Human land-use impacts viral diversity and abundance in a New Zealand river

Rebecca French[1], Justine Charon[1], Callum Le Lay[1], Chris Muller[2,3], Edward C. Holmes[1*]

[1]Sydney Institute for Infectious Diseases, School of Life and Environmental Sciences and Sydney Medical School, The University of Sydney, Sydney, NSW 2006, Australia.

[2]Wildbase, School of Veterinary Sciences, Massey University, Palmerston North 4442, New Zealand.

[3]Wildlife and Ecology Group, School of Agriculture and Environment, Massey University, Palmerston North 4442, New Zealand.

*Corresponding author:

Prof. Edward C. Holmes

Sydney Institute for Infectious Diseases, School of Life and Environmental Sciences and Sydney Medical School, The University of Sydney,

Sydney, NSW 2006, Australia.

Tel: +61 2 9351 5591

Email: edward.holmes@sydney.edu.au

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Abstract

Although water-borne viruses have important implications for the health of humans and other animals, little is known about the impact of human land-use on viral diversity and evolution in water systems such as rivers. We used metagenomic next-generation sequencing to compare the diversity and abundance of viruses at sampling sites along a single river in New Zealand that differed in human land use impact, ranging from pristine to urban. From this we identified 504 putative virus species, of which 97% were novel. Many of the novel viruses were highly divergent, and likely included a new subfamily within the *Parvoviridae*. We identified at least 63 virus species that may infect vertebrates – most likely fish and water birds – from the *Astroviridae, Birnaviridae, Parvoviridae* and *Picornaviridae*. No putative human viruses were detected. Importantly, we observed differences in the composition of viral communities at sites impacted by human land-use (farming and urban) compared to native forest sites (pristine). At the viral species level, the urban sites had higher diversity (327 virus species) than the farming (n=150) and pristine sites (n=119), and more viruses were shared between the urban and farming sites (n=76) than between the pristine and farming or urban sites (n=24). The two farming sites had a lower viral abundance across all host types, while the pristine sites had a higher abundance of viruses associated with animals, plants and fungi. We also identified viruses linked to agriculture and human impact at the river sampling sites in farming and urban areas that were not present at the native forest sites. Overall, our study shows that human land-use can impact viral communities in rivers, such that further work is needed to reduce the impact of intensive farming and urbanization on water systems.

1. **Introduction**

As viruses likely infect all life forms, and often at high abundance, they can be considered an integral part of global ecosystems (Zhang et al. 2018; French and Holmes 2020; Sommers et al. 2021). Until recently, however, there has been a strong bias toward studying viruses in the context of individual disease-causing pathogens, particularly in humans, domestic animals and plants (Zhang et al. 2018). Although understandable, such a bias limits our understanding of their ecology and evolution, how viral abundance and diversity might be shaped by anthropogenic activities, and their role at the ecosystem scale (French and Holmes 2020; Sommers et al. 2021). Clearly, a better understanding of these processes will enable virus evolution and disease emergence to be placed in its true ecological context. As most viruses do not cause disease in their hosts (Roossinck 2015), characterising non-pathogenic viruses will greatly expand our understanding of the composition of the global virosphere.

Metagenomic next-generation sequencing (mNGS) enables the entire virome of a sample to be characterised in an unbiased manner, giving studies of RNA virus diversity and evolution a new perspective (Zhang et al. 2018; Wolf et al. 2020; Nayfach et al. 2021). In particular, mNGS enables the comparison of viral abundance and diversity between groups (animal populations, environments etc.) that was previously not possible on large scales. To date, however, most metagenomic studies of viromes have focused on describing viral diversity without placing it in an appropriate ecological context (Zhang et al. 2018; French and Holmes 2020; Sommers et al. 2021).

Rivers collect water from the land they flow through. As such, their microbial community necessarily reflects the ecological properties of this adjacent land (Van Rossum et al. 2015).

Run-off from farmland, urban areas and sewage discharge directly introduce human and livestock-infecting microbes into rivers, sometimes causing water-borne disease (Ferguson et al. 2003; Alegbeleye and Sant'Ana 2020). For bacteria, it is well understood that human activity on land impacts the environment within rivers, in turn affecting bacterial abundance and diversity (Van Rossum et al. 2015; Chen et al. 2018; Phiri et al. 2020; Qiu et al. 2020). However, even though some water-borne viruses have important implications for human health, such as enteroviruses (Amvrosieva et al. 2001), hepatitis E virus (Sedyaningsih-Mamahit et al. 2002; Martolia et al. 2009) and norovirus (Jack et al. 2013; Sekwadi et al. 2018), we know little about how human land-use impacts viral abundance and diversity in rivers. A study of an agricultural river basin in Ontario, Canada, found that higher levels of human viruses and coliphages were associated with greater upstream human land development (Jones et al. 2017). Similarly, land use in Singapore was the main driver of viral community structure in reservoirs used for potable water supplies and recreational activities (Gu et al. 2018). However, because such comparisons involved different catchments that are likely to have contrasting viral communities, it may not be possible to isolate the effect of human activity on viral ecology. To date there has been no study directly comparing the viral ecology of river water flowing through different land-use types within the same river catchment.

New Zealand freshwater communities have been isolated since New Zealand split from Gondwanaland approximately eighty million years ago (Mortimer et al. 2019). Freshwater communities within New Zealand are also generally isolated from each other, with little opportunity for non-migratory species to colonize new water catchments (Burridge and Waters 2020). This is reflected in the evolution of freshwater plant, vertebrate and invertebrate species. For example, there are high levels of endemism within New Zealand

non-migratory galaxiid fish, with many species are found only in one water catchment (Dunn et al. 2018; Burridge and Waters 2020). It might therefore be expected that the freshwater communities of New Zealand would similarly contain many highly divergent viruses and locally unique viruses that have co-evolved with their isolated hosts. In contrast, human activity has had a large impact on New Zealand freshwater communities, including run-off from intensive agriculture and urbanization and the introduction of invasive species such as rainbow trout (*Oncorhynchus mykiss*). It is likely that these changes would also have affected the viral community in the rivers, introducing viruses associated with plants and animals grown for food, as well as viruses that infect humans.

Very little is known about the viral ecology of New Zealand rivers, with research generally limited to targeted testing for known pathogens. Two river sites – the Waikato River in the North Island and the Oreti River in the South Island – that supply drinking water to urban populations have been screened for enteric viruses, with positive results in 97% of samples (Williamson et al. 2011). In the Manawatū region, the Manawatū and Pohangina rivers and Turitea creek have been screened for plant viruses, with three tombusviruses detected (Mukherjee 2011; Mukherjee et al. 2012). New variants of Tobacco mosaic virus and Tomato mosaic virus were also identified (Mukherjee 2011), and human polyomaviruses have been found in the Matai river in Nelson (Kirs et al. 2011). *Sclerotinia sclerotiorum* Hypovirulence-Associated Virus-1 (which infects a fungus often found in agricultural plants) was detected in a Christchurch river using metagenomic sequencing of DNA from sediment samples (Kraberger et al. 2013). To our knowledge, viral meta-transcriptomics has not yet been performed on a river system in New Zealand.

The core aim of this study was to compare the viral (particularly RNA virus) abundance and diversity between sites with differing human land use impact in a New Zealand river catchment and from this determine how virome ecology and evolution are shaped by human activity. Accordingly, six sites on the Manawatū River, North Island, were selected based on their differing land use types. Two sites were at the edge of the Ruahine forest park, containing water that has only flowed through pristine native forest (pristine sites, denoted P1 and P2). Two sites contain water that has flowed through farmland (farmland sites, at least 25 km for F1 and 50 km for F2). The final two sites have flowed first through pristine native forest, then farmland and finally urbanized areas (urban sites) – Feilding and Palmerston North (named U1 and U2, respectively). Water samples were taken at these sites and subjected to total RNA sequencing (i.e., meta-transcriptomics).

2. Methods

2.1 The Manawatū River

The Manawatū River is a 180 km river located in the North Island of New Zealand (Figure 1). Importantly, it flows through three very different land-use types, allowing a direct comparison between them. The river begins in the Ruahine forest park that encompasses the Ruahine mountain range (Department of Conservation 2021b). The park is dominated by native vegetation, including podocarp forest at lower altitudes and sub-alpine shrubland and tussock grasslands at higher altitudes. Between the 1800s and 1970s there was considerable forest clearing and logging, but since 1976 the area has been protected as a forest park with no farming or logging (Department of Conservation 2021a). There is little to no human habitation or activity in these ranges, with the exception of recreational hikers, hunters and rangers. A variety of endemic New Zealand animals inhabit these ranges, including parrots

(kakariki *Cyanoramphus novaezelandiae*, kaka *Nestor meridionalis*), ducks (whio *Hymenolaimus malacorhynchos*), New Zealand long-tailed bats (*Chalinolobus tuberculatus*) and large carnivorous land snails (*Powelliphanta marchanti*). Introduced pest species are also found there, including red deer (*Cervus elaphus*), feral pigs (*Sus scrofa)* and goats (*Capra hircus)*, brush tailed possums (*Trichosurus vulpecula*), stoats (*Mustela erminea*) and rainbow trout (*Oncorhynchus mykiss*) (Department of Conservation 2021b).

After flowing through the Ruahine Forest Park, the river passes through intensively farmed areas, primarily consisting of sheep, beef and dairy farming. This land use is known to impact the river, with the Manawatū region having among the highest nitrogen and phosphorus concentrations (nutrients associated with pastoral agriculture) in New Zealand (Roygard et al. 2012). It then flows through two urban centres: Feilding (town, population 17,050), and Palmerston North (city, population 81,500), before flowing out into the Tasman Sea. Both these urban centres discharge treated wastewater into the Manawatū River, and in some years this negatively impacts aquatic life through discharge of nutrients and a corresponding increase in periphyton cover (Hamill 2012).

2.2 Sample collection

Two-litre (L) water samples were collected at each of the six sites in the Manawatū River catchment (Figure 1). For consistency, all samples were collected on the same day (13th July 2019). At each site, 1 L was collected from the water's edge and 1 L was collected 1.2 metres from the bank in the main flow of the river using a sampling pole, with the aim of obtaining a representative sample of the river water. In the case of P2, which was less than 1.2 metres wide, the second sample was taken by hand in the main flow of the river. These samples were

168 combined to obtain 2 L of water per site and 12 L in total. Once collected, the water samples
169 were kept at approximately 4°C using icepacks until processing.
170
171 At each site, a separate 250 ml sample was collected from the main flow of the river to
172 measure additional variables (temperature, salinity, conductivity, pH, total dissolved solids,
173 turbidity). These were measured using a multiparameter tester (Waterproof PCSTestr 35,
174 Thermo Scientific) and a Turbidimetre (2100P Turbidimetre, Hach). Temperature was
175 measured on-site and the remaining variables were measured in the laboratory.
176

177 2.3 Sample processing and sequencing

178 Filtering of all samples was completed within thirty hours of sample collection. Samples with
179 a large amount of silt (farming and urban sites) were first filtered through a glass fibre filter,
180 of 47 mm diameter and 0.7 µm pore size (Microscience). All samples were then filtered
181 through polyether sulphone (PES) membrane filters, of 47mm diameter and 0.2µm pore size
182 (Microscience).
183
184 Samples were concentrated from 2 L to ~100 µL in two steps using tangential ultra-filtration
185 and ultra-centrifugation. The samples were first concentrated from 2 L down to 40 mL using
186 the vivaflow 200 (Sartorius). The samples were then concentrated from 40 mL to ~2 mL
187 using vivaspin 20 ultrafiltration units (Sartorius), and further concentrated to ~100 µL using
188 vivaspin 2 ultrafiltration units (Sartorius). All units had polyether sulphone filters with a
189 molecular weight cut-off of 10 kDa. Samples were stored at -80°C until nucleic acid
190 extraction.
191

RNA and DNA were extracted from the concentrated water samples using the AllPrep® PowerViral® DNA/RNA Kit. DNA was removed by DNase digestion (Qiagen RNase-Free DNase I Set), then in the same column the RNA was concentrated to 15 µL using the MN NucleoSpin RNA Clean-up XS (Macherey-Nagel). The same process was conducted on 2 x 200 µL of sterile water to create two blank control libraries. cDNA libraries were prepared using the SMARTer® Universal Low Input RNA Kit for Sequencing (Takara Bio), without rRNA depletion. Libraries were sequenced on the Illumina Novaseq platform (150bp, paired end sequencing). The corresponding sequencing data have been deposited in the Sequence Read Archive (SRA) under accession numbers SRR17234948-53. The trimmed alignment fasta files used to infer the phylogenetic trees are available at https://github.com/RKFrench/Viral-Diversity-NZ-River.

2.4 Quality control, assembly and virus identification

TruSeq3 adapters were trimmed using Trimmomatic (0.38) (Bolger et al. 2014). Bases below a quality score of five were trimmed with a sliding window approach (window size of four). Bases at the beginning and end of the reads were similarly excluded if below a quality of three. SMART adapters were trimmed using bbduk in BBtools (bbmap 37.98) (Bushnell 2018). Sequences below an average quality of ten were removed.

Sequence reads were assembled *de novo* using Trinity (2.5.1) (Grabherr et al. 2011), with a kmer size of 32 and a minimum contig length of 300. BLASTN (BLAST+ 2.9.0) and Diamond BLASTX (Diamond 0.9.32) were used to identify viruses by comparing the assembled contigs to the NCBI nucleotide database (nt) and non-redundant protein database (nr) (Camacho et al. 2009; Buchfink et al. 2021). Contigs with hits to viruses were retained.

To avoid false-positives, sequence similarity cut-off values of 1E-5 and 1E-10 e-value were used for the nt and nr databases, respectively. Virus abundances were estimated using RSEM (1.3.0), allowing us to determine the expected count according to the Expectation-Maximization algorithm for each contig (Li and Dewey 2011). This was expressed as the percentage of the total number of reads in each library. Eukaryotic and prokaryotic diversity was characterized using CCMetagen (v 1.2.4) and the NCBI nucleotide database (nt) (Clausen et al. 2018; Marcelino et al. 2020).

2.5 Evolutionary and ecological analysis

Using the nucleotide sequences identified as viral replication proteins (i.e., as identified by BLAST), the getorf program from EMBOSS (6.6.0) was used to find and extract open reading frames and translate them into amino acid sequences using the standard genetic code with a minimum size of 100 amino acids (Rice et al. 2000). Amino acid sequences were aligned using the E-INS-i algorithm in MAFFT (7.402) (Katoh and Standley 2013) and trimmed using Trimal, (1.4.1) (Capella-Gutiérrez et al. 2009) with a gap threshold of 0.9 and at least 20% of the sequence conserved. See Supplementary Table S1 for more details. Maximum likelihood phylogenetic trees for each virus family were then estimated using IQ-TREE (1.6.12) (Nguyen et al. 2015), with the best fit substitution model determined by the program and employing 1000 bootstrap replications to assess node robustness. Any sequences with >95% amino acid similarity to each other or known species were assumed to represent the same virus species, with only one representative of each then included in the phylogenetic analysis. All novel viruses identified were given names that include the word ‘flumine’ (Latin for ‘of the river’) to convey where the virus was found.

APE (5.4) and ggtree (2.4.1) were used to visualize the phylogenetic trees and produce figures (Paradis and Schliep 2019; Yu 2020). Alpha diversity (i.e., diversity within each sample) was analysed using richness (number of viral families), Shannon index and the Shannon effective number of species (ENS). The Shannon index reflects the number of taxa and the evenness of the taxa abundances. The Shannon ENS is the effective number of taxa present in the community if the abundances were equal (Hill 1973). The beta diversity (i.e., diversity across land-use types) was analysed using a principal co-ordinate analysis with a Bray–Curtis dissimilarity matrix, presented as an ordination plot. Alpha and Beta diversity analyses were conducted using Phyloseq (v1.34.0) in R (v 4.0.5) (McMurdie and Holmes 2013; R Core Team 2021). Other graphs were generated using ggplot2 (Wickham 2016) and venneuler (Wilkinson 2011).

2.6 Identifying possible reagent contamination

Any virus found in the blank negative control libraries (i.e., a sterile water and reagent mix) was assumed to have resulted from contamination likely associated with laboratory reagents. Accordingly, these viruses were removed from the river sample libraries and excluded from all analyses. Additionally, any viruses that fell into the same clades as those found in blank libraries were conservatively assumed to be contaminants (Porter et al. 2021) and similarly removed. These included six circo-like viruses in the *Circoviridae* (single-strand DNA viruses) and 19 tombus-like viruses from the *Tombusviridae* (single-strand, positive-sense RNA viruses).

3. Results

We characterized the RNA viromes from six Manawatū River water samples using total RNA sequencing.

3.1 Water measurements

Our water measurements indicated that the two pristine sites had a different abiotic environment from the farming and urban sites, while the farming and urban sites were similar to each other (Figure 2). Specifically, the pristine sites had lower salinity, pH, total dissolved solids, turbidity, conductivity and temperature than the farming and urban sites. The farming and urban sites also had a larger variation between sites, with the exception of pH and temperature.

3.2 Virus identification

The six sequencing libraries generated had an average of 90 million reads per library, and on average 0.7% of these were derived from viruses. This is within the usual range found in faecal samples, cloacal swabs and invertebrate tissue, but higher than commonly observed in vertebrates (Zhang et al. 2018; Campbell et al. 2020; Le Lay et al. 2020; Mahar et al. 2020; Wille et al. 2020; Wille et al. 2021) in studies using similar metagenomic techniques. However, it was lower than observed in urban streams in Ecuador (Guerrero-Latorre et al. 2018). P2 and U2 had the highest number of reads and the highest number of viral reads (Figure 3). Notably, the two pristine sites had the highest percentage of viral reads, at 1.31 and 1.35%. F1, F2 and U1 all had lower total reads, total viral reads and percentage viral reads. Analysis of eukaryotic and prokaryotic diversity showed that all samples primarily

consisted of bacteria (accounting for 71-86% of assembled contigs) followed by eukaryotes (5-19%).

In total, we identified 504 putative virus species from 27 viral families, of which 491 (97%) were novel using a cut-off of 95% amino acid similarity, primarily in replication-associated proteins (although these await formal verification by the International Committee on Taxonomy of Viruses). These included multiple members of the *Nodaviridae* (n=74 novel viruses), *Tombusviridae* (n=64) and *Dicistroviridae* (n=61). If a more conservative <90% amino acid sequence similarity is used to define a novel virus species, then the samples analysed here contain 470 novel viruses. We also detected previously described viruses (i.e., with more than 95% amino acid similarity to viruses detected previously), including *Sclerotinia sclerotiorum* hypovirulence associated DNA virus 1 (*Genomoviridae*), White clover mosaic virus (*Alphaflexiviridae)*, Rhopalosiphum padi virus (*Dicistroviridae),* Norway luteo-like virus 4 (*Luteoviridae),* Carnation Italian ringspot virus *(Tombusviridae)* and Pepper mild mottle virus (*Virgaviridae)*. Importantly, we identified at least 63 virus species from the *Astroviridae, Birnaviridae, Parvoviridae* and *Picornaviridae* that may infect vertebrates. No likely human viruses were detected. Below we describe, in more detail, those families with high virus diversity in our study and those containing viruses that may infect vertebrates.

3.3 High Phylogenetic Diversity Families

3.3.1 Tombusviridae

We identified a high diversity and abundance of novel *Tombusviridae,* a family of single-strand positive-sense RNA viruses that infect plants (Sit and Lommel 2015). Of the viruses identified 18 fell into the subfamily *Procedovirinae*, found in all land-use types. Of these, one

309 virus in U2 clustered within the genus *Betacarmovirus* and was most closely related to
310 Cardamine chlorotic fleck virus (Skotnicki *et al.* 1993), although with only 45% amino acid
311 similarity. Another, found in U2 and F2, belonged to the genus *Gammacarmovirus* and is
312 most closely related to Melon necrotic spot virus with 71-73% amino acid similarity (Riviere
313 and Rochon 1990). We also found Carnation Italian Ringspot virus (genus *Tombusvirus*) at
314 both farming sites, with 98-99% amino acid similarity. The *Procedovirinae* also contain a
315 clade of closely related novel tombus-like viruses that appear basal to any currently described
316 genera and were found at the pristine sites. Another clade was found in the urban and farming
317 sites and appears to fall within the subfamily *Regressovirinae*.
318
319 *3.3.2 Dicistroviridae*
320 We similarly found a high diversity of viruses belonging to the *Dicistroviridae*, a family of
321 single-strand positive-sense RNA viruses commonly associated with arthropods (Valles et al.
322 2017). Thirty-nine viruses were part of a highly divergent clade that fell basal to the three
323 currently recognized genera, to which it exhibited less than 50% amino acid similarity. There
324 were also 15 newly identified viruses that fell into the genus *Cripavirus* found across all the
325 land-use types. In addition, we detected two previously described cripaviruses -
326 Rhopalosiphum padi virus (97-100% amino acid similarity) and Cricket paralysis virus (96-
327 97%), both only at the urban site U2.
328
329 *3.3.3 Nodaviridae*
330 We identified 13 novel viruses from the genus *Alphanodavirus* of the *Nodaviridae* (single-
331 strand positive-sense RNA viruses). This included Black beetle virus (*Alphanodavirus;* 95-
332 98% amino acid similarity) in all farming and urban sites, but not at the pristine sites.

Although we did not find any viruses belonging to the genus *Betanodavirus*, the only other genus of *Nodaviridae*, we did identify 61 other novel viruses from a divergent clade that fell outside of the *Alphanodavirus* and *Betanodavirus* genera. Many of these were most closely related to Barns Ness serrated wrack noda-like virus 2 isolated from marine algae (Waldron et al. 2018), although with less than 50% amino acid similarity.

3.4 Vertebrate-infecting families

3.4.1 Astroviridae

The *Astroviridae* are a family of single-stranded positive-sense RNA viruses that infect mammals and birds (Lukashov and Goudsmit 2002). We identified 28 novel astroviruses, including three new species within the *Bastrovirus* clade that were found in the farming and urban sites (Figure 4). These three viruses were most closely related to a bastrovirus found in sewage in Brazil (Dos Anjos et al. 2017), although with only 57-67% amino acid similarity. Twenty-four novel viruses fell into a divergent clade outside of the genus *Avastrovirus* and the *Bastrovirus* clade: these were most closely related to '*Astroviridae* sp.' viruses found in metagenomic studies of grassland soil in California, USA (Starr et al. 2019). The viruses found on our study that fell into this clade (denoted Flumine astrovirus 1-24) all had less than 62% amino acid similarity with the soil viruses. Notably, they were found across all our river sites, with a higher diversity at the farming and urban sites (n=11 and 21 species, respectively) than the pristine sites (n=4). However, flumine astrovirus 3 found at a pristine site had the highest abundance, representing 0.04% of the total reads in the library.

355 *3.4.2 Birnaviridae*

356 The *Birnaviridae* are a family of double-stranded RNA viruses that infect fish, birds and
357 insects. We identified one virus from this family at a pristine site (P1), that was most closely
358 related (although with only 30% amino acid similarity) to blotched snakehead virus (Da
359 Costa et al. 2003) and Lates calcarifer birnavirus (Chen et al. 2019), both of which are
360 associated with fish.

361

362 *3.4.3 Parvoviridae*

363 The *Parvoviridae* are a family of small double-stranded DNA viruses that infect both
364 vertebrates and invertebrates. We identified 21 parvoviruses: all were present at the Feilding
365 urban site (U1), with one (Flumine parvovirus 17) found at both P1 and U1. Most (n=18) of
366 these viruses fell into a distinct clade, separate from other previously described subfamily and
367 genera (Figure 5). This clade had less than 18% amino acid similarity with other
368 parvoviruses, but up to 81% similarity (average of 41%) with each other. A new subfamily
369 *Hamaparvovirinae* was created in 2020 that exhibits less than 20% amino acid sequence
370 identity with other parvoviruses (Pénzes et al. 2020). This is comparable to the level of
371 sequence similarity observed in the novel clade identified here, indicating this may also
372 represent a new subfamily that we have tentatively called the *Flumenparvovirinae*. The
373 remaining three viruses fell into the subfamily *Densovirinae*. These viruses most likely infect
374 invertebrates as they were most closely related to Planococcus citri densovirus (Thao et al.
375 2001) (76% amino acid identity) and Blattella germanica densovirus (Kapelinskaya et al.
376 2011) (55-61%) that infect mealy bugs and cockroaches, respectively.

377

3.4.4 Picornaviridae

The *Picornaviridae* are a large family of single-stranded RNA viruses that infect both vertebrates and invertebrates. We identified 15 novel picornaviruses, most (n=11) from the urban sites, although they were also identified at both farming and pristine sites. The pristine sites had the lowest diversity of picornaviruses (n=2), but the highest abundance. Eight of the picornaviruses fell into a clade with fur seal picorna-like virus (Krumbholz et al. 2017) and Ampivirus A1 associated with the smooth newt *Lissotriton vulgaris* (Reuter et al. 2015). This clade is most closely related to a cluster of genera *Tremovirus, Harkavirus* and *Hepatovirus* found in birds and mammals.

3.4.5 Genomoviridae

The *Genomoviridae* are a family of single-stranded DNA viruses. Although they are commonly associated with fungi, they have also identified them in mammals and birds (Varsani and Krupovic 2021). With the exception of one virus species (Flumine genomovirus 1), members of the *Genomoviridae* were only found at the urban sites. Nine of the 14 viruses were most closely related to viruses found in sewage or faeces. Interestingly, a number of these viruses are closely related to viruses previously documented in New Zealand. Flumine genomovirus 11 fell into a clade of five sewage-derived gemycircularviruses, exhibiting 85% amino acid similarity to its closest match Sewage-associated gemycircularvirus-7a sampled from a sewage oxidation pond in Christchurch, New Zealand (Kraberger et al. 2015). Similarly, Flumine genomovirus 8 and 9 share 85% and 86 % amino acid similarity, respectively, with Sewage associated gemycircularvirus 3 also isolated from a New Zealand oxidation pond (Kraberger et al. 2015), while Flumine genomovirus 12, 4 and 6 share 87%, 76% and 82% amino acid similarity, respectively, with faeces-associated gemycircularvirus 21 isolated from llama faeces in New Zealand (Steel et al. 2016). Finally, the single

403 *Genomoviridae* virus found in the pristine sites (Flumine genomovirus 1) was most closely
404 related (81% amino acid similarity) to a virus isolated from minnow tissue in the USA.
405

406 ## 3.5 Host relationships and patterns of virus diversity

407 We next characterized each viral family according to their usual assigned host (as identified
408 in previous studies) and used this to visualize patterns of virus abundance and diversity
409 (Figure 6). This revealed that the two farming sites had a lower viral abundance across all
410 host types, while the two pristine sites had a higher abundance of viruses with animal,
411 plant/fungi (such as the *Astroviridae* and *Tombusviridae*) and unknown hosts. In turn, U1 had
412 a very high abundance of prokaryote-infecting viruses but a lower abundance of all other
413 virus types (Figure 6). Virus abundance was highest for those viral families where the host
414 was unknown, accounting for 2.35% of reads. Plant-infecting viruses were the next-most
415 abundant (1%), followed by animal-infecting viruses (0.56%). At the family level, we found
416 a high diversity of plant- and animal-infecting viruses (18 and 17 families, respectively).
417 There was a surprisingly low abundance and diversity of known prokaryote infecting viruses:
418 these were the least abundant (0.096%), and the least diverse (six families) group. All sites
419 had a proportionally high abundance of *Nodaviridae, Tombusviridae* and 'unclassified
420 *Picornavirales'* compared to other viral families.
421

422 ## 3.6 Alpha and beta virus diversity

423 Sites P1 and U2 had the highest richness, manifesting as 38 and 40 virus families,
424 respectively, while P2 had the lowest at 16 (Table 1). U1 had higher Shannon and Shannon
425 ENS values than any other site, but only the third highest richness. The two pristine sites had
426 very different levels of diversity, with P1 having much higher richness (with more than twice

the number of viral families), Shannon and Shannon ENS than P2. The two farming sites had a similar richness, Shannon and Shannon ENS to each other, and the two urban sites were also similar with respect to richness (with U1 having 20% fewer virus families than U2).

At the level of virus family, we used principal co-ordinate analysis to examine the differences between viral communities, examining 'intra-type' (i.e., comparing sites with the same land-use type) and 'inter-type' (comparing sites across different land-use types) differences (Figure 7). Accordingly, the pristine and farming sites had high intra-type similarity and low inter-type similarity, such that their viral communities were more similar within a land-use type than to viral communities from different land-use types (Figure 7). The two pristine sites displayed the most similar viral communities. In contrast, the two urban sites differed markedly from each other and all other sites and hence had a very different viral community, both from each other and to that at any other site (i.e., both intra- and inter-type). The urban site U2 (Palmerston North) was closer to F1 and F2 than to U1, and hence had a viral community that was more similar to the farming sites than to the other urban site. At the viral species level, the urban sites had a higher diversity (n=327 species) than the farming (n=150) and pristine sites (n=119). There were many more viruses shared between the urban and farming sites (n=76) than between the pristine and farming or urban sites (n=24). Finally, only eight of 504 species were found in all land-use types, indicative of a relatively high level of local differentiation.

4. Discussion

Using a mNGS approach we identified a high diversity of novel and highly divergent viruses in a single New Zealand river system and revealed differences of virome composition in the river between sampling sites associated with different land-use types. In total, we observed

504 putative virus species, of which 491 (97%) were potentially novel and including a new subfamily within the *Parvoviridae*. Notably, there were considerable differences in the viral community structure between the land-use types. In particular, the two pristine sites had a higher abundance of viruses that infect animals, plants and fungi, while at the viral species level, the urban sites had the highest virome diversity. In addition, there were many more viruses shared between the urban and farming sites (n=76) than between the pristine and farming or urban sites (n=24).

The abiotic environment within the Manawatū River system differed considerably between sampling sites that had different land-use types, with the pristine sites having lower measurements of all variables. Total dissolved solids (TDS), salinity, conductivity and turbidity represent different ways of measuring the presence of dissolved solids and ions in the water, and generally the lower they are then the higher the water quality (Davies-Colley 2013). Increases in total dissolved solids can adversely affect plants and animals in freshwater environments due to changes in osmotic conditions, making this an important indicator of the health of the freshwater ecosystem (Chapman et al. 2000). These measures are all elevated in effluents, and their presence in freshwater can therefore indicate contamination from fertilizers, urban run-off, and animal and human waste (Chapman et al. 2000; de Sousa et al. 2014). They can also naturally be elevated due to changes in climate and differing geology, which impacts the amount of dissolved substance from the weathering of rocks (Davies-Colley 2013). However, as the sites in this study were all in the same river catchment and within 50km of each other, the climate and geology are unlikely to be markedly different. Water temperature was also lower in the pristine sites than in the farming and urban sites, likely due to the pristine sites being at higher altitude, closer to the Ruahine Ranges (a source of snow melt), and also the thicker vegetation cover blocking solar

476 radiation. The pristine sites had a pH closer to neutral, which may be related to differences in
477 dissolved solids from the surrounding soils (Baldisserotto 2011). All these indicators show
478 that the riverine environment was very different between the pristine and farming/urban sites,
479 but not substantially different between the farming and urban sites.
480
481 A key observation of our study was the high diversity of viruses in the Manawatū River
482 system, with an average of 30 virus families detected per site. Indeed, we found a very high
483 diversity of novel viruses, and only a small number (n=13) described previously, again
484 indicating that only a tiny fraction of the virosphere has been described to date. Environments
485 such as freshwater have received relatively little viral metagenomic research, although our
486 study suggests that they may have highly diverse viromes. In particular, we show that isolated
487 New Zealand freshwater environments can harbour many novel and highly divergent viruses.
488 Perhaps of most note, we have identified a novel clade of parvoviruses that was
489 phylogenetically distinct from other previously described subfamily and genera, and which
490 may be a new subfamily of the *Parvoviridae*. This may in part reflect New Zealand's
491 distinctive ecological history, including high levels of endemic species (Walker et al. 2021).
492 These novel viruses may therefore be naturally present in this water catchment and reflect the
493 'undisturbed' diversity of the river, rather than the result of human land-use. A high diversity
494 of bacteria have also been found in New Zealand freshwater (Phiri et al. 2021). Indeed, it is
495 notable that the pristine sites had the highest abundance of viruses that infect animals, plants
496 and fungi, and had little overlap (in terms of species shared) with the farming and urban sites
497 (Figures 6 and 7). This may reflect a higher abundance of native flora and fauna in these
498 pristine sections of the river that are surrounded by native forest. The two pristine sites had
499 very different levels of diversity, with P1 having more than twice the number of viral families
500 than P2, despite P2 having a higher number of viral reads. This may be because the P2 stream

501 was smaller in volume with a smaller catchment, with less opportunity for a high diversity of
502 terrestrial viruses to enter the river from the surrounding land.
503
504 Virus families that infect fungi, plants and algae were found in particularly high abundance,
505 with the highest being the *Tombusviridae* associated with plants. This presumably reflects a
506 high abundance of plant and algae matter in the water, including both aquatic plants living in
507 the river and terrestrial plants from the surrounding land. However, there was a surprisingly
508 low abundance and diversity of viruses associated with prokaryotes. This may be because we
509 filtered out bacteria prior to RNA extraction (using PES membrane filters of 0.2µm pore size)
510 which would also have removed any lysogenic phages. However, a previous study of urban
511 streams in Ecuador using a similar filtering system found that prokaryote infecting viruses
512 had the highest abundance, followed by plant viruses (Guerrero-Latorre et al. 2018).
513 Interestingly, these Ecuadorian streams are known to be contaminated with untreated sewage
514 (Guerrero-Latorr*e et al. 2*018), which may increase bacterial load (and therefore phage
515 abundance). It is also possible that the high abundance of divergent viruses belonging to
516 families in which the host is unknown (Figure 6) are in fact phage.
517
518 Metagenomics frequently identifies highly divergent viruses in environmental samples and in
519 most such studies the host is unknown. We observed a similar pattern. The novel viruses
520 identified often clustered into clades that fell in basal positions on family-level phylogenies
521 and were most closely related to viral species found in other metagenomic studies (Zhang et
522 al. 2018; Starr et al. 2019). As a case in point, many of the *Tombusviridae*-like viruses were
523 highly divergent and fell into the 'tombus-like' virus clade. This is similar to the findings of
524 Wolf et al. (2020), who identified 199 tombus-like viruses in seawater from the Yangshan
525 Deep-Water Harbour, Shanghai, China. We also found many divergent viruses from the

526 *Astroviridae* most closely related to viruses found using metagenomics from grassland soil,
527 California, USA (Starr et al. 2019). As the *Astroviridae* is routinely associated with
528 vertebrates it is surprising to find these viruses in soil and water. Hence, it is likely that these
529 were shed from a vertebrate host although this clearly needs to be studied in greater detail.
530
531 As the sampling sites were all from the same river catchment, in some cases the water from
532 one site would flow to other sampling sites downstream. For example, water from P1 and P2
533 would flow through F1 and U2. However, there did not appear to be a trend in the number of
534 reads and number of viral hits when comparing upstream and downstream sites (Figure 3). In
535 particular, very few viruses (n=17) were found at both the pristine sites and the downstream
536 farming and urban sites, suggesting that viruses were generally not being carried downstream
537 for long distances. Notably, for the pristine and farming sites, there was considerably more
538 overlap in viral community at sites of the same land-use type: P1 and P2 were very similar to
539 each other, as were and F1 and F2 (Figure 7). The exception to this was the urban site U2
540 (Palmerston North) that was more similar to the two farming sites than to U1 (Feilding). This
541 difference appears to be driven by the high abundance and diversity of prokaryote infecting
542 viruses in U1, and the lower abundance of animal- and plant-infecting viruses. Indeed, U1
543 was on a different tributary of the Manawatū River than the other sites (Figure 1), so the viral
544 ecology in that part of the river may be different. Feilding (population 17,050) is also a much
545 smaller urban centre than Palmerston North (population 81,500). In addition, while the
546 sample sites from both urban sites were downstream of the urban area at the edge of the
547 town/city, Feilding releases wastewater far downstream in a rural area, whereas Palmerston
548 North releases wastewater upstream of our sampling site. However, although it might be
549 expected that the presence of wastewater would increase the abundance of phage, Feilding

had the higher phage abundance. Possibly changes in the bacterial diversity/structure in this urban site have resulted in differences in the phage diversity and structure.

Some novel viruses were found only in the sites adjacent to human land-use (i.e., farming and urban sites), including all the bastroviruses (*Astroviridae*), all of which were most closely related to a bastrovirus found in sewage (Dos Anjos et al. 2017). The pattern of presence/absence in our study and the link to sewage suggests the presence of these viruses are likely related to human land-use. Similarly, the presence of viruses from the *Genomoviridae* appears to be related to urban land-use: nine of the 14 viruses were most closely related to viruses found in sewage or faeces. Despite the generally large differences in viral community between the two urban sites, they both contained viruses from the *Genomoviridae*: 13 viral species were identified in U1 and four in U2 (including two viruses found in both sites), suggesting a link between human land-use and the presence of these viruses. Notably, however, we did not detect any human viruses. This contrasts with a PCR study that detected enteric viruses (adenovirus, norovirus, enterovirus, rotavirus, and hepatitis E virus) in two other rivers in New Zealand (Williamson et al. 2011), which may reflect the greater sensitivity of PCR assays specifically designed to detect these viruses. In general, more viruses were found in common between urban and farming sites. For example, 11 *Astroviridae*-like viruses were found in both urban and farming sites, but no *Astroviridae*-like viruses found in either of the pristine sites were identified in urban or farming sites. Hence, within viral families at the species level, the farming and urban sites had a more similar viral community to each other than to the pristine sites, which may be a result of human land-use adjacent to the river.

574 The only viruses we identified that were previously described were all found in the urban and
575 farming sites, again indicative of an anthropogenic influence, including agriculture and
576 introduced species. For example, we identified the Black beetle virus in all farming and urban
577 sites. The host (*Heteronychus arator*) is a major invasive pasture pest species that was
578 introduced from Africa in 1930s (Wilson et al. 2016), and the virus was first identified in
579 1975 in New Zealand (Longworth and Carey 1976). Similarly, at the Palmerston North urban
580 site (U2) we detected Rhopalosiphum padi virus that infects the bird cherry-oat aphid, a pest
581 of cereal crops that was first recorded in New Zealand in 1921 (Bulman et al. 2005).
582 Surprisingly, we found Norway luteo-like virus 4 with 96-100% amino acid identity in one
583 farming and one urban site (F1 and U2), previously associated with the castor bean tick
584 *Ixodes ricinus* (Pettersson et al. 2017). As this species of tick has not been described in New
585 Zealand, this result suggest that this virus has a wider host range than is currently known.
586 Similarly, we identified Pepper mild mottle virus in U2. This virus has previously been
587 proposed as a water quality indicator and an indicator of the presence of human faeces in
588 freshwater, as it is the most abundant RNA virus in human faeces but rarely found in animal
589 faecal matter (Kitajima et al. 2018; Rosario et al. 2009). Our ability to detect these viruses
590 suggests this technique could be used for ongoing monitoring, including detecting the
591 presence of pest species in the surrounding area and human-related viruses indicating
592 contamination of faecal matter in the river. That none of these viruses were found in the
593 pristine sites suggests these viruses are being introduced into the river from agricultural and
594 urban run-off, and possibly also discharge of treated wastewater into the river. Indeed, at
595 pristine sites we would generally expect viruses associated with New Zealand native plants
596 and animals, most of which are yet to be described.
597

598 This study represents the first characterization of the virome of a New Zealand river. We
599 observed a high abundance and diversity of viruses, including many that are both novel and
600 highly divergent. Within the same river catchment, we identified viruses linked to agriculture
601 and human presence (including possible links to sewage) in the farming and urban sites that
602 were not present in the pristine sites. More broadly, this work provides the foundation for
603 more detailed research on the impacts of human land-use on river viromes which will require
604 larger sample sizes across multiple river systems. Our results show that human land-use
605 impacted the viral community in the river, suggesting that further work is needed to reduce
606 the impact of intensive farming and urbanization on the land and rivers.

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Data

Sequence data have been deposited in the Sequence Read Archive (SRA) under accession numbers SRR17234948-53. The trimmed alignment fasta files used to inder the phylogenetic trees are available at https://github.com/RKFrench/Viral-Diversity-NZ-River.

5. References

Alegbeleye, O. O., and Sant'Ana, A. S. (2020) 'Manure-borne pathogens as an important source of water contamination: An update on the dynamics of pathogen survival/transport as well as practical risk mitigation strategies', *International Journal of Hygiene and Environmental Health,* 227: 113524.

Amvrosieva, T. et al. (2001) 'Viral water contamination as the cause of aseptic meningitis outbreak in Belarus', *Central European Journal of Public Health* 9: 154-7.

Baldisserotto, B. (2011) 'Water pH and hardness affect growth of freshwater teleosts', *Brazilian Journal of Animal Science* 40: 138-44.

Bolger, A.M., Lohse, M., and Usadel, B. (2014) 'Trimmomatic: a flexible trimmer for Illumina sequence data', *Bioinformatics* 30: 2114-20.

Buchfink, B., Reuter, and K., Drost, H.-G. (2021) 'Sensitive protein alignments at tree-of-life scale using DIAMOND', *Nature Methods* 18: 366-8.

Bulman, S. et al. (2005) 'Rhopalosiphum aphids in New Zealand. I. RAPD markers reveal limited variability in lineages of *Rhopalosiphum padi'*. *New Zealand Journal of Zoology* 32: 29-36.

Burridge, C. P., and Waters, J. M. (2020) 'Does migration promote or inhibit diversification? A case study involving the dominant radiation of temperate Southern Hemisphere freshwater fishes', *Evolution* 74: 1954-65.

Bushnell, B. (2018) 'BBMap short-read aligner, and other bioinformatics tools', University of California, Berkeley, CA.

643 Camacho, C. et al. (2009) 'BLAST+: architecture and applications', *BMC Bioinformatics* 10:
644 1-9.

645 Capella-Gutiérrez, S., Silla-Martínez, J. M., and Gabaldón, T. (2009) 'trimAl: a tool for
646 automated alignment trimming in large-scale phylogenetic analyses', *Bioinformatics* 25:
647 1972-3.

648 Campbell, S. J. et al. (2020). 'Red fox viromes in urban and rural landscapes', *Virus*
649 *Evolution* 6: veaa065.

650 Chapman, P. M., Bailey, H., and Canaria, E. (2000) 'Toxicity of total dissolved solids
651 associated with two mine effluents to chironomid larvae and early life stages of rainbow
652 trout', *Environmental Toxicology and Chemistry* 19: 210-14.

653 Chen, J. et al. (2019) 'Detection and characterization of a novel marine birnavirus isolated
654 from Asian seabass in Singapore', *Virology Journal* 16: 1-10.

655 Chen, W. et al. (2018) 'Aquatic bacterial communities associated with land use and
656 environmental factors in agricultural landscapes using a metabarcoding approach', *Frontiers*
657 *in Microbiology* 9: 2301.

658 Clausen, P. T., Aarestrup, F.M., and Lund, O. (2018) 'Rapid and precise alignment of raw
659 reads against redundant databases with KMA', *BMC Bioinformatics* 19: 1-8.

660 Da Costa, B. et al. (2003) 'Blotched snakehead virus is a new aquatic birnavirus that is
661 slightly more related to avibirnavirus than to aquabirnavirus' *Journal of Virology* 77: 719-25.

662 Davies-Colley, R. J. (2013) 'River water quality in New Zealand: an introduction and
663 overview', *Ecosystem Services in New Zealand: Conditions and Trends.* Manaaki Whenua
664 Press, Lincoln: 432-447.

665 de Sousa, D. N. R. et al. (2014) 'Electrical conductivity and emerging contaminant as
666 markers of surface freshwater contamination by wastewater', *Science of the Total*
667 *Environment* 484: 19-26.

668 Department of Conservation (2021a). 'History and culture', https://www.doc.govt.nz/parks-
669 and-recreation/places-to-go/manawatu-whanganui/places/ruahine-forest-park/history-and-
670 culture/ (Accessed 1st June, 2021).

671 Department of Conservation (2021b). 'Nature and conservation',
672 https://www.doc.govt.nz/parks-and-recreation/places-to-go/manawatu-
673 whanganui/places/ruahine-forest-park/nature-and-conservation/ (Accessed 1st June, 2021).

674 Dos Anjos, K., Nagata, T., and de Melo, F. L. (2017) 'Complete genome sequence of a novel
675 bastrovirus isolated from raw sewage', *Genome Announcements* 5: e01010-01017.

676 Dunn, N. R. et al. (2018) '*Conservation status of New Zealand freshwater fishes, 2017'*,
677 Publishing Team, Department of Conservation.

678 Ferguson, C., et al. (2003) 'Fate and transport of surface water pathogens in watersheds'
679 *Critical Reviews in Environmental Science and Technology* 33: 299-361.

680 French, R. K., and Holmes, E. C. (2020) 'An ecosystems perspective on virus evolution and
681 emergence', *Trends in Microbiology* 28: 165-75.

682 Grabherr, M. G. et al. (2011) 'Full-length transcriptome assembly from RNA-Seq data
683 without a reference genome', *Nature Biotechnology* 29: 644.

684 Guerrero-Latorre, L. et al. (2018) 'Quito's virome: Metagenomic analysis of viral diversity in
685 urban streams of Ecuador's capital city', *Science of the Total Environment* 645: 1334-43.

686 Gu, X. et al. (2018) 'Geospatial distribution of viromes in tropical freshwater ecosystems',
687 *Water Research* 137: 220-32.

688 Guerrero-Latorre, L. et al (2018) 'Quito's virome: Metagenomic analysis of viral diversity in
689 urban streams of Ecuador's capital city', *Science of the Total Environment* 645: 1334-43.

690 Hamill, K. (2012) 'Effects of Palmerston North City's Wastewater Treatment Plant discharge
691 on water quality and aquatic life in the Manawatu River', Palmerston North City Council and
692 Horizons Regional Council.

693 Hill, M. O. (1973) 'Diversity and evenness: a unifying notation and its consequences',
694 *Ecology* 54: 427-32.

695 Jack, S., Bell, D., and Hewitt, J. (2013) 'Norovirus contamination of a drinking water supply
696 at a hotel resort', *New Zealand Medical Journal* 126: 98-107.

697 Jones, T. H., et al. (2017) 'Waterborne viruses and F-specific coliphages in mixed-use
698 watersheds: microbial associations, host specificities, and affinities with environmental/land
699 use factors', *Applied and Environmental Microbiology 83*: e02763-02716.

700 Kapelinskaya, T. V., et al. (2011) 'Expression strategy of densonucleosis virus from the
701 German cockroach, *Blattella germanica*', *Journal of Virology* 85: 11855-70.

702 Katoh, K., and Standley, D. M (2013) 'MAFFT multiple sequence alignment software
703 version 7: improvements in performance and usability', *Molecular Biology and Evolution* 30:
704 772-80.

705 Kirs, M., et al. (2011) 'Source tracking faecal contamination in an urbanised and a rural
706 waterway in the Nelson-Tasman region, New Zealand', *New Zealand Journal of Marine and*
707 *Freshwater Research* 45: 43-58.

708 Kitajima, M., Sassi, H. P., and Torrey, J. R. (2018) 'Pepper mild mottle virus as a water
709 quality indicator', *NPJ Clean Water* 1: 1-9.

710 Kraberger, S., et al. (2015) 'Characterisation of a diverse range of circular replication-
711 associated protein encoding DNA viruses recovered from a sewage treatment oxidation
712 pond', *Infection, Genetics and Evolution* 31: 73-86.

713 Kraberger, S., et al. (2013) 'Discovery of Sclerotinia sclerotiorum hypovirulence-associated
714 virus-1 in urban river sediments of Heathcote and Styx Rivers in Christchurch City, New
715 Zealand', *Genome Announcements* 1: e00559-00513.

716 Krumbholz, A. et al. (2017) 'Genome sequence of a novel picorna-like RNA virus from feces
717 of the Antarctic fur seal (*Arctocephalus gazella*)', *Genome Announcements* 5: e01001-17.

718 Lamberto, I. et al. (2014) 'Mycovirus-like DNA virus sequences from cattle serum and
719 human brain and serum samples from multiple sclerosis patients', *Genome Announcements* 2:
720 e00848-00814.

721 Le Lay, C., et al. (2020) 'Unmapped RNA virus diversity in termites and their symbionts',
722 *Viruses* 12: 1145.

723 Li, B., and Dewey, C. N. (2011) 'RSEM: accurate transcript quantification from RNA-Seq
724 data with or without a reference genome', *BMC Bioinformatics* 12: 1-16.

725 Longworth, J., and Carey, G. (1976) 'A small RNA virus with a divided genome from
726 *Heteronychus arator* (F.)[*Coleoptera: Scarabaeidae*]', *Journal of General Virology* 33: 31-
727 40.

728 Lukashov, V. V., and Goudsmit, J. 2002. Evolutionary relationships among *Astroviridae*.
729 *Journal of General Virology 83*: 1397-1405.

730 Marcelino, V. R., et al. (2020) 'CCMetagen: comprehensive and accurate identification of
731 eukaryotes and prokaryotes in metagenomic data', *Genome Biology* 21: 1-15.

732 Martolia, H. C. S. et al. (2009) 'An outbreak of hepatitis E tracked to a spring in the foothills
733 of the Himalayas, India, 2005', *Indian Journal of Gastroenterology* 28: 99-101.

734 Mahar, J. E., et al. (2020) 'Comparative analysis of RNA virome composition in rabbits and
735 associated ectoparasites' *Journal of Virology* 94: e02119-02119.

736 McMurdie, P. J., and Holmes, S. (2013) 'phyloseq: an R package for reproducible interactive
737 analysis and graphics of microbiome census data', *PLoS One* 8: e61217.

738 Mortimer, N. et al. (2019) 'Late Cretaceous oceanic plate reorganization and the breakup of
739 Zealandia and Gondwana', *Gondwana Research* 65: 31-42.

740 Mukherjee, S. S. (2011) '*Identification and characterization of tobamo and tombusviruses*
741 *isolated from New Zealand waters*', PhD thesis, State University of New York College of
742 Environmental Science and Forestry.

743 Mukherjee, S. S. et al. (2012) 'New tombusviruses isolated from surface waters in New
744 Zealand', *Australasian Plant Pathology* 41: 79-84.

745 Nayfach, S. et al. (2021) 'Metagenomic compendium of 189,680 DNA viruses from the
746 human gut microbiome', *Nature Microbiology* 6: 960-70.

747 Nguyen, L.-T. et al. (2015) 'IQ-TREE: a fast and effective stochastic algorithm for estimating
748 maximum-likelihood phylogenies' *Molecular Biology and Evolution* 32: 268-74.

749 Paradis, E., and Schliep, K. (2019) 'ape 5.0: an environment for modern phylogenetics and
750 evolutionary analyses in R' *Bioinformatics* 35: 526-28.

751 Pénzes, J. J. et al. (2020) 'Reorganizing the family *Parvoviridae*: a revised taxonomy
752 independent of the canonical approach based on host association', *Archives of Virology* 165:
753 2133-2146.

754 Pettersson, J. H.-O. et al. (2017) 'Characterizing the virome of *Ixodes ricinus* ticks from
755 northern Europe', *Scientific Reports* 7: 1-7.

756 Phiri, B. J. et al. (2020) 'Does land use affect pathogen presence in New Zealand drinking
757 water supplies?', *Water Research* 185: 116229.

758 Phiri, B. J. et al (2021) 'Microbial diversity in water and animal faeces: a metagenomic
759 analysis to assess public health risk', *New Zealand Journal of Zoology* 48: 188-201.

760 Porter, A. F. et al. (2021) 'Metagenomic identification of viral sequences in laboratory
761 reagents', *Viruses* 13: 2122

762 Qiu, H. et al. (2020) 'Metagenomic analysis revealed that the terrestrial pollutants override
763 the effects of seasonal variation on microbiome in river sediments', *Bulletin of*
764 *Environmental Contamination and Toxicology* 105: 892-8.

765 R Core Team. (2021) 'R: A language and environment for statistical computing', R
766 Foundation for Statistical Computing, Vienna, Austria.

767 Reuter, G. et al. (2015) 'A highly divergent picornavirus in an amphibian, the smooth newt
768 (*Lissotriton vulgaris*)'. *Journal of General Virology* 96: 2607–13.

769 Rice, P., Longden, I., and Bleasby, A. (2000) 'EMBOSS: the European molecular biology
770 open software suite', *Trends in Genetics* 16: 276-7.

771 Riviere, C., and Rochon, D. (1990) 'Nucleotide sequence and genomic organization of melon
772 necrotic spot virus', *Journal of General Virology* 71: 1887-96.

773 Roossinck, M. J. (2015) 'Plants, viruses and the environment: ecology and mutualism',
774 *Virology* 479: 271-7.

775 Rosario, K. et al. (2009) 'Pepper mild mottle virus as an indicator of fecal pollution', *Applied*
776 *and Environmental Microbiology* 75: 7261-7.

777 Roygard, J., McArthur, K., and Clark, M. (2012) 'Diffuse contributions dominate over point
778 sources of soluble nutrients in two sub-catchments of the Manawatu River, New Zealand'
779 *New Zealand Journal of Marine and Freshwater Research* 46: 219-41.

780 Sedyaningsih-Mamahit, E. et al. (2002) 'First documented outbreak of hepatitis E virus
781 transmission in Java, Indonesia', *Transactions of the Royal Society of Tropical Medicine and*
782 *Hygiene* 96: 398-404.

783 Sekwadi, P. et al. (2018) 'Waterborne outbreak of gastroenteritis on the KwaZulu-natal coast,
784 South Africa, December 2016/January 2017', *Epidemiology and Infection* 146: 1318-25.

785 Sit, T. L., and Lommel, S. A. (2015) '*Tombusviridae*' *eLS*: 1-9.

786 Skotnicki, M. et al. (1993) 'The genomic sequence of cardamine chlorotic fleck carmovirus',
787 *Journal of General Virology* 74: 1933-7.

788 Sommers, P. et al. (2021) 'Integrating viral metagenomics into an ecological framework',
789 *Annual Review of Virology* 8: 133-58.

790 Starr, E. P. et al. (2019) 'Metatranscriptomic reconstruction reveals RNA viruses with the
791 potential to shape carbon cycling in soil', *Proceedings of the National Academy of Sciences*
792 *USA* 116: 25900-8.

793 Steel, O. et al. (2016) 'Circular replication-associated protein encoding DNA viruses
794 identified in the faecal matter of various animals in New Zealand', *Infection, Genetics and*
795 *Evolution* 43: 151-64.

796 Thao, M. L. et al. (2001) 'Genetic characterization of a putative Densovirus from the
797 mealybug *Planococcus citri*', *Current Microbiology* 43: 457-458.

798 Valles, S. et al. (2017) 'ICTV virus taxonomy profile: *Dicistroviridae*', *Journal of General*
799 *Virology 98*: 355-6.

800 Van Rossum, T. et al. (2015) 'Year-long metagenomic study of river microbiomes across
801 land use and water quality', *Frontiers in Microbiology* 6: 1405.

802 Varsani, A., and Krupovic, M. (2021) 'Family *Genomoviridae*: 2021 taxonomy update',
803 *Archives of Virology* 166: 2911–26

804 Waldron, F.M., Stone, G. N., and Obbard, D. J. (2018) 'Metagenomic sequencing suggests a
805 diversity of RNA interference-like responses to viruses across multicellular eukaryotes',
806 *PLoS Genetics* 14: e1007533.

807 Walker, S., Monks, A. and Innes, J. G (2021) 'Life history traits explain vulnerability of
808 endemic forest birds and predict recovery after predator suppression', *New Zealand Journal*
809 *of Ecology* 45: 3447.

810 Wickham, H. (2016) 'ggplot2: Elegant Graphics for Data Analysis', Springer-Verlag New
811 York.

812 Wilkinson, L. (2011) 'venneuler: Venn and Euler Diagrams', R package version 1.1-0.
813 Available at http://CRAN.R-project.org/package=venneuler.

814 Wille, M. et al. (2020) 'Sustained RNA virome diversity in Antarctic penguins and their
815 ticks', *The ISME Journal* 14: 1768-82.

816 Wille, M. et al. (2021) 'RNA virome abundance and diversity is associated with host age in a
817 bird species', *Virology* 561: 98-106.

818 Williamson, W. et al. (2011) 'Enteric viruses in New Zealand drinking-water sources' *Water*
819 *Science and Technology* 63: 1744-51.

820 Wilson, M. J. et al. (2016) 'Developing a strategy for using entomopathogenic nematodes to
821 control the African black beetle (*Heteronychus arator*) in New Zealand pastures and
822 investigating temperature constraints', *Biological Control* 93: 1-7.

823 Wolf, Y. I. et al. (2020) 'Doubling of the known set of RNA viruses by metagenomic analysis
824 of an aquatic virome', *Nature Microbiology* 5: 1262-70.

825 Yu, G. (2020) 'Using ggtree to visualize data on tree-like structures', *Current Protocols in*
826 *Bioinformatics* 69: e96.

827 Zhang, Y.-Z., Shi, M., and Holmes, E. C. (2018) 'Using metagenomics to characterize an
828 expanding virosphere', *Cell* 172: 1168-72.

829 **Figure legends**

830

831 **Figure 1.** Map of the Manawatū River catchment in blue and the six sampling sites as
832 coloured circles, with the inset showing the river catchment location on the North Island of
833 New Zealand as a red square. P1 and P2 are 'pristine' sites having only flowed through native
834 bush (seen as dark green areas on the satellite image). F1 and F2 are 'farming' sites flowing
835 through intensive agricultural land (light green on the satellite image). U1 and U2 are 'urban'
836 sites, flowing through two urban areas – Feilding and Palmerston North (grey on the satellite
837 image). The water flows from the east and south towards the sea on the west coast, as
838 indicated by the white arrows. Satellite image created using Google Earth.

839

840 **Figure 2**. Measurements of salinity, pH, total dissolved solids, turbidity, conductivity and
841 temperature at the six different sites on the Manawatū River, grouped into the different site
842 types (pristine, farming and urban). For all measurements except temperature there are three
843 values per sampling site which have been jittered to reduce overplotting.

844

845 **Figure 3.** Read counts and the percentage of viral reads (%) for libraries from six sites on the
846 Manawatū River.

847

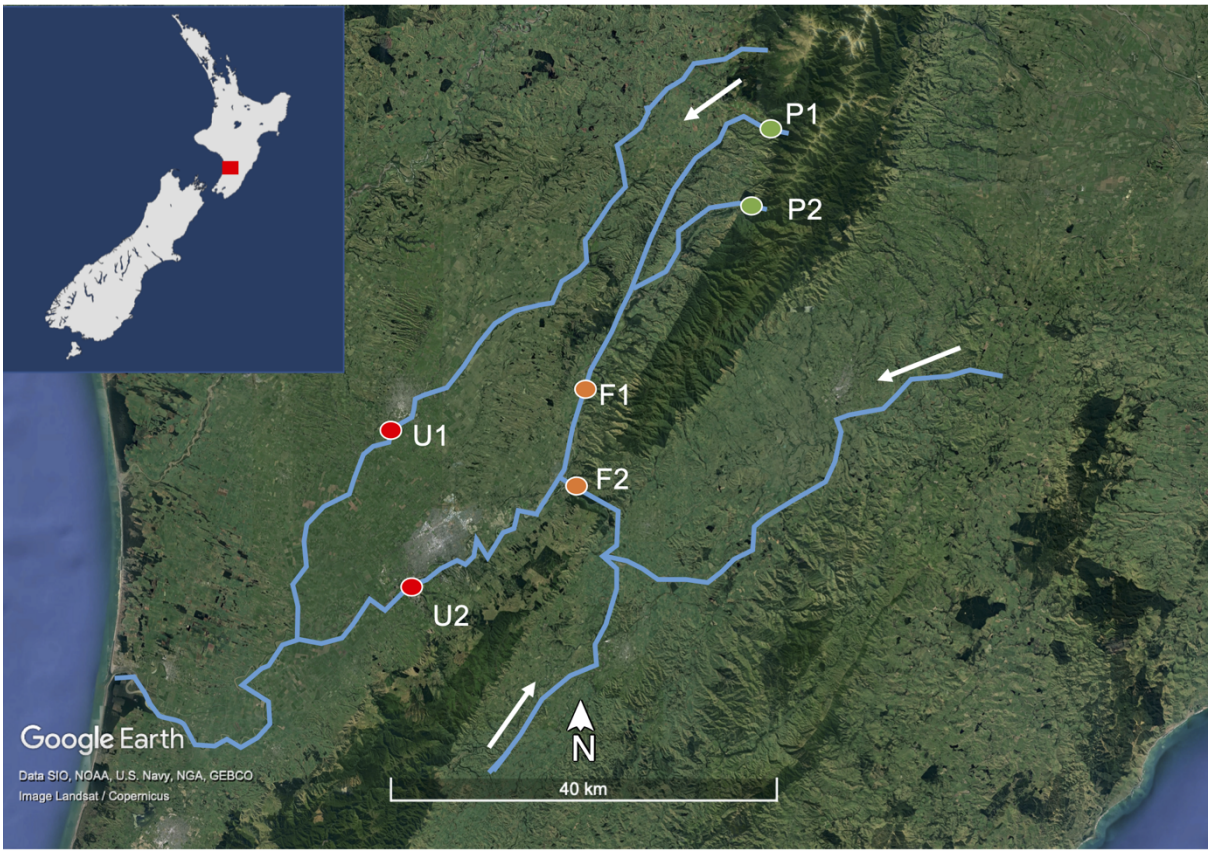
848 **Figure 4.** Phylogeny of the *Astroviridae* based on the non-structural polyprotein sequence
849 (alignment length of 733 amino acids). Viruses obtained in this study are shown in blue and
850 have a '+' after their name. Related viruses are shown in black. Virus abundance is expressed
851 as the percentage of the total number of reads and represented by the size of each coloured
852 symbol. The colour of each symbol refers to the site type. Black circles on nodes show

853 bootstrap support values greater than 90%. Branches are scaled according to the number of
854 amino acid substitutions per site, shown in the scale bar. The tree is midpoint rooted.
855
856 **Figure 5.** Phylogeny of the *Parvoviridae* based on the non-structural protein sequence
857 (alignment length of 452 amino acids). Viruses obtained in this study are shown in blue and
858 have a '+' after their name. Related viruses are in black. Virus abundance is expressed as the
859 percentage of the total number of reads and represented by the size of each coloured symbol.
860 The colour of each symbol refers to the site type. Black circles on nodes show bootstrap
861 support greater than 90%. Branches are scaled according to the number of amino acid
862 substitutions per site, shown in the scale bar. The tree is midpoint rooted.
863
864 **Figure 6.** Virus abundance (as a percentage of the total number of reads) of viral families
865 from each site on the Manawatū River. Virus families are divided into each panel depending
866 on their usual host - animal, plant/fungi, prokaryote and unknown. Unclassified viruses did
867 not have a classification according to the current NCBI taxonomy.
868
869 **Figure 7.** Viral community similarities and differences at the taxonomic family level (left)
870 and species level (right). Left - A Principal Coordinates Analysis (PCoA) applying the Bray–
871 Curtis dissimilarity matrix for viral abundance and virus family diversity, showing the
872 relative similarity/differences in viral community between sites with differing land-use types
873 – pristine (green), farming (orange), and urban (red). Points closer to one another are more
874 similar in virome structure than those further away. Right – a Venn diagram showing the
875 number of virus species shared between the three land-use types (with an amino acid
876 similarity >95%).

877 **Table 1.** The richness, Shannon, and Shannon effective number of species (ENS) values for
878 each site on the Manawatū River. These were calculated at a family level and include
879 unclassified virus groups such as 'unclassified Riboviria'.

	Richness	Shannon	Shannon ENS
P1	38	2.13	8.39
P2	16	1.88	6.54
F1	28	2.21	9.07
F2	24	2.11	8.28
U1	32	2.52	12.45
U2	40	2.00	7.39

880



881

882 **Figure 1**

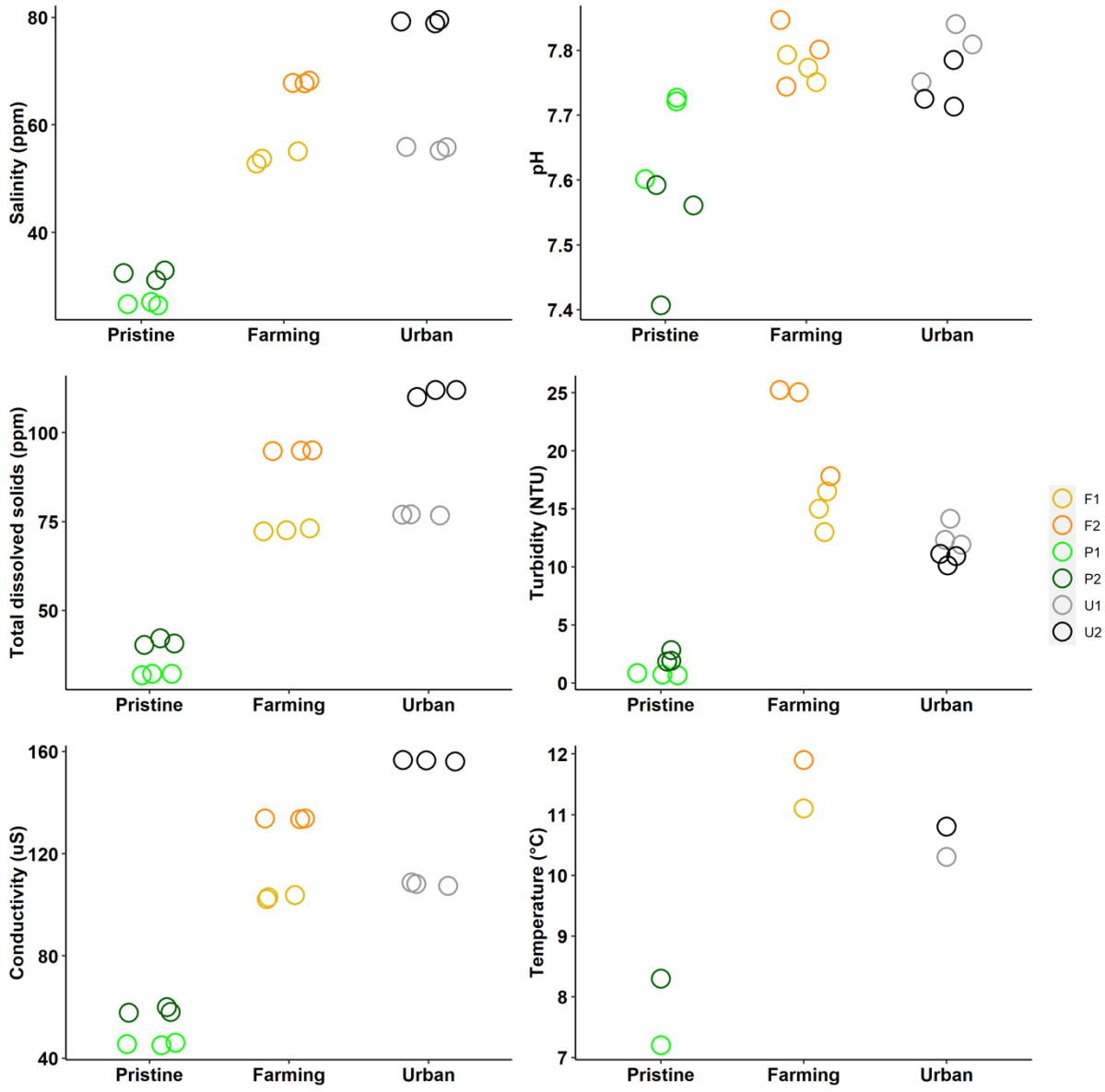
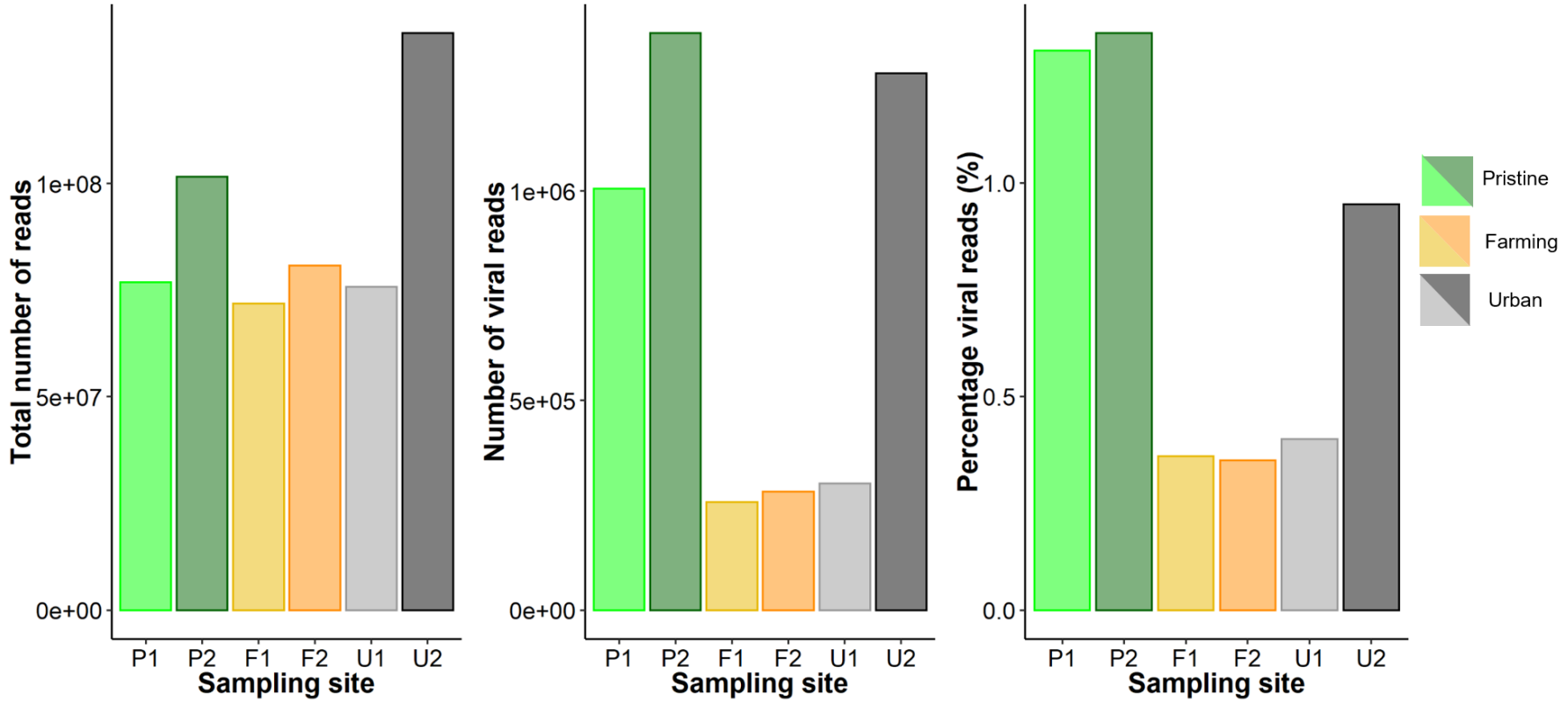


Figure 2



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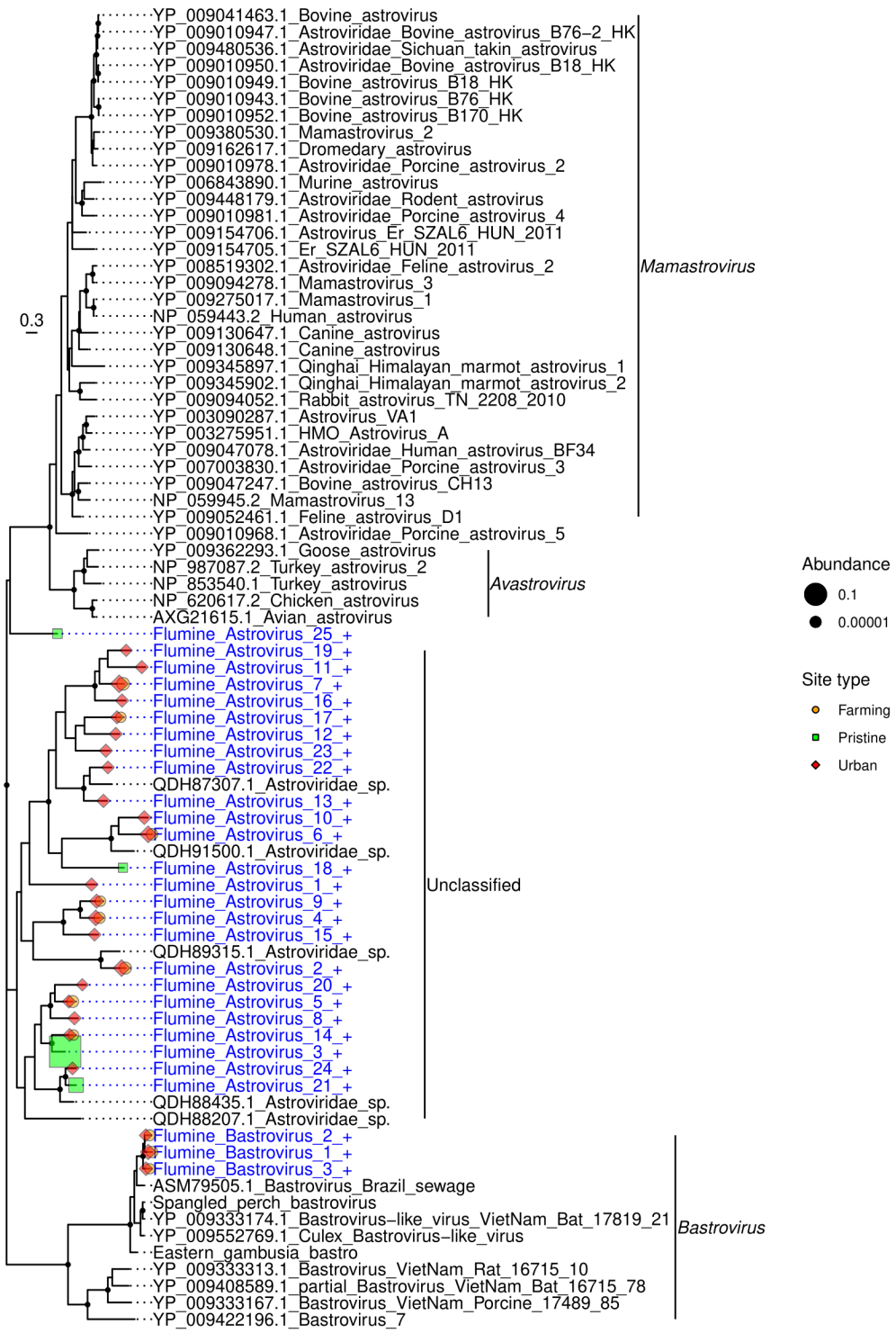
886 **Figure 3**



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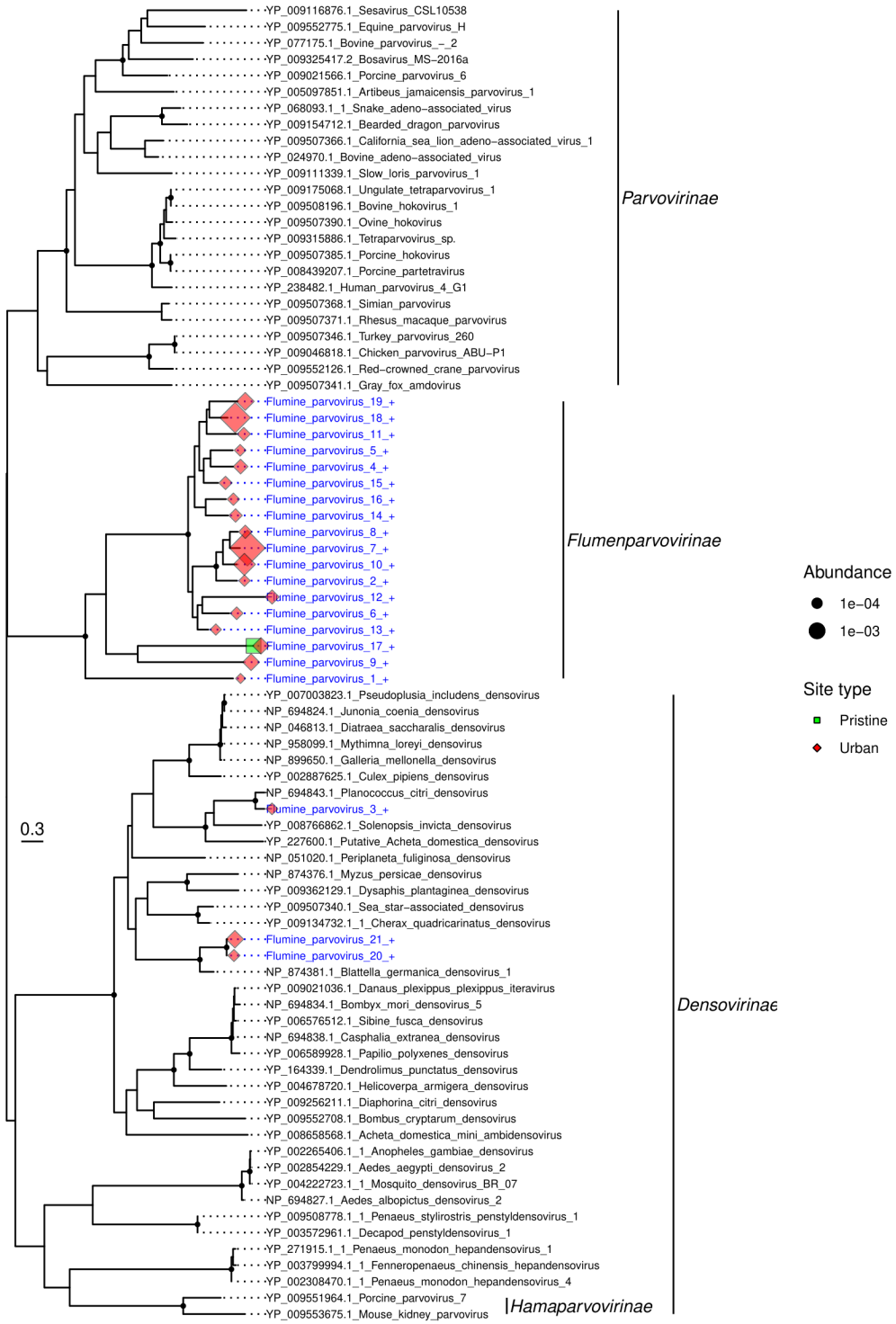
888 **Figure 4**



889

890 **Figure 5**

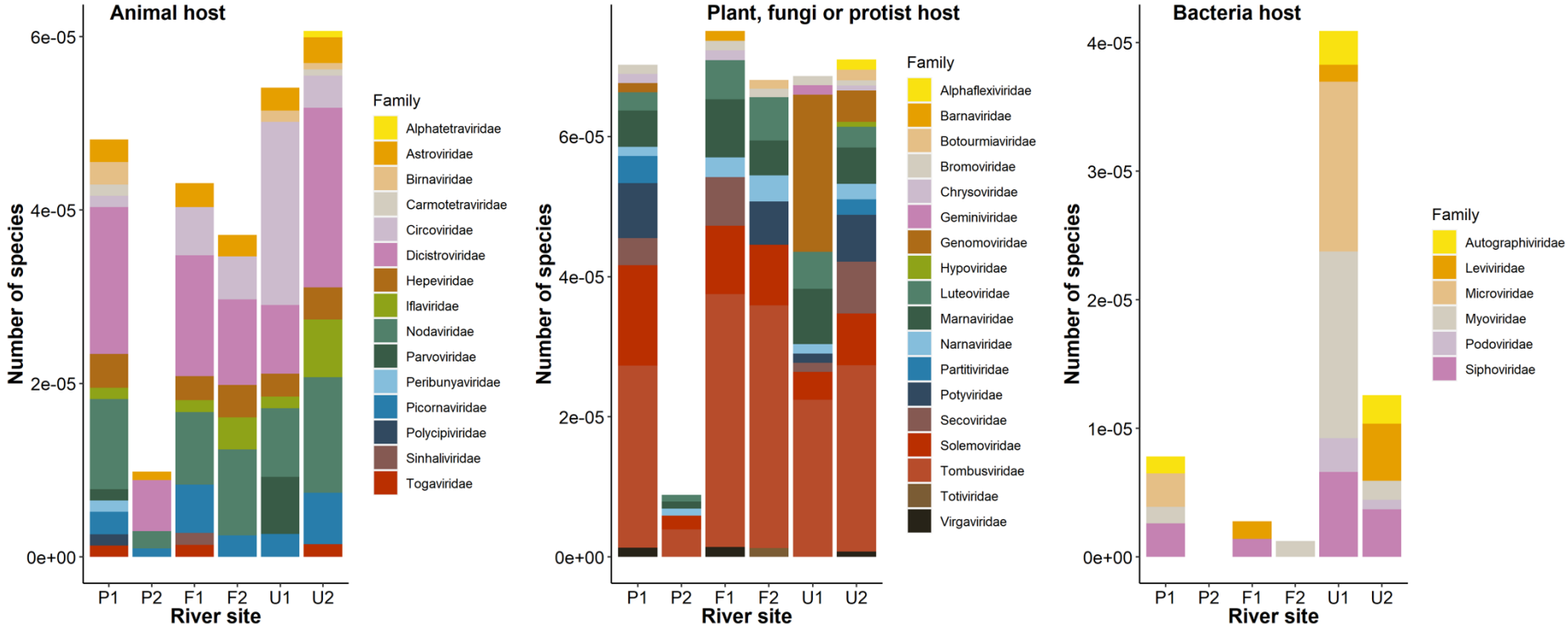
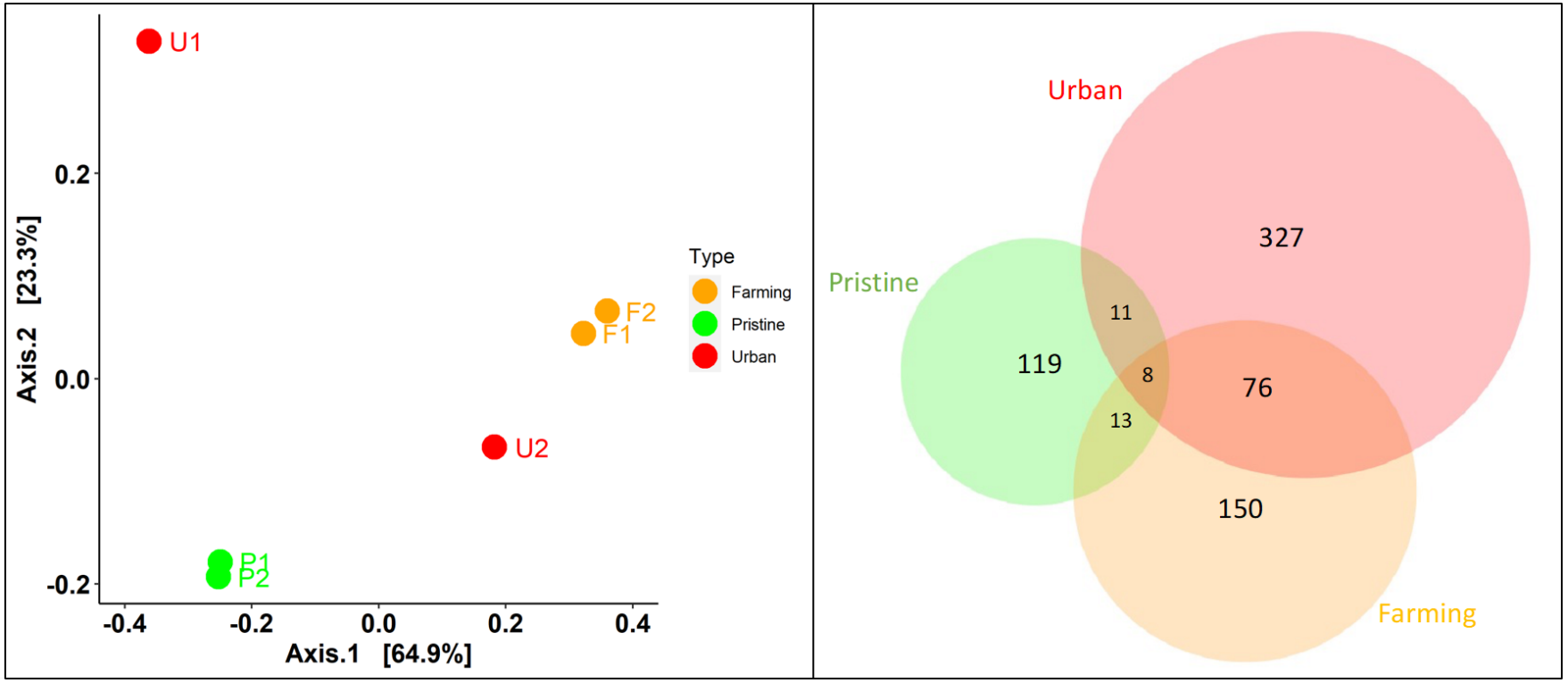

Animal host
Number of species
6e-05
4e-05
2e-05
0e+00
P1
P2
F1
F2
U1
U2
River site
Family
Alphatetraviridae
Astroviridae
Birnaviridae
Carmotetraviridae
Circoviridae
Dicistroviridae
Hepeviridae
Iflaviridae
Nodaviridae
Parvoviridae
Peribunyaviridae
Picornaviridae
Polycipiviridae
Sinhaliviridae
Togaviridae
Plant, fungi or protist host
Alphaflexiviridae
Barnaviridae
Botourmiaviridae
Bromoviridae
Chrysoviridae
Geminiviridae
Genomoviridae
Hypoviridae
Luteoviridae
Marnaviridae
Narnaviridae
Partitiviridae
Potyviridae
Secoviridae
Solemoviridae
Tombusviridae
Totiviridae
Virgaviridae
Bacteria host
4e-05
3e-05
2e-05
1e-05
Autographiviridae
Leviviridae
Microviridae
Myoviridae
Podoviridae
Siphoviridae

Figure 6



893

894 **Figure 7**