Unique community structure of viruses in a glacier soil of the Tianshan Mountains, China

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Abstract

Purpose Glaciers of the Tianshan Mountains have been geographically isolated for a long time with little human interference. This study aimed to understand the viral community structures and their ecological functions in a unique glacier soil.

Materials and methods The abundances of purified virus particles and bacteria in the glacier soil were examined using epifluorescence microscopy and quantitative PCR of the 16S rRNA gene, respectively. Metagenomic analysis was employed to investigate the taxonomic and functional compositions as well as the phylogenetic relationship of the functional genes of the viruses in the glacier soil.

Results and discussion A total of eight double-stranded DNA (dsDNA) virus families were identified in the glacier soil, with *Siphoviridae*, *Podoviridae*, and *Myoviridae* being the more abundant families. The diverse dsDNA viruses and few ssDNA and other types of viruses formed the unique community structure of viruses in the Tianshan Mountains glacier soil. The functional genes identified from the virome mainly

belonged to phage-related proteins. The phage terminase of *Caudovirales* presented high diversity, and its amino acid sequences were different from those in other environments.

Conclusions The results showed a unique viral diversity and taxonomic composition in the glacier soil, which suggests the significant ecological role of *Caudovirales* in this environment.

Keywords Functional composition · Taxonomic diversity · Terminase · Virome · Virus

1 Introduction

Viruses are the most abundant biological entities in soil, reaching 10^9 virus particles g^{-1} soil (Williamson et al. 2005). They can affect the community composition of other microbial groups (Vos et al. 2009; Koskella and Brockhurst 2014); manipulate the life histories and evolution of their host (Rohwer and Thurber 2009); mediate biogeochemical cycles in aquatic environments (Jover et al. 2014); and even threaten plant, animal, and human health. As viruses lack shared phylogenetic biomarkers that can be used for the investigation of their community structures and evolutionary history, such as the 16S ribosomal ribonucleic acid (rRNA) gene of bacteria and archaea, viral ecology research is far behind other microbial ecology studies. Following the development of next-generation sequencing platforms, most of the metagenomic studies have focused on viruses from many environments. Nevertheless, although a large number of new viruses and their genes have been identified, which has significantly enriched the virus gene database and contributed to the development of the field of viral ecology, our understanding of the soil viruses in different soil types is still in its infancy.

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Over the past years, studies on viral ecology have mainly focused on air (Whon et al. 2012), marine (Breitbart et al. 2002; Angly et al. 2006; Mizuno et al. 2013; Hurwitz et al. 2014; Jover et al. 2014), freshwater (Lopez-Bueno et al. 2009; Rodriguez-Brito et al. 2010; Roux et al. 2012; Cai et al. 2016), soil (Prigent et al. 2005; Prestel et al. 2008; Zablocki et al. 2014, 2016; Adriaenssens et al. 2015), and sediment (Biddle et al. 2011; Yoshida et al. 2013; Dell’Anno et al. 2015) ecosystems. These studies have demonstrated that viral diversity is consistently high in aquatic and soil environments and that double-stranded DNA (dsDNA) viruses are widespread and in relatively high proportion in aquatic and soil ecosystems.

Xinjiang Uygur Autonomous Region is isolated from other regions of China by the Gobi desert in the east, the Altai Mountains in the northeast, and the Qunlun Mountains in the south and is divided into two main parts by the Tianshan Mountains. As a result, Xinjiang has formed its own unique ecological regions. Owing to its significant distance from the oceans, Xinjiang is a well-studied independent territory, which has a typical arid continental climate, with higher evaporation rates than precipitation, wide temperature range between day and night, and abundant sunshine (Han et al. 2009). The Tianshan Mountains no. 51 glacier region is significantly affected by dust activity and anthropogenic sources. The major ions, especially Na^+ , originate from the dust sources of central Asia and the ocean and are transported by the westerly winds (Dong et al. 2011). To the best of our knowledge, up till now, only a few studies have focused on glacier soil microorganisms, especially viral community structure.

Viruses not only transfer genetic material from one organism to another but also from one ecosystem to another. For instance, some phage sequences have been found to be ubiquitously spread throughout the biosphere (Breitbart et al. 2004; Short and Suttle 2005). Furthermore, there is also evidence that phage can successfully infect and replicate among unrelated environments (Sano et al. 2004). Accordingly, the population composition of viruses in the glacier soil that has been geographically isolated for a long time with little human interference might differ from those in other regions. Therefore, in the present study, we investigated the community composition and the phylogenetic relationship of the functional genes of the viruses in Tianshan Mountains no. 51 glacier soil in Xinjiang.

2 Materials and methods

2.1 Soil sample collection

Soil sample was collected from no. 51 glacier (43° 43' N, 84° 24' E), located at the headwaters of the Kuyntun River in the Tianshan Mountains with an area of about 1.48 km² and maximum length of about 1.7 km. The glacier is about 3610 m

above sea level and surrounded by Gobi desert. To study the viral diversity in the soil under the glacier, approximately 2 kg of soil sample was collected in August 2015, transported at 4 °C to our laboratory, and immediately suspended and filtered.

2.2 Virus extraction and purification

A total of 250 g of the glacier soil sample was suspended in 1.5 l of glycine solution (250 mM, pH = 8.5), shaken for 30 min, and centrifuged at 3500×g for 10 min at 4 °C to precipitate the soil particles. Then, the supernatant was filtered sequentially through 1-mm, 0.45-μm, 0.20-μm, and 100-kDa tangential flow filters (QuixStand, GE Healthcare Life Sciences, Pittsburgh, PA, USA). Subsequently, the viruses in the filtrate were concentrated using 30-kDa centrifugal ultrafiltration tubes (Merck Millipore Ltd., Tullagreen, Ireland) until the final sample volume was less than 1 ml. Finally, the viral concentrates were treated with DNase I (10 u DNaseI/100 μl) and incubated at 37 °C for 1 h to remove free, non-encapsulated DNA. The presence of free and bacterial DNA contaminant was checked by PCR amplification of the 16S rRNA gene with primers 27F/1492R (DeLong 1992).

2.3 Examination of bacterial and viral abundances

The viral abundances in the soils were estimated by direct counting of virus-like particles under epifluorescence microscope (EFM). Aliquots of the final viral concentrates were suspended in 900 μl of sterile deionized water and vacuum filtered through a 25-mm-diameter filters consisting of a Whatman Anodisc with a pore size of 0.02 μm at a pressure less than 62 kPa for each sample. The filters (with the sample side up) were stained with 100 μl of SYBR Gold working solution for 15 min in dark. The slides were counted immediately using three-dimensional structured illumination microscopy at the Center for Biological Imaging, Institute of Biophysics, Chinese Academy of Sciences (Beijing, China).

The soil total DNA was extracted from 0.25 g of the soil sample using a MoBio Powersoil® DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. The bacterial abundances were quantified by qPCR of the 16S rRNA gene. The 16S rRNA gene was analyzed using 1369f (5'-CGGTGAATACGTTTCYCGG-3') and 1492r (5'-GGWTACCTTGTTACGACTT-3') primers (Suzuki et al. 2000). The qPCRs were performed on an iQTM5 Thermocycler (Bio-Rad Laboratories, CA, USA) with 25 μl of the reaction mixture containing 1 μl of the extracted DNA, 12.5 μl of Power SYBR Green Master Mix (TaKaRa Biotechnology, Dalian, China), and 0.25 μM of each primer. The amplification program is as follows: 94 °C for 5 min, 30 cycles of 94 °C for 45 s, 55 °C for 30 s, and 72 °C for 45 s, followed by a plate read at 83 °C. The specificity of the

amplification products was confirmed by melting curve analysis. After measuring the DNA concentration with NanoDrop ND-2000 UV–Vis Spectrophotometers (NanoDrop Technologies, USA), a known copy number of the plasmid DNA was serially diluted in tenfold steps and subjected to real-time PCR in triplicate to generate an external standard curve. The threshold values obtained from sample amplification were interpolated from the standard curve to determine the number of bacteria found per gram of soil.

2.4 Viral DNA extraction and high-throughput sequencing

The viral DNA was extracted from the collected sample using PowerViral Environmental RNA/DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA). The REPLI-g Single Mini Kit (MDA) (Qiagen, Hilden, Germany) was employed to amplify the total viral DNA to obtain the concentration and quantity needed for high-throughput sequencing. For each sample, 1 µg of DNA was fragmented by sonication to a size of 350 bp, and the obtained DNA fragments were end-polished, A-tailed, and ligated with the full-length adaptor for Illumina sequencing with further PCR amplification. Clustering of the index-coded samples was performed on a cBot Cluster Generation System according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on the HiSeq platform (Illumina, San Diego, CA, USA) at Novogene, Beijing, China.

2.5 Sequence data analysis

The original sequences of the glacier soil (named BC virome) were uploaded into Metagenomics Rapid Annotation using Subsystem Technology (MG-RAST) v3.6 (Meyer et al. 2008) to perform quality control and automated annotation as well as produce taxonomic and functional assignments. The MG-RAST generates functional categories based on the SEED subsystem database by hierarchical classification. The metagenomes are available on the MG-RAST server under the accession number 4696287.3 for the soil viruses. The rarefaction curve of the BC virome computed by MG-RAST was compared with M5NR using a maximum e-value of $1e-5$, minimum identity of 60 %, and minimum alignment length of 15 (measured in aa for protein and bp for RNA databases).

Subsequently, the original sequence reads were also performed for quality control and then loaded into Soapdenovo (Luo et al. 2012) with kmer = 59, no read trimming, and a minimum of 100 reads per contig for sequence assembly. The assembly contigs were uploaded into Metavir2 server (Roux et al. 2014) to annotate viral metagenomic sequences and produce taxonomic assignments. Taxonomic composition was computed from BLAST comparison with the Refseq complete viral genome protein sequence database from NCBI (released on January 19, 2016) using BLASTp (29.65 % of the virome

sequences presented a significant hit; the e-value threshold was 10^{-5}) and displayed through Krona.

2.6 Phylogenetic analysis

Phylogenetic analysis of the phage terminase was reconstructed using MEGA6 software (Tamura et al. 2013). For the phage terminase tree, reference amino acid sequences were downloaded from the Metavir2 server (Roux et al. 2014). All the amino acid sequences (Table S1, Electronic Supplementary Material) were aligned using ClustalW, and then, the gaps and ambiguously aligned positions were deleted. After alignment, the phylogeny tree was constructed using the Jones–Taylor–Thornton model, maximum likelihood method, and bootstrapping using 1000 replicates for each sequence.

3 Results

3.1 Bacterial and viral abundance

The abundances of purified virus particles and bacteria from the glacier soil were examined using EFM and qPCR of the 16S rRNA gene, respectively. Fluorescence enumeration showed that the dsDNA virus abundance was 4.35×10^9 virus particles g^{-1} soil (dry weight). Quantitative PCR assays revealed that the 16S rRNA gene abundance was 2.76×10^9 copies g^{-1} soil (dry weight). The dsDNA virus to bacteria abundance ratio in the glacier soil was 1.6.

3.2 Sequence analysis of the BC virome

Pyrosequencing of the BC virome resulted in 13,327,792 raw reads, with an average length of 153 bp. The metagenomic preliminary analysis results were computed by MG-RAST, and a total of 1,836,368 reads with a 144-bp average length passed the quality control. The mean GC percent was 48 % for clean data, and most of the predicted protein sequences (93.6 %) were orphans (no significant matches to translated gene in a BLASTX search of the SEED non-redundant database). In addition, only 0.08 % of the annotated sequences were identified as rRNA features, and 62.2 % of the identified protein features were functional categories. The rarefaction curve of the BC virome (Fig. S1, Electronic Supplementary Material) computed by MG-RAST showed 3050 species count cluster at a minimum identity of 60 % for the 13,327,000 reads that were uploaded. As the rarefaction curve neared an asymptote, we assumed that a substantial fraction of the viral richness was sampled. The alpha diversity, determined based on a species-level annotation, summarizes the diversity of organisms in a sample with a single number, and the annotated species richness indicates the number of distinct

species annotations in the combined MG-RAST dataset. The species diversity of the BC virome was 263.5 species, which are relatively lower than that of open soil and hypolith (344.5 and 1058.4 species, respectively) (Zablocki et al. 2014).

Contig assembly was performed to circumvent the short read lengths generated with the Illumina platform for open reading frame (ORF) prediction and annotation. A total of 3723 contigs larger than 500 bp were assembled with Soapdenovo software, and the longest contig was 14,975 bp.

3.3 Viral diversity and taxonomic composition

A total of 3723 contigs larger than 500 bp were uploaded for analysis of the BC viral taxonomic composition. According to the Metavir2 annotation, 1104 contigs had homology to known viral sequences through the GAAS (genome length normalization) taxonomic composition plot (Fig. 1) with an e-value threshold of 10^{-5} . The majority (96 %) of the contigs showing similarity to dsDNA viruses belonged to the order *Caudovirales*, with *Siphoviridae* being the most abundance family (37 %), followed by *Podoviridae* (23 %) and *Myoviridae* (22 %). Furthermore, 3 % of the contigs shared identity with unclassified *Caudovirales*. The other dsDNA viruses identified at an abundance of 0.09–3 % included *Phycodnaviridae*, *Mimiviridae*, *Iridovirus*, *Tectivirus*, and *Chordopoxvirinae* (Fig. S2, Electronic Supplementary Material). In addition, there were also smaller numbers of contigs that matched the single-stranded DNA (ssDNA) virus families (0.9 %) in the BC virome, including *Inovirus*, *Alphatorquevirus*, and *Circovirus*-like. All these formed the unique community structure of viruses in Tianshan Mountains no. 51 glacier soil.

3.4 Functional composition analysis

Functional annotation of the BC virome was performed by MG-RAST and resulted in 546,018 functional hits. A total of 28 functional categories were assigned to the BC libraries; the majority (65.8 %) of which belonged to phages, prophages, transposable elements, and plasmids (Fig. 2a). Other relatively prominent subsystems (>1 %) included clustering-based subsystems, DNA metabolism, cell wall and capsule, membrane transport, nucleosides and nucleotides, and fatty acids, lipids, and isoprenoids. Furthermore, there were several metabolic pathways involving nitrogen, phosphorus, sulfur, potassium, iron, aromatic compound, RNA, and protein metabolism-related subsystems. However, only 1.4 % of the virulence, disease, and defense subsystem was functionally annotated, which were pathogenic bacteria or phage infection-related proteins.

The subsystem of phages, prophages, transposable elements, and plasmids was further investigated and identified

as 19 phage-related proteins (Fig. 2b); most of them are phage tail fiber proteins (37.2 %), r1t-like streptococcal phage (23.4 %), and phage tail proteins 2 (21.3 %). In this analysis, phage-related functional genes in the BC virome were over-represented compared to functional categories in other types of viruses.

3.5 Phylogenetic analysis

As dsDNA viruses presented the highest abundance in the glacier soil examined, we constructed the phylogenetic tree with terminase amino acid sequences annotated in the Metavir2 analysis, which resembled the phage terminase of *Caudovirales*, and compared it with the terminase of a wide range of known *Caudovirales* viruses and related metagenomic samples. Figure 3 shows an abridged version of the phylogenetic tree for clarity, and the complete tree is illustrated in Fig. S3, Electronic Supplementary Material. The reference sequences of *Caudovirales* were distinguished by different colors according to Metavir2 annotation, such as purple for *Myoviridae*, green for *Siphoviridae*, and blue for *Podoviridae*. A total of 19 terminase amino acid sequences (Table S1, Electronic Supplementary Material) were collected from the BC contigs. According to gene annotation information from Metavir2 database, 14 genes formed four clusters dispersed with the *Siphoviridae* group (light green background in Fig. 3), three genes clustered with *Podoviridae* viruses (light blue background in Fig. 3), and two genes belonged to the *Myoviridae* group (light purple background in Fig. 3). Furthermore, the sequences from the BC virome were very much dispersed within the *Caudovirales* group. These results indicated that diverse dsDNA viruses occurred in no. 51 glacier soil with less human inference.

4 Discussion

The present study showed that the dsDNA viral abundance in the glacier soil was 4.4×10^9 virus particles g^{-1} soil (dry weight). However, it should be noted that only dsDNA could be labeled by fluorescent through EFM method, while ssDNA viruses cannot be detected. Also, the nucleic acid extraction method applied in the present study excluded RNA viruses, and therefore, our data may not reflect the complete viral abundance in the glacier soil. Previous study showed that there were approximately $1.3\text{--}4.2 \times 10^9$ virions in forest soils and 8.7×10^8 to 1.1×10^9 agricultural soils (Williamson et al. 2005), $1.5\text{--}6.4 \times 10^8$ virus particles in arid desert soils (Zablocki et al. 2016), and $1.1\text{--}1.2 \times 10^9$ virions in wheat rhizosphere soil (Swanson et al. 2009); all these viral particles were tested in per gram dry weight soil by transmission electron microscopy (TEM). In the analysis of virus abundance under EFM and TEM, the extraction procedure may have

Fig. 1 Taxonomic composition of the BC virome. Composition type was GAAS (genome length normalization) with an e-value threshold of 10^{-5} , as generated by Metavir2. The most abundant viral taxa are indicated in *red*, while *yellow*, *green*, *blue*, and *purple* indicate the subsequent abundant viral taxa, respectively

Fig. 2 Major functional subsystems in the BC virome. **a** Relative abundance of the functional genes based on the predicted ORFs identified by the MG-RAST server. **b** Relative abundance of the functional genes of phages and prophages determined by MG-RAST

Fig. 3 Phylogenetic tree of phage terminase amino acid sequences determined by Metavir2. The tree is bootstrapped with 1000 subreplicates, and confidence levels >50 % are indicated at the internodes. The *scale bar* represents an amino acid substitution per site. The *purple-colored* sequence names indicate *Myoviridae*, *green-colored* sequence names stand for *Siphoviridae*, and *blue-colored* sequence names represent *Podoviridae*

easily resulted in phage tail breakage (Williamson et al. 2005), and sample transportation from Tianshan to our lab in Beijing may have caused a small portion of virus degradation; therefore, the number of virus particles determined by EFM and TEM might have been underestimated. Thus, future work will focus on developing new protocols for viral enumeration, such as PCR-based analysis.

Moreover, diverse dsDNA viruses and few ssDNA and other types of viruses were identified from the glacier soil sample. A total of eight dsDNA virus families were identified; *Caudovirales* was the most abundant viral order, and *Phycodnaviridae* and *Mimiviridae* viruses were the second-

most abundant taxa. A recent study showed that viruses are predominantly found in similar habitats, regardless of their geographic proximity according to the global maps of viral biogeography (Paez-Espino et al. 2016). In this study, the virus diversity observed in the glacier soil showed similar taxonomic composition but different abundance to that found in Antarctic open soil (Zablocki et al. 2014) and Namib desert (Adriaenssens et al. 2015). The viral community structure in glacier soil characterized by high levels of bacterial lysogens and low extracellular virus counts may be derived by multiplicity factor, such as temperature, water availability, geographic distances, and habitat type. Until now, no clear

Table 1 Taxonomic composition of metagenomes in different types of samples by Metavir analysis

Systems	Location	Percent of total sequences						Reference
		Group		Order		Family		
Air	Industrial complex in Seoul, South Korea	ssDNA	78	Unclassified	37	Circoviridae	24	Whon et al. (2012)
	Residential district in Seoul, South Korea	ssDNA	39	Unclassified	35			
Seawater	Iquique, Chile (10 m)	dsDNA	81	Caudovirales	66	Myoviridae	39	Cassman et al. (2012)
	Iquique, Chile (90 m)	dsDNA	56	Caudovirales	39			
		ssDNA	43	Circoviridae	33			
	Iquique, Chile (200 m)	dsDNA	89	Caudovirales	73	Myoviridae	39	
Seawater	Northern Line Islands	dsDNA	100	Caudovirales	83			Dinsdale et al. (2008)
Seawater	Indian Ocean	dsDNA	99	Caudovirales	79	Myoviridae	27	Williamson et al. (2012)
						Podoviridae	32	
						Siphoviridae	17	
Freshwater	Lake Limnopolar, Antarctica	ssDNA	50	Microviridae	40			Lopez-Bueno et al. (2009)
Freshwater	Lake Bourget in France	dsDNA	67	Caudovirales	54	Myoviridae	18	Roux et al. (2012)
						Podoviridae	15	
						Siphoviridae	19	
	Lake Pavin in France	ssDNA	37	Microviridae	30			
						Myoviridae	25	
						Podoviridae	13	
Freshwater	Salton Sea (Kent SeaTech) in USA	dsDNA	98	Caudovirales	93	Siphoviridae	29	Rodriguez-Brito et al. (2010)
						Myoviridae	19	
						Podoviridae	46	
Freshwater	Jiulong River in China	dsDNA	99	Caudovirales	84	Siphoviridae	27	Cai et al. (2016)
						Myoviridae	26	
						Podoviridae	31	
Sediment	Izu-Ogasaware Trench	ssDNA	81	Microviridae	74			Yoshida et al. (2013)
	Mariana Trench	ssDNA	76	Circoviridae	36	Gokushovirinae	32	
	Shimokita Peninsula	ssDNA	97	Microviridae	36	Circoviridae	50	
Sediment	Arctic Ocean	ssDNA	68	Inoviridae	45	dsDNA	29	Dell'Anno et al. (2015)
Sediment	Brazos-Trinity Basin in Mexico	dsDNA	95	Caudovirales	51	Phycodnaviridae	20	Biddle et al. (2011)
Soil	Antarctic open soil	dsDNA	76	Caudovirales	70	Myoviridae	25	Zablocki et al. (2014)
						Podoviridae	15	
						Siphoviridae	27	
Soil	Namib desert	dsDNA	80	Caudovirales	75	Myoviridae	9	Adriaenssens et al. (2015)
						Podoviridae	10	
						Siphoviridae	48	
Soil	Coastal soil in Scotland	ssDNA	88	Microviridae	84.6			Reavy et al. (2015)
	Brown Earth in Scotland	ssDNA	71.6	Microviridae	50.3			
Soil	Tianshan Mountains no. 51 glacier	dsDNA	96	Caudovirales	85	Myoviridae	22	This study
						Podoviridae	22	
						Siphoviridae	38	

explanation for this division could be offered, and the dispersal pattern was not quite obvious in soils owing to the limitation of samples.

We compared the glacier soil viral composition with selected viromes in Metavir2 (Table 1), spanning different types of samples, including air, seawater, freshwater, sediment, and

soil. The prevalence of *Caudovirales* as the dominant taxon was observed in most of the seawater (Dinsdale et al. 2008; Cassman et al. 2012; Williamson et al. 2012) and freshwater (Rodriguez-Brito et al. 2010; Roux et al. 2012; Cai et al. 2016) samples and in some of the soil samples (Biddle et al. 2011; Zablocki et al. 2014; Adriaenssens et al. 2015). On comparing the families within the *Caudovirales*, *Podoviridae* and *Myoviridae* represented the major fraction in aquatic environments, and *Siphoviridae* were dominant in soil environments, including the glacier soil sample examined in the present study.

An alternative approach to investigate the community composition and diversity of viruses is to assign the phylogeny of signature genes. The marker gene with the most hits in the BC virome, as determined by Metavir2, was the terminase gene, which was mainly present in the *Caudovirales* order. In current study, the terminase gene *terL*, required for packaging initiation in members of the *Caudovirales*, was the most common in many soil habitats (Black 1995; Zablocki et al. 2016). A phylogenetic tree was generated from the contig sequences, which showed that most of the BC virome *terL* gene amino acid sequences clustered separately from the reference sequences of the cultured tailed phages and related metagenomic samples (Fig. S2, Electronic Supplementary Material). These results are consistent with taxonomic classification in the BC virome. It has been hypothesized that despite the very high local viral diversity, the total global diversity is limited by worldwide movement of virions, because viruses can move throughout the world and contribute to a global genetic pool (Breitbart and Rohwer 2005). The present study showed that the viruses in the glacier soil had high local viral diversity and their functional genotypes differed from those in other environments, which might be owing to the blocking of communications among bacteria and restriction of virus movement, resulting in the formation of a specific viral community structure in the glacier soil.

Our study could lead to a more comprehensive understanding of the viral distribution pattern in the glacier soil, as well as facilitate building global maps of soil viral biogeography in the nearest future. Further efforts on detailed sequencing using third-generation sequencing technology may benefit to identify new virus species and achieve longer viral contigs that probably represent more abundant viral populations.

5 Conclusions

The results of the present study demonstrated a unique viral diversity and taxonomic composition as well as the significant ecological role of *Caudovirales* in no. 51 glacier soil. The functional genes identified from the BC virome mainly belonged to phage-related proteins. Furthermore, the phage terminase of *Caudovirales* exhibited high diversity in the

BC virome, and its terminase amino acid sequences were different from those in other environments. The observed abundance as well as taxonomic and functional diversities of the viruses in the glacier soil could aid future soil viromic studies on a larger spatial scale and enhance our understanding of the ecological functions of soil viruses.

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