

# **1 Viral spillover risk in High Arctic increases in a 2 glacierised watershed**

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## 12 Abstract

13 While many viruses have a single natural host, host restriction can be incomplete, hereby  
 14 leading to spillovers to other host species. However, such spillover risks are difficult to  
 15 quantify. As climate change is rapidly transforming environments, it is becoming critical  
 16 to quantify the potential for spillovers. To address this issue, we resorted to an unbiased  
 17 metagenomics approach, and focused on two environments, soil and lake sediments from  
 18 Lake Hazen, the largest High Arctic freshwater lake in the world. We used DNA and  
 19 RNA sequencing to reconstruct the lake's virosphere and its range of eukaryotic hosts,  
 20 and estimated the spillover risk by measuring the congruence between the viral and the  
 21 eukaryotic host phylogenetic trees. We show that spillover risk is higher in lake sediments  
 22 than in soil and increased with runoff from glacier melt, a proxy for climate change.  
 23 Should climate change also shift species range of potential viral vectors and reservoirs  
 24 northwards, the High Arctic could become fertile ground for emerging pandemics.

# 1 Introduction

Viruses are ubiquitous and are often described as the most abundant replicators on Earth [1–3]. In spite of having highly diverse genomes, viruses are not independent “organisms” or replicators [4], as they need to infect a host’s cell in order to replicate. These virus/host relationships seem relatively stable within superkingdoms, and can hence be classified as *archaeal*, *bacterial* (also known as *bacteriophages*), and *eukaryotic* viruses [5–7]. However, below this rank, viruses may infect a novel host from a reservoir host by being able to transmit sustainably in this new host, a process known as viral spillover [8, 9]. Indeed, in the past years, many viruses such as the Influenza A [10], Ebola [11], and SARS-CoV-2 [12] viruses spilled over to humans and caused significant diseases. While these three viruses have non-human wild animal reservoirs as natural hosts, others have a broader host range, or their reservoir is more challenging to identify. For instance, iridoviruses are known to infect both invertebrates and vertebrates [13], and *Picornavirales* are found in vertebrates, insects, plants, and protists [2]. Such host restrictions (or alternatively, spillover risks) are to date poorly defined and hence, difficult to assess without resorting to expert opinion [14].

Numerous factors can influence such a viral spillover risk. For instance, viral particles need to attach themselves to specific receptors on their host’s cell to invade it [15–17]. The conservation of those receptors across multiple species allows these hosts to be more predisposed to becoming infected by the same virus [17, 18]. Indeed, from an evolutionary standpoint, viruses are more prone to infecting hosts that are phylogenetically close to their natural host [15, 19], potentially because it is easier for them to infect and colonize species that are genetically similar [20]. Alternatively, but not exclusively, high mutation rates might explain why RNA viruses spill over more often than other viruses [15], as

most lack proofreading mechanisms, making them more variable and likely to adapt to a new host [17].

While more studies are starting to characterize the communities and genomes of viruses in extreme environments [21–23], only few, if any, describe their spillover risk. The High Arctic is of special interest as it is particularly affected by climate change, warming faster than the rest of the world [24–27]. Warming climate and rapid transitions of the environment increase the risks of spillover events by varying the global distributions and dynamics of viruses, and their reservoirs and vectors [28, 29], as shown for arboviruses [30] and the Hendra virus [31]. Furthermore, as the climate changes, the metabolic activity of the Arctic’s microbiosphere also shifts, which in turns affects numerous ecosystem processes such as the emergence of new pathogens [32]. It has now become critical to quantify the risk of these spillovers. An intuitive approach to do this is to focus on the cophylogenetic relationships between viruses and their hosts [33–37]. Conceptually, if both viruses and their hosts cospeciate, the topologies of their respective phylogenetic trees should be identical or *congruent*. On the other hand, the occurrence of spillovers would result in incongruent virus/host phylogenies, so it can be postulated that measuring phylogenetic congruency can be used to assess spillover risk.

To test this hypothesis in the context of a changing High Arctic environment, we resorted to a combination of metagenomics and of cophylogenetic modelling by sampling, in an unbiased manner, both the virosphere and its range of hosts [3], focusing on eukaryotes, which are critically affected by viral spillovers [38]. We contrasted two local environments, lake sediments and soil samples of Lake Hazen, to test how viral spillover risk is affected by glacier runoff, and hence potentially by global warming, which is expected to increase runoff with increasing glacier melt at this specific lake [24, 25]. While microbial eukaryotes have been identified in Lake Hazen and other Arctic freshwater ecosystems [39–42], the

Arctic multicellular macro-eukaryotes have yet to be sufficiently characterized. We show here that the risk of spillovers increases with warming climate, but is likely to remain low in the absence of “bridge vectors” and reservoirs.

## 2 Methods

### (a) Data acquisition

An overview of data acquisition and analytical pipeline is shown in figure S1. Between the 10<sup>th</sup> of May and the 10<sup>th</sup> of June, 2017, sediments and soil cores were collected from Lake Hazen (82°N, 71°W; Quttinirpaaq National Park, northern Ellesmere Island, Nunavut, Canada), the largest High Arctic lake by volume in the world, and the largest freshwater ecosystem in the High Arctic [25]. Sampling took place as the lake was still completely covered in ice (table S1), as previously described [24]. The sediment accumulation at the bottom of the Lake is caused by both allochthonous and autochthonous processes. The former are characterised by meltwaters that flow between late June and the end of August, and run from the outlet glaciers along the northwestern shoreline through poorly consolidated river valleys, while the latter refer to the sedimentation process within the lake.

To contrast soil and sediment sites, core samples were paired, whenever possible, between these two environments. Soil samples were taken at three locations (figure S2; C-Soil, L-Soil, and H-Soil) in the dried streambeds of the tributaries, on the northern shore, upstream of the lake and its sediments. The corresponding paired lake sediment samples were also cored at three locations, separated into hydrological regimes by seasonal runoff volume: negligible, low, and high runoff (figure S2; C-Sed, L-Sed, and H-Sed). Specifically, the C (for *Control*) sites were both far from the direct influence of glacial

inflows, while L sites were at a variable distance from Blister Creek, a small glacial inflow, and the H sites were located adjacent to several larger glacial inflows (Abbé River and Snow Goose). The water depth at L-Sed and H-Sed was respectively 50 m and 21 m, and the overlying water depth for site C-Sed was 50 m.

Before sample collection, all equipment was sterilised with 10% bleach and 90% ethanol, and non-powdered latex gloves were worn to minimise contamination. Three cores of  $\sim$  30 cm length were sampled at each location, and the top 5 and 10 cm of each sediment and soil core, respectively, were then collected and homogenized for genetic analysis. DNA was extracted on each core using the DNeasy PowerSoil Pro Kit, and RNA with the RNeasy PowerSoil Total RNA Kit (MO BIO Laboratories Inc, Carlsbad, CA, USA), following the kit guidelines, except that the elution volume was 30  $\mu$ L. DNA and RNA were thereby extracted three times per sampling site, and elution volumes were combined for a total volume of 90  $\mu$ L instead of 100  $\mu$ L.

To sequence both DNA and RNA, a total of 12 metagenomic libraries were prepared ( $n = 6$  for DNA,  $n = 6$  for RNA), two for each sampling site, and run on an Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA) at Génome Québec, using Illumina's TruSeq LT adapters (forward: AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC, and backward: AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT) in a paired-end 125 bp configuration. Each library was replicated ( $n = 2$  for DNA,  $n = 3$  for RNA) for each sample. Further details, such as DNA and RNA yields following extractions, can be found in Colby et al. [24].

## (b) Data preprocessing and taxonomic assignments

A first quality assessment of the raw sequencing data was made using FastQC v0.11.8 [43]. Trimmomatic v0.36 [44] was then employed to trim adapters and low-quality reads and bases using the following parameters: phred33, ILLUMINACLIP:adapters/TruSeq3-PE-2.

121 fa:3:26:10, LEADING:3, TRAILING:3, SLIDINGWINDOW:4:20, CROP:105, HEADCROP:15,  
122 AVGQUAL:20, MINLEN:36. A second round of quality check was performed with FastQC  
123 to ensure that Illumina’s adapter sequences and unpaired reads were properly removed.  
124 Reads assembly into contigs was done *de novo* with both SPAdes v3.13.1 [45] and metaS-  
125 PAdes v3.13.1 [46] for DNA, and with Trinity v2.9.0 [47], rnaSPAdes v3.13.1 [48], and  
126 metaSPAdes for RNA. The choice of an assembly tool was based on (i) the number of  
127 contigs generated, (ii) the taxonomic annotations, (iii) the time of assembly, and (iv) the  
128 contig lengths (see electronic supplementary material). In all cases, the pipelines were  
129 used with their default settings.

130 Once assembled, a high-level (superkingdom) taxonomic assignment was determined  
131 based on BLASTn v2.10.0 [49] searches. Those were performed at a stringent  $10^{-19}$   
132 *E*-value threshold against the partially non-redundant nucleotide (nr/nt) database from  
133 NCBI v5 [50] (<ftp.ncbi.nlm.nih.gov/blast/db/nt.tar.gz>; downloaded on June 17, 2020).  
134 We chose this threshold to increase the significance of our hits, as our preliminary results  
135 showed less ambiguity with smaller *E*-values, starting at a  $10^{-19}$  cut-off. The proportions  
136 of taxonomic annotations (“Archaea,” “Bacteria,” “Eukaryota,” or “Viruses”) were cal-  
137 culated, and a 95% consensus was taken to assign a superkingdom rank for each contig.  
138 When no such 95% consensus could be determined, the contigs were classified as “Other.”

139 To refine the taxonomic assignment of “viruses,” GenBank’s viral nucleotide sequences  
140 v238.0 [51] were retrieved ([ftp.ncbi.nlm.nih.gov/genbank/gbvr1\\*seq.gz](ftp.ncbi.nlm.nih.gov/genbank/gbvr1*seq.gz); downloaded on  
141 23<sup>rd</sup> of July, 2020), concatenated, converted into FASTA with seqret v6.6.0 [52], and  
142 used to create a local database for BLASTn alignments. For each sampling location, after  
143 combining the DNA and RNA contigs classified as viral in the previous step, BLASTn  
144 searches were again conducted at the same stringent  $10^{-19}$  *E*-value threshold, and the  
145 accession numbers of all the High-scoring Segment Pairs (HSPs) were used to retrieve

146 their corresponding taxonomy identifiers (IDs) and their full taxonomic lineages with the R  
147 package `taxonomizr` v0.5.3 [53]. The viral contigs were also mapped with Bowtie2 v2.3.5.1  
148 [54], using default settings to compare BLASTn and Bowtie2 efficiencies in refining these  
149 taxonomic annotations. As searches were found to be more sensitive with BLASTn than  
150 with Bowtie2 (see electronic supplementary material), only BLASTn results are shown  
151 hereafter, as our goal was to find as many similar sequences as possible in more than  
152 one species to eventually infer the virosphere from the virome. Eukaryotic contigs were  
153 processed as above, based off the nr/nt database. To increase specificity considering that  
154  $> 100$  hits were found *per* contig, results were filtered by keeping a maximum of 12 HSPs  
155 whose  $E$ -value  $< 10^{-100}$  *per* contig, for which lineages were obtained.

156 All samples were filtered to remove non-eukaryotic and uncultured hosts as well as  
157 viral and eukaryotic sequences with no taxonomy information. The ViralZone [55] and  
158 International Committee on Taxonomy of Viruses (ICTV) [56] databases were consulted  
159 to obtain host range information on each viral family. These taxonomic assignments were  
160 then used to retrieve their phylogenetic placements according to the Tree of Life (ToL)  
161 ([tolweb.org](http://tolweb.org)), hence generating two trees: one for known viruses and one for known eu-  
162 karyotes. For this, we used the `classification` and `class2tree` functions from the R  
163 package `taxize` v0.9.99 [57, 58]. In each environment, vertices of the viral and eukary-  
164 otic trees were then put in relation with each other according to the Virus-Host DB  
165 (downloaded on the 29<sup>th</sup> of September, 2020) [59]. These relations were saved in a bi-  
166 nary association matrix (0: no infection; 1: infection), one for each environment. To  
167 simplify downstream computations without losing any information, only eukaryotic hosts  
168 associated to at least one virus were kept in the non-viral tree.



## 169 (c) Spillover quantification

170 To quantify viral spillovers based on the viral and eukaryotic hosts identified, we employed  
 171 the Random Tanglegram Partitions algorithm (Random TaPas) [60]. This algorithm com-  
 172 putes the cophylogenetic signal or congruence between two phylogenetic trees, the viral  
 173 and the host trees, with the normalised Gini coefficient ( $G^*$ ). When congruence is large,  
 174 or “perfect,” the two trees are identical and hence, there is strong cophylogenetic signal –  
 175 and absence of spillover. On the other hand, weak congruence is evidence for the existence  
 176 of spillovers. Random TaPas quantifies congruence in two ways: a geodesic distance (GD)  
 177 [61], or a Procrustes distance (Procrustes Approach to Cophylogeny: PACo) [62], the lat-  
 178 ter measuring the distance between two trees geometrically transformed to make them  
 179 as identical as possible. To partially account for phylogenetic non-independence when  
 180 measuring congruence, Random TaPas further implements a resampling scheme where  
 181  $N = 10^4$  subtrees of about 20% of the total number of virus/hosts links are randomly  
 182 selected. This selection is used to generate a distribution of the empirical frequency of  
 183 each association, measured by either GD or PACo.

184 Each empirical frequency is then regressed against a uniform distribution, and the  
 185 residuals are used in two ways: (i) to quantify co-speciation, which is inversely propor-  
 186 tional to spillover risk; and (ii) to identify those virus/host pairs that contributed the  
 187 least to the cophylogenetic signal, *i.e.*, the most to spillover risk. This risk is finally  
 188 quantified by the shape of the distribution of residuals (for GD or PACo), with  $G^*$  that  
 189 takes its values between 0 (perfect congruence, no spillover) to 1 (maximal spillover risk),  
 190 with a defined threshold of 2/3 indicating a “large” value of  $G^*$  or large incongruence.  
 191 To account for phylogenetic uncertainty, the process is repeated  $n = 1,000$  times, each  
 192 replicate being a random resolution of the multifurcating virus/host trees of life into a  
 193 fully bifurcating tree.

### 3 Results and Discussion

#### (a) Plant and fungal viruses are overrepresented

Based on our most sensitive annotation pipeline (see electronic supplementary material), viruses represented less than 1% of all contigs, and our samples were dominated by bacteria, with low proportions of eukaryotes (proportions of bacterial and eukaryotic contigs being respectively  $> 89.2\%$  and  $< 6.4\%$ , in 11 out of 12 samples) (see electronic supplementary material). These results could be due to our extraction process, which might have been biased towards microbial nucleic acids. For instance, an overrepresentation of bacteria was also found in a shotgun-metagenomics based study that also used soil extraction kits [63]. To assess the impact of this potential bias, the extraction process should be taken into consideration by future studies.

RNA viral contigs of all kinds (*i.e.*, dsRNA, +ssRNA, and -ssRNA viruses) were found to be significantly more abundant than DNA viral contigs in all samples, as 70.5% to 87.9% of viral families had a RNA genome (binomial tests,  $P < 2.48 \times 10^{-7}$ ; figure 1, table 1). This dominance of RNA viruses is not unexpected, as fungi biomass for instance surpasses that of bacteria in Arctic environments by 1-2 orders of magnitude [64], and eukaryotes are known to be the main targets of RNA viruses [2, 5–7].

Our results are however difficult to compare with previous studies in the High Arctic, as most were solely based on DNA metagenomics sequencing [22, 65, 66], probably because RNA viruses are thought to be unstable [23], or due to inadequate sampling strategies to extract RNA viruses [67]. Two studies have been able to recover RNA viruses but one had not intended to characterise the RNA viral community, rather randomly finding sequences related to ssRNA viruses [68], and while the other also identified RNA and DNA viruses from RNA-seq, the environments were slightly different: although they included a

freshwater lake, more abundant in ssDNA phages, the Baltic Sea contains varying levels of salinity [69] unlike Laze Hazen. Nonetheless, our results and those of this previous study [69] both show that it is possible to recover RNA viruses from RNA-seq metagenomics.

All viral genomes confounded, in all samples, known plants and/or fungi viral families were overrepresented compared to those infecting animals and protists, as proportions of the former ranged between 69.8% to 87.1% (binomial tests,  $P < 2.48 \times 10^{-7}$ ; table 1). This overrepresentation might reflect a preservation bias, due to the constitutive defences found in plants and fungi offered by their waxy epidermal cuticles and cell walls [70], even if most plant viruses lack a protective lipoprotein envelope as found in animal viruses [71]. But irrespective of such a preservation bias, this imbalance could imply a high spillover potential among plants and fungi in the High Arctic for two reasons. First, RNA viruses are the most likely pathogens to switch hosts, due to their high rates of evolution [15, 72]. Second, plant biomass has been increasing over the past two decades in the High Arctic due to regional warming [73], and is likely to keep doing as warming continues.

## (b) Spillover risk increases with glacier runoff

Given these viral and eukaryotic host representations, can spillover risk be assessed in these environments? To address this question, we resorted to the novel global-fit model Random TaPas, which computes the congruence between the virus and the eukaryotic host trees, with large and weak congruent topologies indicating low and high spillover risk, respectively. The stability of its results was assessed by running this algorithm three times, and by combining the results for the normalised Gini coefficients ( $G^* \in [0, 1]$ ), a direct measure of spillover risk (see Methods).

When the runoff volume was negligible (the C sites; figure 2a), spillover risk's median  $G^*$  ranged between 0.675 and 0.725, thus exceeding the 2/3 threshold, and was

significantly higher in soil than in lake sediments for both GD and PACo (Dunn test, Benjamini-Hochberg [BH] correction,  $P < 0.001$ ). However, in the presence of a low runoff volume (the L sites), spillover risk was higher in lake sediments than in soil for GD, but lower for PACo, with  $G^* \in [0.70, 0.75]$  (Dunn test, BH correction,  $P < 0.001$ ; figure 2*b*). Finally, in the high runoff regime (the H sites), for both GD and PACo, spillover risk was higher in lake sediments than in soil, with values of  $G^* > 0.75$  (Dunn test, BH correction,  $P < 0.001$ ; figure 2*c*). Altogether, these results show that as runoff volume increases from almost non-existent to high, spillover risk increased with runoff, and shifted from higher in soil, to higher in lake sediments.

This pattern is consistent with the predictions of the Coevolution Effect hypothesis [74], and provides us with a mechanism explaining the observed increase in spillover risk with runoff. Lake Hazen was recently found to have undergone a dramatic change in sedimentation rates since 2007 compared to the previous 300 years: an increase in glacial runoff drives sediment delivery to the lake, leading to increased turbidity that perturbs anoxic bottom water known from the historical record [25]. Not only this, but turbidity also varies within the water column throughout the season [75], hence fragmenting the lake habitat every year, and more so since 2007. This fragmentation of the aquatic habitat creates conditions that are, under the Coevolution Effect, favourable to spillover. Fragmentation creates barriers to gene flow, that increases genetic drift within finite populations, accelerating the coevolution of viruses and of their hosts. This acceleration leads to viral diversification which, should it be combined with “bridge vectors” (such as mosquitoes in terrestrial systems) and/or invasive reservoir species, increases spillover risk [74]. Lake sediments are environmental archives: over time, they can preserve genetic material from aquatic organisms but also, and probably to a lesser extent, genetic material from its drainage basin. The coevolutionary signal detected in lake sediments reflects

interactions that may have happened in the fragmented aquatic habitat but also elsewhere in the drainage basin. Regardless of where the interaction occurred, our results show that spillover risk increases with runoff, a proxy of climate warming (figure 2).

To our knowledge, this is the first attempt to assess the complete virosphere of both DNA and RNA viruses, and their spillover capacity in any given environments, leading us to show that increased glacier runoff, a direct consequence of climate change, is expected to increase viral spillover risk of Lake Hazen. However, as this is the first study applying the Random TaPas algorithm, we do not have yet any comparators in order to gauge the efficacy of  $G^*$  in assessing spillover capacity, both qualitatively and quantitatively. Additional studies including more runs of the algorithm and multiple environmental settings of the High Arctic would be necessary to further reinforce our results, and to calibrate the “true” risk of viral spillovers.

### (c) Spillovers might already be happening

To go one step further and identify the viruses most at risk of spillover, we focused on the model predictions made by Random TaPas. Under the null model, the occurrence of each virus/host association is evenly distributed on their cophylogeny (when sub-cophylogenies are drawn randomly, from a uniform law). Departures from an even distribution are measured by the residuals of the linear fit. Positive residuals indicate a more frequent association than expected, that is pairs of host/virus species that contribute the most to the cophylogenetic signal. On the other hand, negative residuals indicate a less frequent association than expected, and hence pairs of host/virus species that contribute little to the cophylogenetic signal, because they tend to create incongruent phylogenies, a signature of spillover risk.

For both soil and lake sediments, the magnitude of the largest residuals tended either to

291 decrease (Soil; figure 3a) or to stay the same (Sediment; figure 3b). This means that with  
 292 increasing runoff, the strength of the cophylogenetic signal may remain steady, or may  
 293 even weaken. On the other hand, the magnitude of the most negative residuals either  
 294 remained globally unchanged (Soil; figure 3a, 6a), or tended to become more negative  
 295 (Sediment; figure 3b, 6b). This latter pattern indicates that as runoff increases, the  
 296 strength of the cophylogenetic signal deteriorates, potentially implying a higher spillover  
 297 risk in lake sediments.

298 With this, Random TaPas can identify the viruses driving the spillover signal. For  
 299 both GD (figure 4) and PACo (figure S7), the 5 most negative residuals of each sample  
 300 ( $n = 60$ ) suggest that viruses are most likely to spill over in fungi ( $n = 19$ ), plants  
 301 ( $n = 16$ ), and protists ( $n = 15$ ; including different species of microalgae), the other 10  
 302 species being mostly insects (animals:  $n = 8$ ; oomycetes:  $n = 2$ ).

303 Altogether, we provided here a novel and unbiased approach to assessing spillover  
 304 risk. This is not the same as predicting spillovers or even pandemics, because as long as  
 305 “bridge vectors” and/or invasive reservoir species [74] are not present in the environment,  
 306 the likelihood of dramatic events probably remains low. But as climate change leads to  
 307 shifts in species ranges and distributions, new interactions can emerge [76], bringing in  
 308 vectors that can mediate viral spillovers [77]. This twofold effect of climate change, both  
 309 increasing spillover risk and leading to a northward shift in species ranges [78], could  
 310 have dramatic effect in the High Arctic. Disentangling this risk from actual spillovers and  
 311 pandemics will be a critical endeavour to pursue in parallel with surveillance activities,  
 312 in order to mitigate the impact of spillovers on economy and health-related aspects of  
 313 human life, or on other species [9].

**Data accessibility.** The raw data used in this study can be found at [www.ncbi.nlm.nih.gov/bioproject/556841](http://www.ncbi.nlm.nih.gov/bioproject/556841) (DNA-Seq) and at [www.ncbi.nlm.nih.gov/bioproject/PRJNA746497](http://www.ncbi.nlm.nih.gov/bioproject/PRJNA746497) (RNA-Seq). The code developed for this work is available from [github.com/sarisbro/](https://github.com/sarisbro/) data.

**Authors' contributions.** S.A.B. and A.J.P. designed research; G.A.C. collected and processed the samples; A.L. performed all analyses and wrote the original draft; A.L. and S.A.B. wrote the manuscript with contributions and suggestions from G.A.C. and A.J.P.; and S.A.B. and A.J.P. supervised this study and acquired funding.

**Competing interests.** We declare we have no competing interests.

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## References

1. Dávila-Ramos, S., Castelán-Sánchez, H. G., Martínez-ávila, L., Sánchez-Carbente, M. D. R., Peralta, R., Hernández-Mendoza, A., Dobson, A. D., Gonzalez, R. A., Pastor, N. & Batista-García, R. A., 2019 A review on viral metagenomics in extreme environments. *Frontiers in Microbiology* **10**, 2403. (doi:10.3389/fmicb.2019.02403).
2. Koonin, E. V., Dolja, V. V. & Krupovic, M., 2015 Origins and evolution of viruses of eukaryotes: the ultimate modularity. *Virology* **479-480**, 2–25. (doi:10.1016/j.virol.2015.02.039).
3. Zhang, Y. Z., Shi, M. & Holmes, E. C., 2018 Using metagenomics to characterize an expanding virosphere. *Cell* **172**, 1168–1172. (doi:10.1016/j.cell.2018.02.043).
4. Dawkins, R., 1989 *The Selfish Gene*. Oxford University Press.

- 338 5. Malik, S. S., Azem-e Zahra, S., Kim, K. M., Caetano-Anollés, G. & Nasir, A., 2017  
339 Do viruses exchange genes across superkingdoms of life? *Frontiers in Microbiology*  
340 **8**, 2110. (doi:10.3389/fmicb.2017.02110).
- 341 6. Nasir, A., Forterre, P., Kim, K. M. & Caetano-Anollés, G., 2014 The distribution  
342 and impact of viral lineages in domains of life. *Frontiers in Microbiology* **5**, 194.  
343 (doi:10.3389/fmicb.2014.00194).
- 344 7. Nasir, A., Kim, K. M. & Caetano-Anollés, G., 2017 Long-term evolution of viruses:  
345 a Janus-faced balance. *BioEssays* **39**, 1700026. (doi:10.1002/bies.201700026).
- 346 8. Alexander, K. A., Carlson, C. J., Lewis, B. L., Getz, W. M., Marathe, M. V.,  
347 Eubank, S. G., Sanderson, C. E. & Blackburn, J. K., 2018 *The Ecology of Pathogen*  
348 *Spillover and Disease Emergence at the Human-Wildlife-Environment Interface*, pp.  
349 267–298. Springer International Publishing. (doi:10.1007/978-3-319-92373-4\_8).
- 350 9. Becker, D. J., Washburne, A. D., Faust, C. L., Pulliam, J. R. C., Mordecai, E. A.,  
351 Lloyd-Smith, J. O. & Plowright, R. K., 2019 Dynamic and integrative approaches to  
352 understanding pathogen spillover. *Philosophical Transactions of the Royal Society*  
353 *B: Biological Sciences* **374**, 20190014. (doi:10.1098/rstb.2019.0014).
- 354 10. Long, J. S., Mistry, B., Haslam, S. M. & Barclay, W. S., 2019 Host and viral  
355 determinants of Influenza A virus species specificity. *Nature Reviews Microbiology*  
356 **17**, 67–81. (doi:10.1038/s41579-018-0115-z).
- 357 11. Rewar, S. & Mirdha, D., 2014 Transmission of Ebola virus disease: an overview.  
358 *Annals of Global Health* **80**, 444–451. (doi:10.1016/j.aogh.2015.02.005).
- 359 12. Zhou, P. & Shi, Z.-L., 2021 SARS-CoV-2 spillover events. *Science* **371**, 120–122.  
360 (doi:10.1126/science.abf6097).
- 361 13. İnce, İ. A., Özcan, O., Ilter-Akulke, A. Z., Scully, E. D. & Özgen, A., 2018  
362 Invertebrate iridoviruses: A glance over the last decade. *Viruses* **10**. (doi:  
363 10.3390/v10040161).
- 364 14. Grange, Z. L., Goldstein, T., Johnson, C. K., Anthony, S., Gilardi, K., Daszak,  
365 P., Olival, K. J., O'Rourke, T., Murray, S., Olson, S. H. *et al.*, 2021 Ranking the  
366 risk of animal-to-human spillover for newly discovered viruses. *Proceedings of the*  
367 *National Academy of Sciences* **118**. (doi:10.1073/pnas.2002324118).
- 368 15. Longdon, B., Brockhurst, M. A., Russell, C. A., Welch, J. J. & Jiggins, F. M.,  
369 2014 The evolution and genetics of virus host shifts. *PLoS Pathogens* **10**, 1–8.  
370 (doi:10.1371/journal.ppat.1004395).



- 371 16. Maginnis, M. S., 2018 Virus–receptor interactions: the key to cellular invasion.  
372 *Journal of Molecular Biology* **430**, 2590–2611. (doi:10.1016/j.jmb.2018.06.024).
- 373 17. Parrish, C. R., Holmes, E. C., Morens, D. M., Park, E.-C., Burke, D. S., Calisher,  
374 C. H., Laughlin, C. A., Saif, L. J. & Daszak, P., 2008 Cross-species virus trans-  
375 mission and the emergence of new epidemic diseases. *Microbiology and Molecular*  
376 *Biology Reviews : MMBR* **72**, 457–470. (doi:10.1128/MMBR.00004-08).
- 377 18. Woolhouse, M. E. J., Haydon, D. T. & Antia, R., 2005 Emerging pathogens: the  
378 epidemiology and evolution of species jumps. *Trends in Ecology & Evolution* **20**,  
379 238–244. (doi:10.1016/j.tree.2005.02.009).
- 380 19. Longdon, B., Day, J. P., Alves, J. M., Smith, S. C. L., Houslay, T. M., McGonigle,  
381 J. E., Tagliaferri, L. & Jiggins, F. M., 2018 Host shifts result in parallel genetic  
382 changes when viruses evolve in closely related species. *PLoS Pathogens* **14**, 1–14.  
383 (doi:10.1371/journal.ppat.1006951).
- 384 20. Geoghegan, J. L., Duchêne, S. & Holmes, E. C., 2017 Comparative analysis esti-  
385 mates the relative frequencies of co-divergence and cross-species transmission within  
386 viral families. *PLoS Pathogens* **13**, 1–17. (doi:10.1371/journal.ppat.1006215).
- 387 21. Gil, J. F., Mesa, V., Estrada-Ortiz, N., Lopez-Obando, M., Gómez, A. & Plácido,  
388 J., 2021 Viruses in extreme environments, current overview, and biotechnological  
389 potential. *Viruses* **13**. (doi:10.3390/v13010081).
- 390 22. Labbé, M., Girard, C., Vincent, W. F. & Culley, A. I., 2020 Extreme viral par-  
391 titioning in a marine-derived High Arctic lake. *mSphere* **5**, e00334–20. (doi:  
392 10.1128/mSphere.00334-20).
- 393 23. Yau, S. & Seth-Pasricha, M., 2019 Viruses of polar aquatic environments. *Viruses*  
394 **11**. (doi:10.3390/v11020189).
- 395 24. Colby, G. A., Ruuskanen, M. O., St.Pierre, K. A., St.Louis, V. L., Poulain, A. J.  
396 & Aris-Brosou, S., 2020 Warming climate is reducing the diversity of dominant  
397 microbes in the largest High Arctic lake. *Frontiers in Microbiology* **11**, 2316. (doi:  
398 10.3389/fmicb.2020.561194).
- 399 25. Lehnher, I., St. Louis, V. L., Sharp, M., Gardner, A. S., Smol, J. P., Schiff,  
400 S. L., Muir, D. C. G., Mortimer, C. A., Michelutti, N., Tarnocai, C. *et al.*, 2018  
401 The world’s largest High Arctic lake responds rapidly to climate warming. *Nature*  
402 *Communications* **9**, 1290. (doi:10.1038/s41467-018-03685-z).

26. Ruuskanen, M. O., Colby, G., St.Pierre, K. A., St.Louis, V. L., Aris-Brosou, S. & Poulain, A. J., 2020 Microbial genomes retrieved from High Arctic lake sediments encode for adaptation to cold and oligotrophic environments. *Limnology and Oceanography* **65**, S233–S247. (doi:10.1002/lno.11334).
27. Meredith, M., Sommerkorn, M., S., C., Derksen, C., Ekaykin, A., A., H., Kofinas, G., Mackintosh, A., Melbourne-Thomas, J., Muelbert, M. *et al.*, 2019 *Polar Regions*, pp. 203–320.
28. Parkinson, A. J., Evengard, B., Semenza, J. C., Ogden, N., Børresen, M. L., Berner, J., Brubaker, M., Sjöstedt, A., Evander, M., Hondula, D. M. *et al.*, 2014 Climate change and infectious diseases in the Arctic: establishment of a circumpolar working group. *International Journal of Circumpolar Health* **73**, 25163. (doi:10.3402/ijch.v73.25163).
29. Waits, A., Emelyanova, A., Oksanen, A., Abass, K. & Rautio, A., 2018 Human infectious diseases and the changing climate in the Arctic. *Environment International* **121**, 703–713. (doi:10.1016/j.envint.2018.09.042).
30. Ciota, A. T. & Keyel, A. C., 2019 The role of temperature in transmission of zoonotic arboviruses. *Viruses* **11**. (doi:10.3390/v11111013).
31. Martin, G., Yanez-Arenas, C., Chen, C., Plowright, R. K., Webb, R. J. & Skerratt, L. F., 2018 Climate change could increase the geographic extent of Hendra Virus spillover risk. *Ecohealth* **15**, 509–525. (doi:10.1007/s10393-018-1322-9).
32. Messan, K. S., Jones, R. M., Doherty, S. J., Foley, K., Douglas, T. A. & Barbato, R. A., 2020 The role of changing temperature in microbial metabolic processes during permafrost thaw. *PLoS ONE* **15**, e0232169. (doi:10.1371/journal.pone.0232169).
33. Bellec, L., Clerissi, C., Edern, R., Foulon, E., Simon, N., Grimsley, N. & Desdevises, Y., 2014 Cophylogenetic interactions between marine viruses and eukaryotic picophytoplankton. *BMC Evolutionary Biology* **14**, 59. (doi:10.1186/1471-2148-14-59).
34. Bennett, A. J., Paskey, A. C., Kuhn, J. H., Bishop-Lilly, K. A. & Goldberg, T. L., 2020 Diversity, transmission, and cophylogeny of Ledanteviruses (*Rhabdoviridae*: *Ledantevirus*) and Nycteribiid bat flies parasitizing Angolan soft-furred fruit bats in Bundibugyo District, Uganda. *Microorganisms* **8**, 750. (doi: 10.3390/microorganisms8050750).
35. Jackson, A. P. & Charleston, M. A., 2004 A cophylogenetic perspective of RNA-virus evolution. *Molecular Biology and Evolution* **21**, 45–57. (doi:10.1093/molbev/msg232).

36. Madinda, N. F., Ehlers, B., Wertheim, J. O., Akoua-Koffi, C., Bergl, R. A., Boesch, C., Akonkwa, D. B. M., Eckardt, W., Fruth, B., Gillespie, T. R. *et al.*, 2016 Assessing host-virus codivergence for close relatives of Merkel cell Polyomavirus infecting African great apes. *Journal of Virology* **90**, 8531–8541. (doi:10.1128/JVI.00247-16).
37. Olival, K. J., Hosseini, P. R., Zambrana-Torrel, C., Ross, N., Bogich, T. L. & Daszak, P., 2017 Host and viral traits predict zoonotic spillover from mammals. *Nature* **546**, 646–650. (doi:10.1038/nature22975).
38. Carrasco-Hernandez, R., Jácome, R., López Vidal, Y. & Ponce de León, S., 2017 Are RNA viruses candidate agents for the next global pandemic? A review. *ILAR Journal* **58**, 343–358. (doi:10.1093/ilar/ilx026).
39. Grossart, H.-P., Massana, R., McMahon, K. D. & Walsh, D. A., 2020 Linking metagenomics to aquatic microbial ecology and biogeochemical cycles. *Limnology and Oceanography* **65**, S2–S20. (doi:10.1002/lno.11382).
40. Jacquemot, L., Kalenitchenko, D., Matthes, L. C., Vigneron, A., Mundy, C. J., Tremblay, J.-É. & Lovejoy, C., 2021 Protist communities along freshwater–marine transition zones in Hudson Bay (Canada). *Elementa: Science of the Anthropocene* **9**. (doi:10.1525/elementa.2021.00111).
41. Marquardt, M., Vader, A., Stübner, E. I., Reigstad, M., Gabrielsen, T. M. & Stams, A. J. M., 2016 Strong seasonality of marine microbial eukaryotes in a High-Arctic fjord (Isfjorden, in West Spitsbergen, Norway). *Applied and Environmental Microbiology* **82**, 1868–1880. (doi:10.1128/AEM.03208-15).
42. Azovsky, A. I., Tikhonenkov, D. V. & Mazei, Y. A., 2016 An estimation of the global diversity and distribution of the smallest eukaryotes: Biogeography of marine benthic heterotrophic flagellates. *Protist* **167**, 411–424. (doi:10.1016/j.protis.2016.07.001).
43. Andrews, S., Krueger, F., Segonds-Pichon, A., Biggins, L., Krueger, C. & Wingett, S., 2010. FastQC.
44. Bolger, A. M., Lohse, M. & Usadel, B., 2014 Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120. (doi:10.1093/bioinformatics/btu170).
45. Prjibelski, A., Antipov, D., Meleshko, D., Lapidus, A. & Korobeynikov, A., 2020 Using SPAdes *de novo* assembler. *Current Protocols in Bioinformatics* (doi:10.1002/cpbi.102).

46. Nurk, S., Meleshko, D., Korobeynikov, A. & Pevzner, P. A., 2017 metaSPAdes: a new versatile metagenomic assembler. *Genome Research* **27**, 824–834. (doi:10.1101/gr.213959.116).
47. Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q. *et al.*, 2011 Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology* **29**, 644–652. (doi:10.1038/nbt.1883).
48. Bushmanova, E., Antipov, D., Lapidus, A. & Prjibelski, A. D., 2019 rnaSPAdes: a *de novo* transcriptome assembler and its application to RNA-Seq data. *GigaScience* **8**, 1–13. (doi:10.1093/gigascience/giz100).
49. Zhang, Z., Schwartz, S., Wagner, L. & Miller, W., 2000 A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* **7**, 203–214. (doi:10.1089/10665270050081478).
50. Nucleotide [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] - [cited 2021 Jul 29]. Available from <https://www.ncbi.nlm.nih.gov/nucleotide/>.
51. Sayers, E. W., Cavanaugh, M., Clark, K., Ostell, J., Pruitt, K. D. & Karsch-Mizrachi, I., 2019 GenBank. *Nucleic Acids Research* **47**, D94–D99. (doi:10.1093/nar/gky989).
52. Rice, P., Longden, L. & Bleasby, A., 2000 EMBOSS: The European Molecular Biology Open Software Suite. *Trends in Genetics* **16**, 276–277. (doi:10.1016/S0168-9525(00)02024-2).
53. Sherrill-Mix, S., 2019. taxonomizr: functions to work with NCBI accessions and taxonomy.
54. Langmead, B. & Salzberg, S. L., 2012 Fast gapped-read alignment with Bowtie 2. *Nature Methods* **9**, 357–359. (doi:10.1038/nmeth.1923).
55. Hulo, C., de Castro, E., Masson, P., Bougueleret, L., Bairoch, A., Xenarios, I. & Le Mercier, P., 2011 ViralZone: a knowledge resource to understand virus diversity. *Nucleic Acids Research* **39**, D576–D582. (doi:10.1093/nar/gkq901).
56. Lefkowitz, E. J., Dempsey, D. M., Hendrickson, R. C., Orton, R. J., Siddell, S. G. & Smith, D. B., 2018 Virus taxonomy: the database of the International Committee on Taxonomy of Viruses (ICTV). *Nucleic Acids Research* **46**, D708–D717. (doi:10.1093/nar/gkx932).

- 504 57. Chamberlain, S., Szoecs, E., Foster, Z., Arendsee, Z., Boettiger, C., Ram, K.,  
505 Bartomeus, I., Baumgartner, J., O'Donnell, J., Oksanen, J. *et al.*, 2020. taxize:  
506 Taxonomic information from around the web.
- 507 58. Chamberlain, S. A. & Szocs, E., 2013 taxize: taxonomic search and retrieval in R.  
508 *F1000Research* **2**. (doi:10.12688/f1000research.2-191.v2).
- 509 59. Mihara, T., Nishimura, Y., Shimizu, Y., Nishiyama, H., Yoshikawa, G., Uehara,  
510 H., Hingamp, P., Goto, S. & Ogata, H., 2016 Linking virus genomes with host  
511 taxonomy. *Viruses* **8**, 66. (doi:10.3390/v8030066).
- 512 60. Balbuena, J. A., Pérez-Escobar, Ó. A., Llopis-Belenguer, C. & Blasco-Costa, I.,  
513 2020 Random tanglegram partitions (Random TaPas): an Alexandrian approach  
514 to the cophylogenetic Gordian knot. *Systematic Biology* **69**, 1212–1230. (doi:  
515 10.1093/sysbio/syaa033).
- 516 61. Schardl, C. L., Craven, K. D., Speakman, S., Stromberg, A., Lindstrom, A. &  
517 Yoshida, R., 2008 A novel test for host-symbiont codivergence indicates ancient  
518 origin of fungal endophytes in grasses. *Systematic Biology* **57**, 483–498. (doi:  
519 10.1080/10635150802172184).
- 520 62. Balbuena, J. A., Míguez-Lozano, R. & Blasco-Costa, I., 2013 PACo: a novel  
521 procrustes application to cophylogenetic analysis. *PLoS ONE* **8**, e61048. (doi:  
522 10.1371/journal.pone.0061048).
- 523 63. Cissell, E. & McCoy, S., 2021 Shotgun metagenomic sequencing reveals the full  
524 taxonomic, trophic, and functional diversity of a coral reef benthic cyanobacterial  
525 mat from Bonaire, Caribbean Netherlands. *Science of the Total Environment* **755**,  
526 142719. (doi:10.1016/j.scitotenv.2020.142719).
- 527 64. Schmidt, N. & Bölter, M., 2002 Fungal and bacterial biomass in tundra soils along  
528 an Arctic transect from Taimyr Peninsula, central Siberia. *Polar Biology* **25**, 871–  
529 877. (doi:10.1007/s00300-002-0422-7).
- 530 65. Aguirre de Cárcer, D., López-Bueno, A., Pearce, D. A. & Alcamí, A., 2015 Bio-  
531 diversity and distribution of polar freshwater DNA viruses. *Science Advances* **1**.  
532 (doi:10.1126/sciadv.1400127).
- 533 66. Gregory, A. C., Zayed, A. A., Conceição-Neto, N., Temperton, B., Bolduc, B.,  
534 Alberti, A., Ardyna, M., Arkhipova, K., Carmichael, M., Cruaud, C. *et al.*, 2019  
535 Marine DNA viral macro- and microdiversity from pole to pole. *Cell* **177**, 1109–  
536 1123.e14. (doi:10.1016/j.cell.2019.03.040).
- 537 67. Wang, D., 2020 5 challenges in understanding the role of the virome in health and  
538 disease. *PLoS Pathogens* **16**, 1–6. (doi:10.1371/journal.ppat.1008318).

- 539 68. de Cárcer, D. A., López-Bueno, A., Pearce, D. A. & Alcamí, A., 2015 Biodiversity  
540 and distribution of polar freshwater DNA viruses. *Science Advances* **1**, e1400127.  
541 (doi:10.1126/sciadv.1400127).
- 542 69. Allen, L. Z., McCrow, J. P., Ininbergs, K., Dupont, C. L., Badger, J. H., Hoffman,  
543 J. M., Ekman, M., Allen, A. E., Bergman, B., Venter, J. C. *et al.*, 2017 The  
544 Baltic Sea virome: Diversity and transcriptional activity of DNA and RNA viruses.  
545 *mSystems* **2**, e00125–16. (doi:10.1128/mSystems.00125-16).
- 546 70. Koziel, E., Otulak-Koziel, K. & Bujarski, J. J., 2021 Plant cell wall as a key player  
547 during resistant and susceptible plant-virus interactions. *Frontiers in Microbiology*  
548 **12**, 495. (doi:10.3389/fmicb.2021.656809).
- 549 71. Stavolone, L. & Lionetti, V., 2017 Extracellular matrix in plants and animals:  
550 Hooks and locks for viruses. *Frontiers in Microbiology* **8**, 1760. (doi:10.3389/fmicb.  
551 2017.01760).
- 552 72. Aris-Brosou, S., Parent, L. & Ibeh, N., 2019 Viral long-term evolutionary strategies  
553 favor stability over proliferation. *Viruses* **11**. (doi:10.3390/v11080677).
- 554 73. Hudson, J. M. G. & Henry, G. H. R., 2009 Increased plant biomass in a High Arctic  
555 heath community from 1981 to 2008. *Ecology* **90**, 2657–2663. (doi:10.1890/09-0102.  
556 1).
- 557 74. Zohdy, S., Schwartz, T. S. & Oaks, J. R., 2019 The Coevolution Effect as a driver  
558 of spillover. *Trends in Parasitology* **35**, 399–408. (doi:10.1016/j.pt.2019.03.010).
- 559 75. St. Pierre, K. A., St. Louis, V. L., Lehnher, I., Schiff, S. L., Muir, D. C. G., Poulain,  
560 A. J., Smol, J. P., Talbot, C., Ma, M., Findlay, D. L. *et al.*, 2019 Contemporary  
561 limnology of the rapidly changing glacierized watershed of the world's largest High  
562 Arctic lake. *Scientific Reports* **9**, 4447. (doi:10.1038/s41598-019-39918-4).
- 563 76. Wallingford, P. D., Morelli, T. L., Allen, J. M., Beaury, E. M., Blumenthal, D. M.,  
564 Bradley, B. A., Dukes, J. S., Early, R., Fusco, E. J., Goldberg, D. E. *et al.*, 2020 Ad-  
565 justing the lens of invasion biology to focus on the impacts of climate-driven range  
566 shifts. *Nature Climate Change* **10**, 398–405. (doi:10.1038/s41558-020-0768-2).
- 567 77. Rocklöv, J. & Dubrow, R., 2020 Climate change: an enduring challenge for vector-  
568 borne disease prevention and control. *Nature Immunology* **21**, 479–483. (doi:10.  
569 1038/s41590-020-0648-y).
- 570 78. Osland, M. J., Stevens, P. W., Lamont, M. M., Brusca, R. C., Hart, K. M., Waddle,  
571 J. H., Langtimm, C. A., Williams, C. M., Keim, B. D., Terando, A. J. *et al.*, 2021  
572 Tropicalization of temperate ecosystems in north america: The northward range

573 expansion of tropical organisms in response to warming winter temperatures. *Global*  
574 *Change Biology* **27**, 3009–3034. (doi:10.1111/gcb.15563).

## 575 Tables

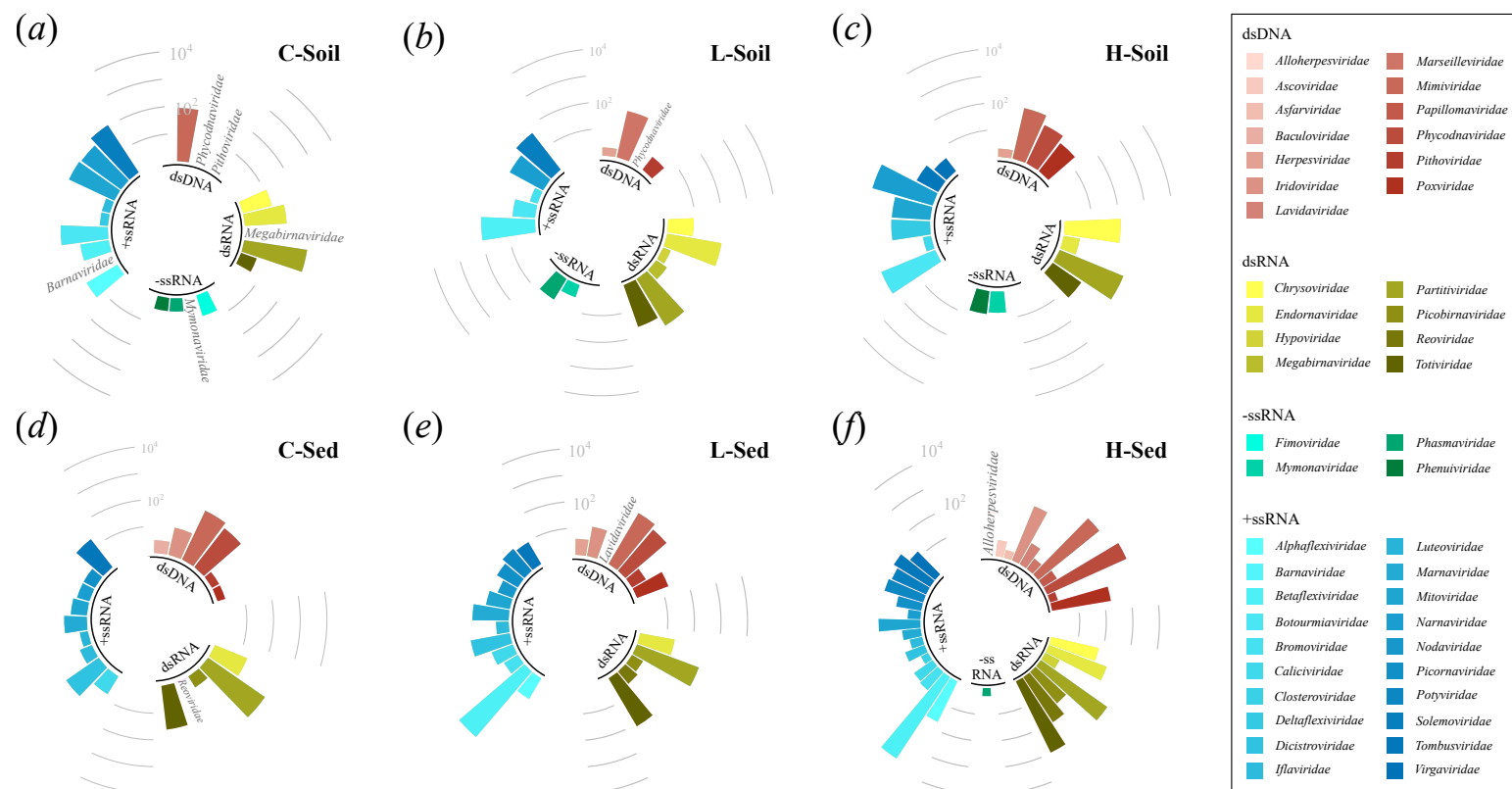


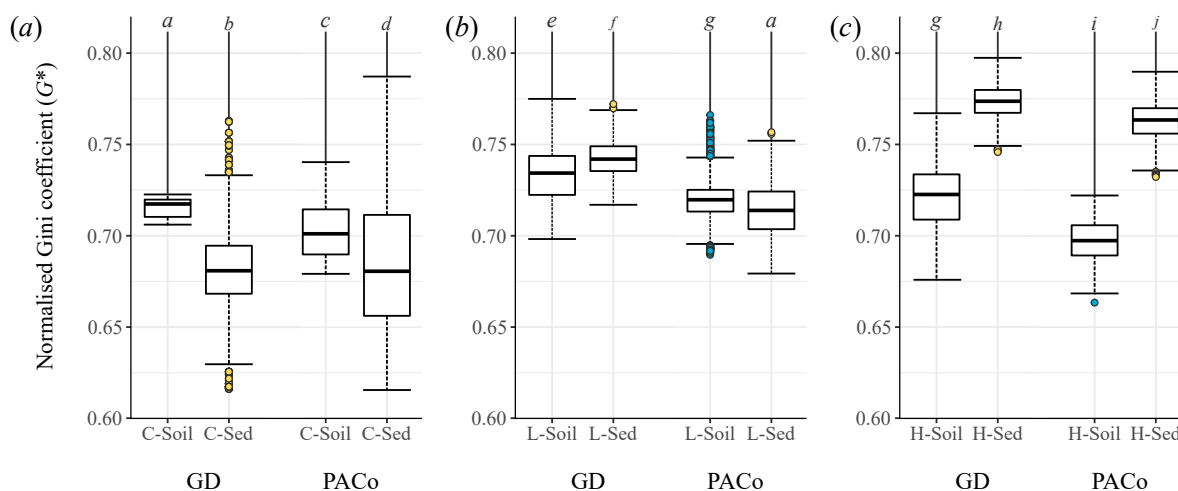
**Table 1.** Abundance of the viral families of the viral HSPs. The host range information was obtained from the ViralZone and International Committee on Taxonomy of Viruses (ICTV) databases. Viruses with no or unknown family were excluded from this table.

Viral family	Known eukaryotic host range	Count of HSP by site					
		Control		Low runoff		High runoff	
		C-Soil	C	L-Soil	L1	H-Soil	H1
dsDNA viruses							
<i>Alloherpesviridae</i>	Fish						1
<i>Ascoviridae</i>	Insects: mainly Noctuids						4
	SfAV: <i>Spodoptera</i> species only						
<i>Asfarviridae</i>	Pigs warthogs bushpigs						2
	Vector: Argasid ticks						
<i>Baculoviridae</i>	Arthropods: Lepidoptora, Hymenoptera, Diptera		3				
	Crustacean: Decapoda (Shrimps)						
<i>Herpesviridae</i>	Vertebrates			2	4	2	
<i>Iridoviridae</i>	Insects		11		13		159
<i>Lavidaviridae</i>	Protists infected by <i>Mimivirus</i>				1		8
<i>Marseilleviridae</i>	Amoeba						3
<i>Mimiviridae</i>	Amoeba	86	105	60	167	82	886
<i>Papillomaviridae</i>	Vertebrates						4
<i>Phycodnaviridae</i>	Alga	1	69	1	108	37	1,858
<i>Pithoviridae</i>	Amoeba	1	2		4		2
<i>Poxviridae</i>	Human, vertebrates, and arthropods		2		18	20	173
dsRNA viruses							
<i>Chrysoviridae</i>	Fungi	12		9		116	69
<i>Endornaviridae</i>	Plants, fungi, and oomycetes	33	20	115	21	4	212
<i>Hypoviridae</i>	Fungi			2			4
<i>Megabirnaviridae</i>	Fungi	1		3			
<i>Partitiviridae</i>	Fungi and plants	206	384	112	304	352	1,140
<i>Picobirnaviridae</i>	Vertebrates and invertebrates		3		3		36
<i>Reoviridae</i>	Vertebrates, invertebrates, plants, and fungi		1		4		106
<i>Totiviridae</i>	<i>Totivirus</i> : Fungi	4	44	45	124	19	957

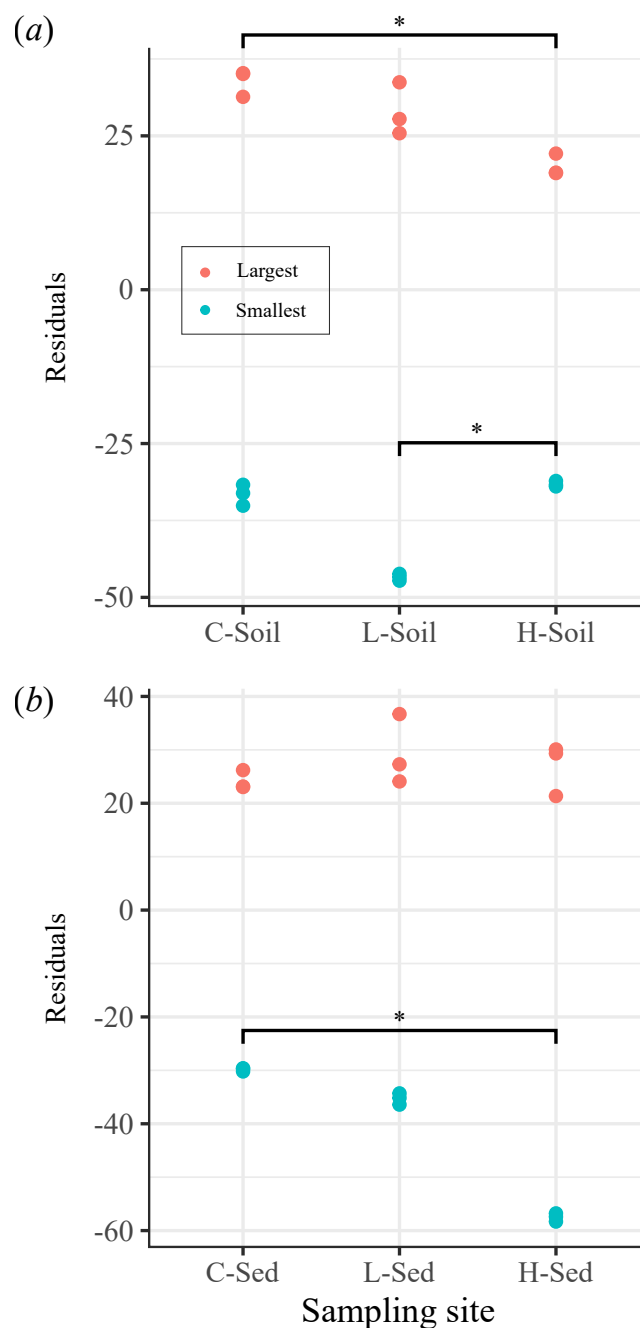
<i>Victorivirus</i> : Fungi							
<b>-ssRNA viruses</b>							
<i>Fimoviridae</i>	Plant: European mountain ash	6					
<i>Mymonaviridae</i>	<i>Sclerotinia sclerotiorum</i> fungi	1		3		6	
<i>Phasmaviridae</i>	Insects (mosquitos, cockroaches, water striders, psyllids, odonates, and drosophilids)	3					2
<i>Phenuiviridae</i>	RVFV: ruminants, camels, and humans Vector: Mosquitoes	3		8		7	
<b>+ssRNA viruses</b>							
<i>Alphaflexiviridae</i>	Plants and fungi	20			8		50
<i>Barnaviridae</i>	Cultivated mushroom ( <i>Agaricus bisporus</i> )	1					
<i>Betaflexiviridae</i>	Plants and fungi	11		103	1,716		4,631
<i>Botourmiaviridae</i>	Plants and fungi	52		7		142	
<i>Bromoviridae</i>	Plants			2	4		8
<i>Caliciviridae</i>	Vertebrates		6		7	2	7
<i>Closteroviridae</i>	Plants						2
<i>Deltaflexiviridae</i>	Fungi and plants	2	30			27	
<i>Dicistroviridae</i>	Invertebrates				31		6
<i>Iflaviridae</i>	Insects	2	3		3		3
<i>Luteoviridae</i>	Plants		2				
<i>Marnaviridae</i>	Phytoplankton <i>Heterosigma akashiwo</i>		7		23		5
<i>Mitoviridae</i>	Fungi	66	4		8	26	37
<i>Narnaviridae</i>	Fungi	59	3	26		207	3
<i>Nodaviridae</i>	Vertebrates and invertebrates		3		4		
<i>Picornaviridae</i>	Vertebrates				8		9
<i>Potyviridae</i>	Plants				12		33
<i>Solemoviridae</i>	Plants (few species of Gramineae)	114		54			26
<i>Tombusviridae</i>	Plants		21		9	8	40
<i>Virgaviridae</i>	Plants					4	16
<b>Total</b>		684	723	552	2,604	1,061	10,502

## 576 **Figures**

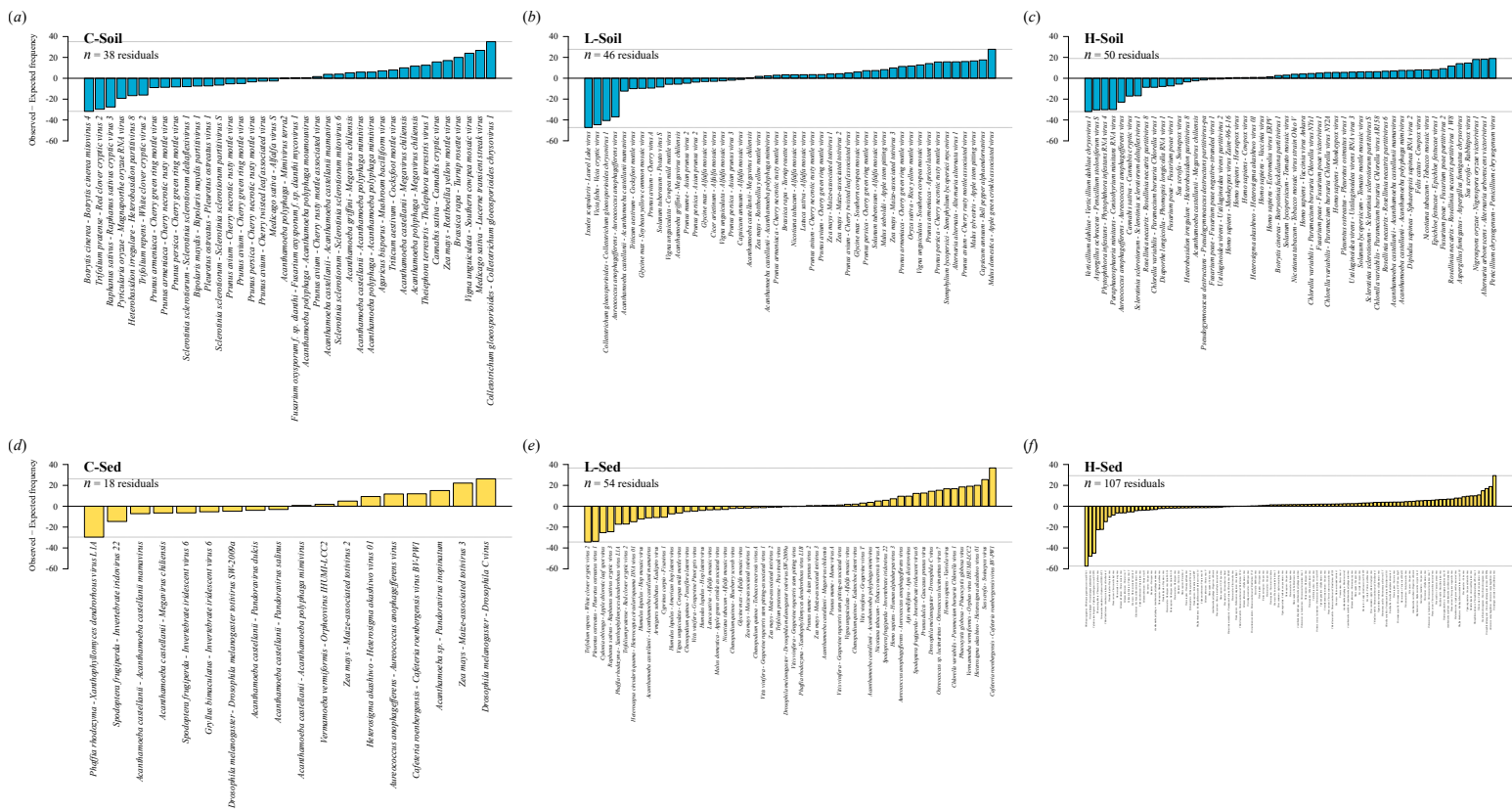




**Figure 2.** Normalised Gini coefficients ( $G^*$ ) obtained with Random TaPas ( $n = 3$  runs). The values are separated by runoff volume: (a) control; (b) low runoff; and (c) high runoff. The two global-fit models used were GD (geodesic distances in tree space) and PACo (Procrustes Approach to Cophylogeny). Significant results (Dunn test, BH correction) are marked with letters from *a* to *j* ( $\alpha = 0.05$ ). Blue represents the soil and yellow, the lake sediments.



**Figure 3.** Largest and smallest residuals per sampling site for (a) soil and (b) lake sediments samples. Residuals were computed by Random TaPas ( $n = 3$  runs) using GD (geodesic distances in tree space). Significant results (Dunn test, BH correction) are marked with an asterisk (\*) ( $\alpha = 0.05$ ). Red represents the largest and blue, the smallest residuals. figure S6 further shows these results to be robust to the distance used to compare trees.



**Figure 4.** Distribution of the residuals computed by Random TaPas ( $n = 1$  run) using GD (geodesic distances in tree space). (a) C-Soil; (b) L-Soil; (c) H-Soil; (d) C-Sed; (e) L-Sed; and (f) H-Sed sites. Blue residuals represent the soil, and yellow the lake sediments.