To investigate virus-mediated metabolic functions in different deep-sea ecosystems, we analyzed the functional genes carried by DNA and RNA viruses across four ecosystems: cold seep, hydrothermal vent, seamount, and ocean basin. The results showed that most viral genes in all four ecosystems were classified under "metabolism" (Supplementary Fig. S2a). When comparing the abundance of different functional genes using RPKM, the RNA viruses had a higher metabolic gene abundance than the DNA viruses in all four ecosystems (Supplementary Fig. S2b). Furthermore, more than 10 % of the metabolic genes in both the

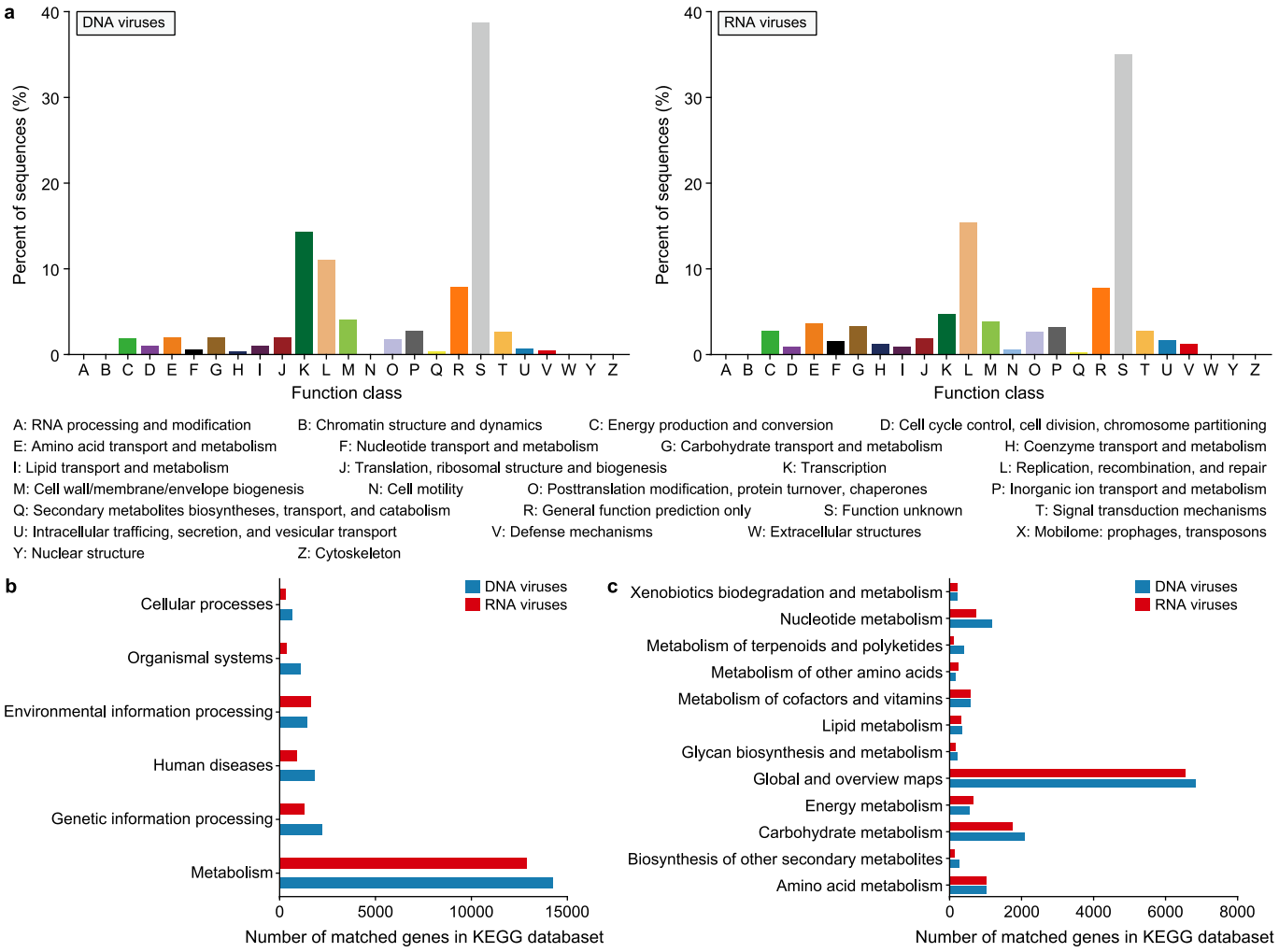

Fig. 2. Roles of deep-sea viruses in metabolisms. **a**, Functional annotation of deep-sea DNA and RNA viromes. Functional annotation of viral proteins was performed by searching the predicted viral proteins against the eggNOG database using Blastp (*E*-value $<1 \times 10^{-5}$). COG functional categories are ordered according to their hit frequency. **b**, Number of deep-sea virus-encoded metabolic genes according to the KEGG pathway database. The viral genes are categorized into six categories of the KEGG pathway database. **c**, Number of viral genes from deep-sea DNA and RNA viruses involved in different metabolisms. The viral genes related to different metabolic functions in the "Metabolism" category of the KEGG pathway database are shown. COG: cluster of orthologous groups.

DNA and RNA viruses may be involved in carbon metabolism across these ecosystems (Supplementary Fig. S2c), highlighting the potential crucial role of deep-sea viruses in carbon metabolism.

Collectively, these findings indicate that deep-sea DNA and RNA viruses may play a vital role in the carbon metabolism of deep-sea ecosystems.

3.3. Involvement of deep-sea viruses in carbon metabolism

To characterize the roles of deep-sea DNA and RNA viruses in deep-sea carbon metabolism, virus-encoded CAZymes were annotated using the dbCAN server based on the CAZyme signature domains. Based on the CAZy database annotation, 1259 DNA virus–encoded genes and 464 RNA virus–encoded genes were identified as CAZyme genes, which were classified into six groups (Fig. 3a). In the DNA viruses, the majority of viral genes (789 genes) were affiliated with CAZymes that had glycosyltransferase (GT) activity, indicating the significant role of viral CAZyme genes in the synthesis of deep-sea organic carbons. In the RNA viruses, most of the viral genes (233 genes) were associated with CAZymes that had glycoside hydrolase (GH) activity, suggesting that RNA viruses can fulfill critical functions in the decomposition of deep-sea organic carbons (Fig. 3a).

Further analysis revealed that the 1259 CAZyme genes in the DNA viruses were classified into 101 CAZyme families, with 21 families containing more than 10 viral genes (Fig. 3b). In the RNA viruses, 789 CAZyme genes were classified into 84 CAZyme families, with 14 families containing more than 10 viral genes (Fig. 3b). Notably, GT Families 2 and 4 (GT2 and GT4) were the most abundant families in both the DNA and RNA viruses (Fig. 3b). These data demonstrate that deep-sea viruses may have a substantial impact on carbohydrate metabolism in deep-sea ecosystems. However, when comparing the abundance of functional genes across different ecosystems using TPM, the relative abundance of viral CAZyme genes varied across the four deep-sea ecosystems (Supplementary Fig. S3). For the DNA viruses, the most abundant CAZyme families were GT9, AA3, GH24, and GT4 in the cold seep, hydrothermal vent, ocean basin, and seamount, respectively. For the RNA viruses, the most abundant CAZyme families were GT51, GH23, GH1, and GH108 in the cold seep, hydrothermal vent, ocean basin, and seamount, respectively (Supplementary Fig. S3). These

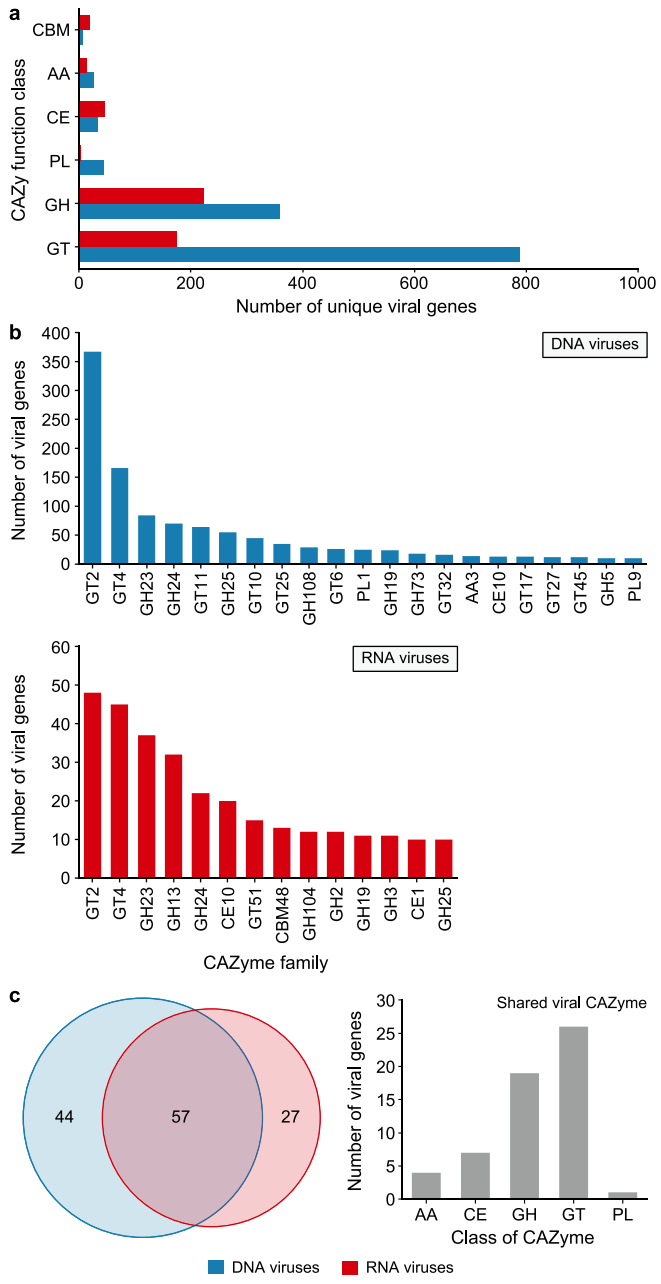

Fig. 3. Involvement of deep-sea viruses in carbon metabolism. **a**, Annotation of viral carbohydrate-metabolism-related genes in the CAZy database. GT: glycosyltransferase; GH: glycoside hydrolases; AA: auxiliary activity; CE: carbohydrate esterase; PL: polysaccharide lyases; CBM: carbohydrate-binding modules. **b**, Abundance of viral carbohydrate-metabolism-related genes at the carbohydrate-active enzyme (CAZyme) family level. The bar charts show the most abundant CAZyme families, which contain more than ten viral genes. **c**, CAZyme genes shared by deep-sea DNA and RNA viruses. A Venn diagram shows the number of viral CAZymes encoded by DNA and RNA viruses. The bar chart indicates the number of viral genes in each class of viral CAZymes.

results demonstrate that viral genes related to deep-sea carbon metabolism are highly diverse across different deep-sea ecosystems.

To explore whether there is a synergistic effect between DNA and RNA viruses in deep-sea carbon metabolism, we compared the CAZyme genes encoded by the DNA and RNA viruses. The results revealed that 57 CAZyme genes were shared by the DNA and RNA viruses (Fig. 3c). These shared CAZyme genes were predominantly classified into the GT (26 genes) and GH (19 genes) families, suggesting the potential synergistic role of DNA and RNA viruses in deep-sea carbon metabolism.

Collectively, these findings indicate that the genes encoded by DNA and RNA viruses may have a synergistic effect on the carbon metabolism of deep-sea ecosystems.

3.4. Bacteriophages and bacterial hosts jointly participate in the deep-sea carbon cycle

To explore the impact of deep-sea viruses on the carbon metabolism of bacterial hosts in the deep sea, we analyzed the microbial metagenomic data of eight deep-sea sediments (DP003, DP004, DP005, DP006, DP008, DP009, and DP010) (National Omics Data Encyclopedia database accession No. OEP002479). The results of the taxonomy classification of deep-sea microbes revealed that the deep sea is home to a diverse array of bacteria (Supplementary Table S3). A total of 3817 bacterial genes were annotated, of which 2214 were associated with "metabolism" (Fig. 4a). Among these metabolic genes, 232 were involved in carbon metabolism, accounting for more than 10 % of the metabolic genes (Fig. 4a). These data suggest that deep-sea bacterial hosts may play an important role in the carbon metabolism of deep-sea ecosystems.

To further assess the roles of deep-sea bacterial hosts in carbon metabolism, we identified microbial genes. The results showed that 8890 bacterial genes belonged to the CAZyme genes (Fig. 4b). These CAZymes were classified into five groups, including 4313 GT genes and 2986 GH genes (Fig. 4b). These data imply that deep-sea bacterial hosts are primarily involved in the synthesis and decomposition of deep-sea organic carbon.

Further analysis revealed that the 8890 CAZyme genes of bacterial hosts were classified into 200 CAZyme families, with 12 families containing more than 200 genes (Fig. 4c). Notably, GT families 2 and 4 (GT2 and GT4) were the most abundant, containing 1767 and 1708 genes, respectively. These results appear similar to those observed in deep-sea viruses, suggesting that both deep-sea bacteriophages and their microbial hosts may play important roles in carbohydrate metabolism in deep-sea ecosystems.

To evaluate the role of deep-sea bacteriophages in the carbon metabolism of bacterial hosts, we characterized the bacteriophage genes and bacterial genes involved in metabolic pathways. The results demonstrated that the deep-sea bacteriophages and bacteria shared 202 metabolic pathways (Fig. 4d), indicating that bacteriophages and co-occurring bacterial hosts were extensively involved in the hosts' metabolic processes. Further analysis revealed that the genes encoded by deep-sea bacteriophages and bacterial hosts were jointly involved in all 15 carbon metabolic pathways (Fig. 4e), suggesting that bacteriophage–host interactions are essential in the deep-sea carbon cycle.

Taken together, these results indicate that bacteriophages and bacterial hosts may jointly participate in the deep-sea carbon cycle.

3.5. Role of viral gene-mediated metabolic compensation in deep-sea carbon metabolism

To explore the underlying mechanism by which deep-sea viruses participate in deep-sea carbon metabolism, we characterized the involvement of these viruses in the carbon metabolic pathways of their hosts using viral and microbial metagenomic data from eight deep-sea sediment samples (DP003, DP004, DP005, DP006, DP007, DP008, DP009, and DP010). The results demonstrated that among the 15 microbial carbon metabolic pathways identified in the eight deep-sea sediments, viral genes

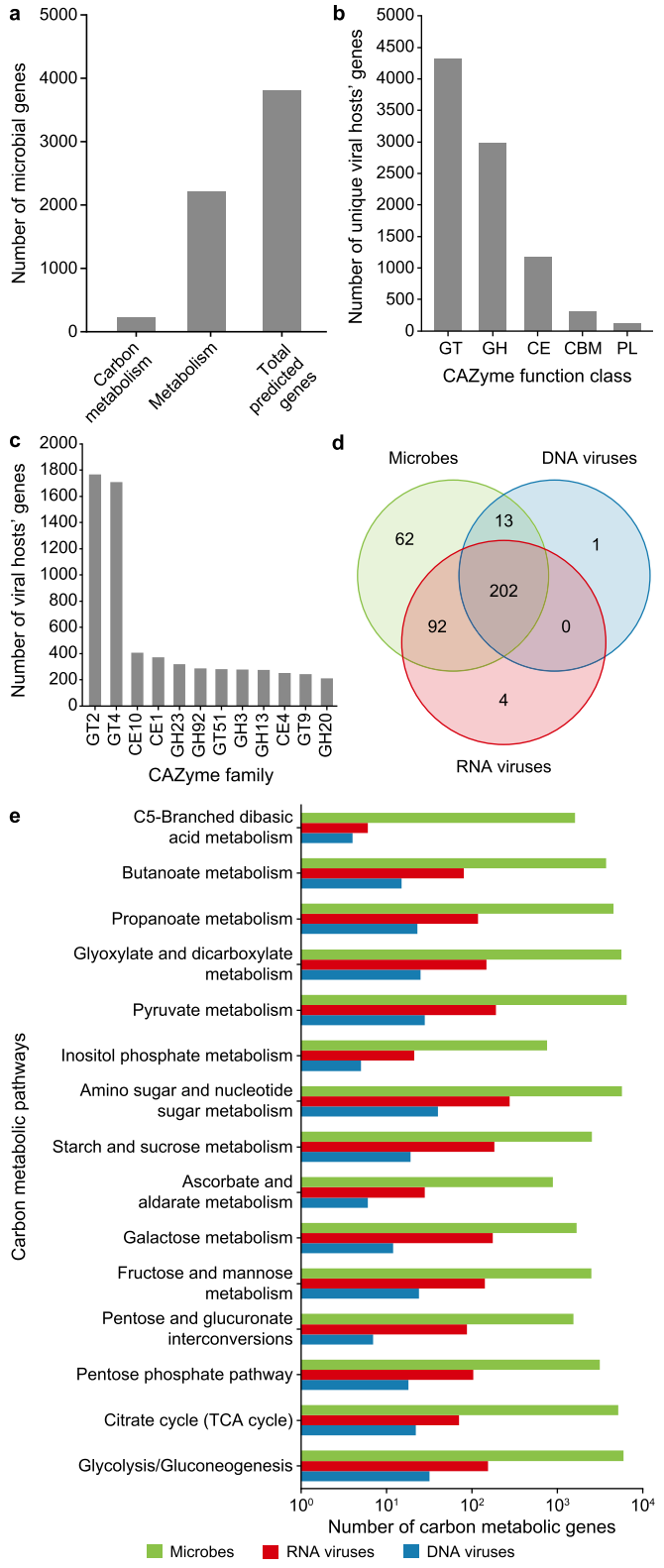

Fig. 4. Bacteriophages and bacterial hosts jointly participate in the deep-sea carbon cycle. **a**, Number of carbon metabolic genes in bacterial hosts in deep-sea ecosystems. **b**, Annotation of carbohydrate-metabolism-related genes of bacterial hosts in the CAZy database. GT: glycosyltransferases; GH: glycoside hydrolases; CE: carbohydrate esterases; PL: polysaccharide lyases; CBM: carbohydrate-binding modules. **c**, Abundance of carbohydrate-metabolism-related genes of bacterial hosts at the carbohydrate-active enzyme (CAZyme) family level. The bar charts show the most abundant CAZyme families, which contain more than 200 genes from viral hosts. **d**, Venn diagram of the metabolic pathways shared by DNA viruses, RNA viruses, and microbes in the deep sea. **e**, Number of viral and microbial genes participating in the carbon metabolic pathways in the deep sea. TCA, tricarboxylic acid cycle.

were involved in 14 pathways (Fig. 5a), which highlights the importance of viruses in deep-sea carbon metabolism. However, only the fructose and mannose metabolism pathway was shared by all eight deep-sea sediment samples (Fig. 5a). Further analysis revealed that 102 unique viral genes participated in carbon metabolism (Fig. 5b). The viral genes most abundantly involved in the amino sugar and nucleotide sugar metabolism pathway were followed by those involved in the fructose and mannose metabolism and starch and sucrose metabolism pathways (Fig. 5b). Only one unique viral gene was associated with the citrate cycle pathway. These findings demonstrate that deep-sea DNA and RNA viruses encode numerous genes that contribute to their hosts' carbon metabolism, indicating a significant role for these viruses in the carbon metabolism of deep-sea environments.

To further elucidate the mechanism by which deep-sea viruses participate in host carbon metabolism, we characterized 15 microbial carbon metabolic pathways that are mediated by both viral and microbial genes. In the fructose and mannose metabolism pathway, deep-sea viruses encoded four unique carbon metabolic genes—mannosyl-3-phosphoglycerate phosphatase (*MpgP*) (EC:3.1.3.70), 2-dehydro-3-deoxy-L-rhamnonate aldolase (*rhmA*) (EC:4.1.2.53), L-sorbose 1-phosphate reductase (*sorE*) (EC:1.1.1), and GDP-4-dehydro-6-deoxy-D-mannose reductase (*rmd*) (EC:1.1.1.281)—which contributed to the hosts' metabolic pathway (Fig. 5c). The enzyme encoded by viral *MpgP* introduced a new branch to produce alpha-mannosylglycerate (Fig. 5c). The viral *sorE* encoded an enzyme that catalyzed the conversion of L-sorbose 1-phosphate to D-glucitol 6-phosphate, forming a new pathway. A new pathway for L-lactaldehyde synthesis in the hosts was created through the involvement of viral *rhmA* (Fig. 5c). In addition, viral *rmd* formed a new pathway to produce GDP-alpha-D-rhamnose (Fig. 5c). These viral genes created new branches in the fructose and mannose metabolism of the hosts, which may have compensated for the carbon metabolism of the hosts and enabled them to adapt to various environments in deep-sea ecosystems.

Moreover, the pyruvate metabolism pathway also showed obvious viral gene-mediated compensation for host metabolism (Fig. 5d). The virus-encoded D-lactate dehydratase (EC:4.2.1.130, hchA) catalyzed the conversion of methylglyoxal into D-lactaldenhyde in a single step, which was more efficient than the host's metabolic pathway (Fig. 5d). The viral unique phosphotransacetylase (EutD) converted acetyl-CoA to acetyl-phosphate (Acetyl-P), introducing a new pathway outside the hosts' metabolic network. The viral alcohol dehydrogenase (EutG) enhanced the hosts' ethanol metabolic pathway (Fig. 5d).

Similar findings of viral metabolic compensation effects were observed for virus-encoded genes in the remaining 12 carbon metabolic pathways: citrate cycle, glycolysis/gluconeogenesis, pentose phosphate pathway, pentose and glucuronate interconversions, galactose metabolism, ascorbate and aldarate metabolism, starch and sucrose metabolism, C5-branched dibasic acid metabolism, amino sugar and nucleotide sugar metabolism, glyoxylate and dicarboxylate metabolism, propanoate metabolism, butanoate metabolism, and inositol phosphate metabolism (Supplementary Fig. S4). The virus-encoded unique metabolic genes compensated for the bacterial host gene-mediated metabolism by forming new pathway branches or completing metabolic processes. These results indicate that deep-sea viruses not only participate extensively in the hosts' carbon metabolism pathways but also form new branches, playing a crucial role in the marine carbon cycle.

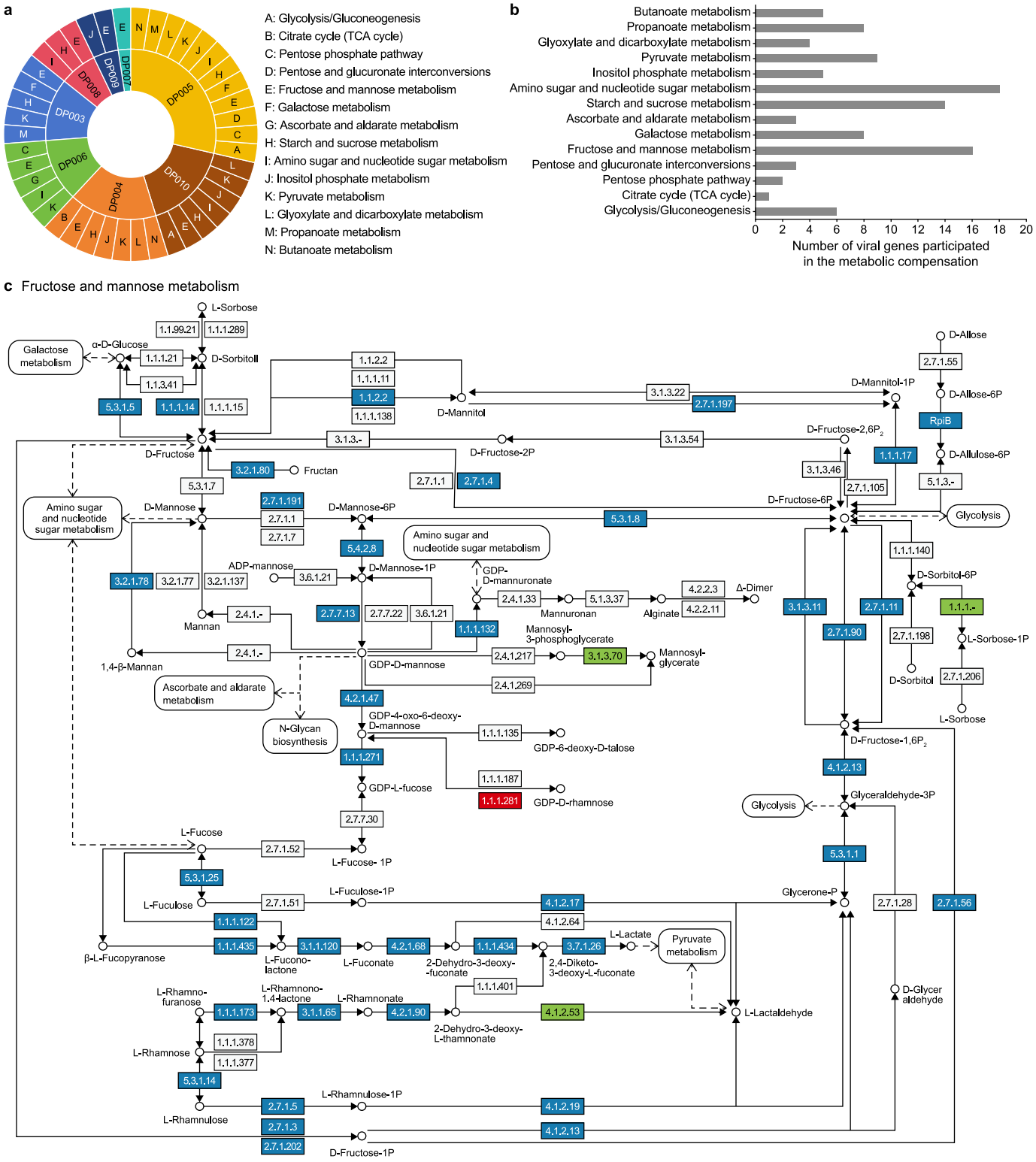

Fig. 5. Role of viral gene-mediated metabolic compensation in deep-sea carbon metabolism. **a**, Microbial carbon metabolic pathways involved by deep-sea viruses. A total of eight deep-sea sediment samples (DP003–DP010) were used. The inner circle represents the microbial carbon metabolic pathways of each sample, while the outer circle shows the pathways in each sample. The center shows the shared pathways across all eight samples. TCA, tricarboxylic acid cycle. **b**, Number of viral genes participating in the carbon metabolism of hosts. The values indicate the count of unique viral genes annotated to carbon metabolic pathways in the host. **c**, Metabolic compensation of viral genes in the host carbon metabolism pathways. The fructose and mannose metabolism pathway was shared by viral and microbial genes across the eight deep-sea samples. **d**, Shared pyruvate metabolism pathway by viral and microbial genes in deep-sea samples. The blue boxes represent host genes, the red boxes indicate unique genes of DNA viruses, the green boxes show unique genes of RNA viruses, and the white boxes represent genes not detected in this study. A solid arrow indicates a molecular interaction or relationship; a dashed arrow denotes a link to or from another map.

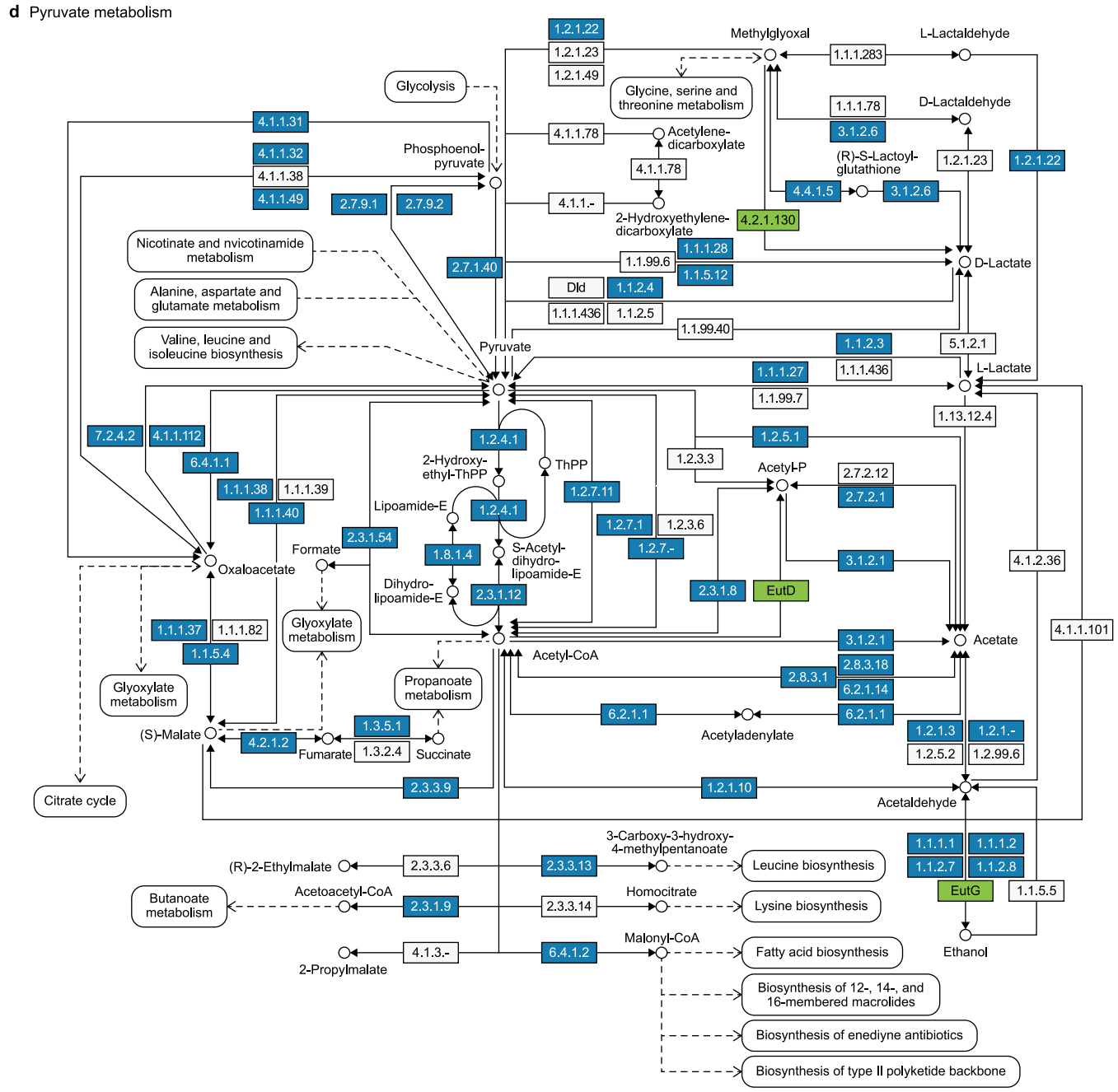

Fig. 5. (*continued*).

Collectively, these findings demonstrate that deep-sea viruses not only participate in their hosts' carbon metabolism pathways but also provide potential metabolic compensation, thus having a significant impact on deep-sea and marine carbon cycles.

4. Discussion

Viruses, characterized by substantial genetic diversity, represent the most numerous biological entities within marine and deep-sea ecosystems [10,23,25]. They infect all living organisms in the ocean [11]. As "pathogens" of marine organisms, viruses play a crucial role in driving marine biogeochemical cycles by releasing cellular carbon and nutrients during the lysis of host cells [13]. Virus-mediated lysis accelerates the release of particulate organic matter and dissolved organic matter in a process known as the "viral shunt" [45]. Most of these organic materials are converted to CO_2 through respiration and photodegradation and then recycled by marine organisms [46–48]. A small portion is deposited into the deep sea via biological pumps, which reduce the carbon content in surface seawater, allowing it to absorb more CO_2 from the atmosphere to restore surface equilibrium [49]. Moreover, microbial infections can alter host metabolism, further influencing the marine carbon cycle [10,11]. Cell-based studies have confirmed that metabolic pathways within cells can be altered [50,51]. However, the ecological role of deep-sea viruses in marine biogeochemical cycles remains poorly understood. In this study, a global analysis of deep-sea sediment viromes identified 411,653 deep-sea viruses (vOTUs), including DNA and RNA viruses, from 66 deep-sea

sediment samples. Of these viruses, less than 12 % could be taxonomically classified. The DNA and RNA viruses were classified into 56 and 18 families, respectively. In the previous largest marine DNA virus dataset, only 195,728 vOTUs were identified [36]. Another investigation focusing on marine RNA viruses revealed only 5504 vOTUs [52]. In this context, the deep-sea viruses revealed in this study possessed high viral diversity. The characterization of virome and microbiome data revealed that deep-sea viruses, including both DNA and RNA viruses, can significantly influence the carbon metabolism of deep-sea ecosystems, thereby providing the first comprehensive insight into the role of these viruses in the marine carbon cycle. In this study, the viruses originated from deep-sea sediments dating back 1900–24,000 years. Recently, it was discovered that benthic viruses purified from 1900 to 17,300-year-old deep-sea sediments can be revived to trigger a viral pandemic in terrestrial soil [53]. The bacteriophages preserved in Qingzang Plateau ice cores from approximately 15,000 years ago encode four auxiliary metabolic genes that regulate methane metabolism, suggesting that these viruses may be linked to global climate changes [54]. These findings closely parallel the results of this study, implying that deep-sea viruses may play a significant role in the marine carbon cycle.

In ocean ecosystems, viruses influence marine biogeochemical cycling by reprogramming host metabolism, including photosynthesis, carbon metabolism, and nitrogen/sulfur cycling [36]. In deep-sea ecosystems, viruses not only regulate microbial diversity and abundance by lysing their hosts but also impact global biogeochemical cycles by participating in the metabolic processes of their hosts [55]. This study revealed that deep-sea DNA and RNA viruses encoded unique metabolic genes, such as *MpgP, sorE, rhmA*, and *rmd*, to create new branches in the carbon metabolism pathways of their hosts. These viral genes may enable hosts to adapt to changing environmental conditions, emphasizing the viruses' potential essential role in the deep-sea carbon cycle. In cyanophages, the viral *psbA* gene encodes the photosystem II core reaction center protein D1, which enables energy generation when host cells are unable to produce the necessary proteins for photosynthesis [56,57]. In hydrothermal plume phages, viral metabolic genes can encode reverse dissimilatory sulfite reductase, potentially infecting sulfur-oxidizing bacteria and suggesting a direct involvement in the sulfur cycle [58]. Additionally, a unique mannose-6-phosphate isomerase gene, *nblA*, identified in the cyanophage S-SCSM1, is involved in the carbon metabolism of hosts [59]. This study highlights the essential role of viral genes in compensating for metabolic processes within deep-sea ecosystems. Future biological assays should investigate the metabolic compensation effects of these viral genes in greater detail.

5. Conclusions

Viruses, including DNA and RNA viruses, have high diversity in the deep-sea biosphere—a distinct ecosystem on Earth. These viruses may play crucial roles in the carbon cycle of deep-sea ecosystems by forming new metabolic branches through viral genes that influence host metabolism. Therefore, viruses can be the key regulators of the carbon cycle in the deep-sea biosphere. Our study employed a viromic analysis of deep-sea DNA and RNA viruses across geological timescales, spanning approximately 19,000 to 24,000 years, to elucidate the critical role of viruses in host carbon metabolism and provided a theoretical framework for understanding Earth's biological evolution. Moreover, the integrated analysis of DNA and RNA viromes in deep-sea sediments revealed their potential complementary roles in carbon metabolism, uncovering a previously unrecognized viral coordination mechanism. These findings expand our fundamental understanding of the involvement of existing viral genes in microbial metabolism.

CRediT authorship contribution statement

Xinyi Zhang: Writing - Original Draft, Methodology, Investigation, Formal Analysis, Data Curation, Software, Validation, Writing - Review & Editing, Conceptualization. **Tianliang He:** Methodology, Formal Analysis, Data Curation, Investigation, Software, Writing - Review & Editing. **Jiyong Zhou:** Writing - Review & Editing, Conceptualization. **Xiaobo Zhang:** Writing - Review & Editing, Resources, Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Project Administration, Supervision.

Data accessibility

The data we obtained from next sequencing were uploaded on National Omics Data Encyclopedia database (accession number OEP002537:
https://www.biosino.org/node/project/detail/OEP00002537,
OEP003296:
https://www.biosino.org/node/project/detail/OEP00003296,
OEP002479:
https://www.biosino.org/node/project/detail/OEP00002479,
OEP003844:
https://www.biosino.org/node/project/detail/OEP00003844).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ese.2025.100609.

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