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Rodent Virus Diversity and Differentiation across Post-Katrina New Orleans

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Abstract: Concern about elevated disease risk following disasters has been growing with the progression of global trends in urbanization and climate, in part because shifts in socioecological conditions can promote greater human contact with pathogen reservoirs in cities. Remarkably little is known, however, about the diversity and distributions of pathogens carried by commensal reservoirs across disaster-affected urban landscapes. To address this deficit, we characterized the assemblage structure of viruses in the serum of three widespread commensal rodents (*Rattus norvegicus*, *Rattus rattus*, and *Mus musculus*) that were trapped in New Orleans (LA, USA) following Hurricane Katrina. We assessed virus diversity and differentiation according to host species identity, co-occurrence and abundance, as well as prevailing landscape features known to shape urban rodent assemblages. We detected ≥34 viruses in total, including several pathogens of concern, through metagenomic analysis of serum taken from ≥149 individuals of each host species. We found that virus richness as well as assemblage composition and spatial differentiation differed by host species. Notably, we detected associations with host species co-occurrence and abundance, and while we found that assemblage structure varied by study area, we did not detect strong associations with landscape features known to influence rodent hosts. Evidence that virus diversity and assemblage structure reflect host identity more so than other factors indicates that biotic benchmarks might serve as prognostic indicators of post-disaster pathogen exposure risk in cities worldwide.

Keywords: abandonment; disaster; emerging infectious disease; pathogens; surveillance; urban; virome

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1. Introduction

Concern about elevated disease risk following disasters has been growing with the progression of global shifts in urbanization and climate that are putting an increasing proportion of the world's population at risk of experiencing an extreme weather event [1–4]. Risk of disease emergence and transmission can rise immediately following a disaster that results in the acute disturbance of the built or natural environment [4–6]. Risks can also become elevated days to decades after a disaster due to response and recovery efforts that exert equivalent or greater pressures on affected areas [4,6]. Disaster-driven disturbance and responses thereof can, for example, reduce demographic constraints of pathogen reservoirs, which may subsequently become more prevalent [4–8]. Greater prevalence can

increase the possibility of human contact, especially in cities where commensal reservoirs and humans live in close proximity [9]. Disease risk may not necessarily parallel the post-disaster demography of reservoirs, however, because pathogen prevalence can be highly heterogeneous in cities [10–13]. Thus, understanding the diversity and distributions of pathogens harbored by commensal reservoirs can better ensure that disaster response efforts foster human well-being by reducing the risk of pathogen transmission.

Characterizing symbiont communities harbored by prevalent commensal hosts can shed valuable light on zoonotic infectious disease risk following a disaster. Studies focusing on commensal rodents illustrate the merits of pathogen characterization to understand post-disaster disease risk in cities [4,8,10,14]. For example, targeted assays of known rodent-associated bacterial and viral pathogens such as *Bartonella* [12,15], *Rickettsia* [15], Hantaviruses [15] and lymphocytic choriomeningitis virus (LCMV) [16,17] provide evidence of infection hotspots. Targeted assays of known pathogens like *Leptospira* also have demonstrated that infection prevalence can parallel external factors like physiography and host co-occurrence [11,17–19]. Notably, exploratory surveys (e.g., unbiased metagenomic assays) have demonstrated that urban rodents can host more microbial and viral diversity than anticipated [20–23]. Exploratory surveys are also starting to reveal the factors shaping the diversity of rodent-associated pathogens in cities [20,21], suggesting that further characterization of symbiont communities harbored by urban rodent hosts is warranted.

Considerable gains could be made through greater study of rodent-associated viruses, which are increasingly being recognized as public health concerns. The transmission of rodent-associated viruses can result in regional disease outbreaks and global pandemics [24,25]. It is also expected that commensal rodents will increasingly serve as primary agents of emerging zoonotic diseases, due in part to global trends in urbanization [14,25]. Additionally, regions of the world with coincidentally dense human and rodent occupancy—including areas in North America and Europe—are considered to be global hotpots of rodent-associated viral diversity [26], heightening concerns about risk of transmission to humans. Apprehension about transmission risk has motivated targeted surveillance of known viral pathogens [15] as well as exploratory surveys to detect and catalogue viruses [20,21], but little work has thus far been done to determine what factors shape virus assemblage structure in urban rodents [27,28].

Some insights about potential determinants of virus assemblage structure in urban rodents can be derived from efforts to catalog rodent-associated viruses, broad comparative assessments of host-virus associations, and ecological studies of viruses in other vertebrate reservoirs of concern. Exploratory surveys indicate that the diversity and composition of virus assemblages differ among rodent host species [20,21,23,29]. Consistent with this, broad comparative assessments indicate that viral diversity and assemblage structure reflect rodent host phylogeny [30,31], with associations contingent on spatial scale [30]. Some evidence suggests that diversity also relates to ecological factors like host range size and sympatry [31], highlighting the potential for cross-species transmission to influence viral assemblage structure. Consistent with this, cross-species transmission was identified as a key ecological factor structuring fecal viral assemblages in vampire bats [27], which also reflected physiography, host demography and resource availability [27]. Targeted assays of viral pathogens in urban rodents provide further support for the premise that infection can be influenced by host co-occurrence [32–34], although evidence of fine-scale geographic heterogeneity [16,17] points to the possibility that neutral processes like dispersal limitation give rise to distance–decay relationships and spatial aggregation [27,30,35–38].

To gain further understanding of how disasters can influence the risk of pathogen transmission to humans, we undertook a metagenomic study of blood-borne viruses found in commensal rodents collected from across New Orleans (LA, USA) following Hurricane Katrina, which struck the city in late August 2005 [2,4,19]. We examined viral assemblages in populations of three cosmopolitan commensal species—Norway rats (*Rattus norvegicus*), roof rats (*Rattus rattus)*, and house mice (*Mus musculus*)—from study areas that span a mosaic of habitat conditions that have arisen due to Katrina-related flooding, discriminatory

post-disaster resettlement policies [39–42], and municipal differences in post-disaster land management [4,8,43]. We first set out to determine whether viral assemblages (1) differ according to host species; (2) reflect host co-occurrence and abundance; and (3) reflect spatial proximity. We also tested the hypothesis that areas undergoing disaster-driven counter-urbanization harbor more diverse pathogen pools [8,9,44] by determining whether (4) viral richness varies according to the severity of Katrina-related flooding and the extent of abandonment across the study areas (Figure 1).

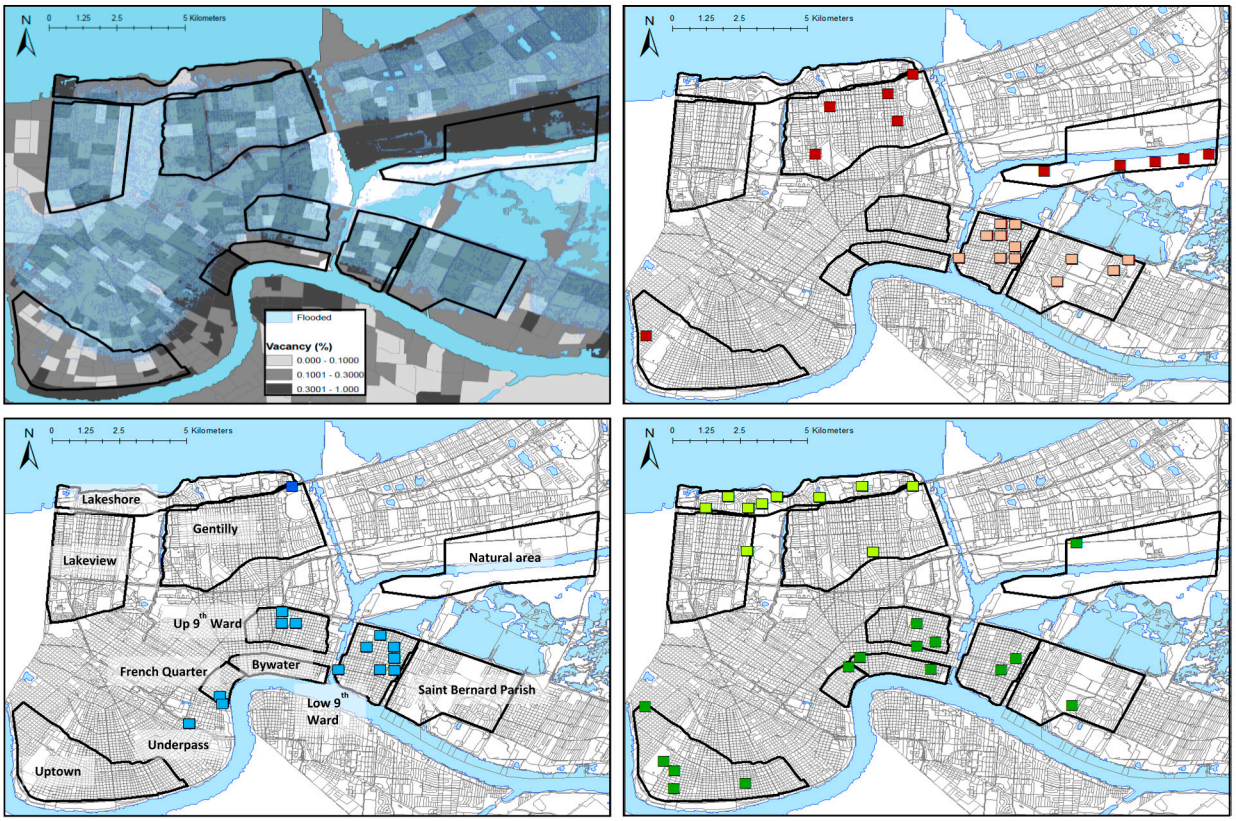

Figure 1. Locations of study areas and trapping sites from which we obtained information of virus assemblages according to the extent of Katrina-related flooding and percent vacancy at the US Census block group level (**top left**) for *M. musculus* (**top right**, red), *R. norvegicus* (**bottom left**, blue) and *R. rattus* (**bottom right**, green) with reference to the street grid. Trapping sites included in spatial analyses are presented in a paler color.

2. Materials and Methods

2.1. Study Areas and Rodent Trapping

We examined blood-borne viruses of rodents that were captured for prior studies [4,8,45] showing that variation in rodent demography, richness, and assemblage structure reflects a mosaic of habitat conditions that have arisen following Hurricane Katrina in 2005 due to flooding, discriminatory post-disaster resettlement policies [39–42], and municipal differences in post-disaster land management [43]. For this study, we examined animals captured at trapping sites located in ten study areas spanning socioecological mosaics encompassing contrasting levels of income, flooding, and post-Katrina landscape management, as follows: eight neighborhoods in the urban footprint of New Orleans, a neighborhood in adjacent St. Bernard Parish, and a nearby undeveloped ("natural") area located within Orleans Parish (Figure 1). All rodents were trapped between May 2014 and February 2017 following Tulane IACUC-approved protocols #0451 and #0460 [8,45]. Rodents were captured using Tomahawk and Sherman traps set on eight to ten randomly selected blocks in each neighborhood and at eight equivalently sized trapping sites in

the nonresidential natural area. Rats included in this study were trapped at a total of 77 sites (Figure 1) across a succession of six alternating summer and winter bouts (Summer 2014-Winter 2016/2017) in the Gentilly, Uptown, Lower 9th, Upper 9th, Bywater, Lakeshore and Lakeview neighborhoods (Figure 1). Rats were trapped in the natural area and French Quarter across a succession of four bouts (Summer 2015–Winter 2016/2017), and a succession of two trapping bouts (Summer 2016–Winter 2016/2017) in St. Bernard Parish. As detailed elsewhere [8,45], 30 Tomahawk live traps (Tomahawk Live Trap, Hazelhurst, WI, USA) were set at each site to capture larger rodents (i.e., rats) during each trapping bout. Mice included in this study were concurrently trapped using an equal number of Sherman traps (H.B. Sherman Traps, Inc., Tallahassee, FL, USA) at 25 sites in five study areas starting in the Summer of 2015 (Figure 1). At each site, all Tomahawk traps were set for a minimum of three continuous nights, with trapping sustained until the trap rate reached an asymptote (i.e., until no individuals were captured). Trapping of small rodents was sustained for four continuous nights, which yielded outcomes statistically indistinguishable from asymptotic trapping [8]. To enable further comparisons, additional rats were collected through opportunistic trapping at one site in the Central Business District (hereafter referred to as the 'Underpass' site) as part of control efforts conducted by the City of New Orleans Mosquito, Termite, Rodent Control Board (NOMTCB) in the Summer of 2014 (Figure 1).

We leveraged information gained from prior work to evaluate how viral assemblage structure relates to trapping site and study area characteristics. As detailed elsewhere [8,43], land use was characterized according to high-resolution Pleiades satellite imagery supported by plot-based estimates of vegetation and ground cover. We also overlaid spatial layers of parcel boundaries onto Google Earth imagery to determine the proportion of vacant lots at each trapping site for each year of the study, with all trapping sites outside of residential areas considered to be 100% vacant [8]. Image-based estimates of vacancy were supported by plot-based assessments of abandonment (unmaintained buildings, unmaintained vegetation, and debris piles) that were conducted over the course of the study period [8].

2.2. Tissue Collection and Specimen Measurements

We euthanized and necropsied all animals using isoflurane anesthesia followed by cardiac puncture according to a standard protocol (Tulane IACUC approved protocols #0451 and #0460) performed at the City of New Orleans Mosquito, Termite Rodent Control Board facility. Blood collected from the cardiac puncture was immediately spun down to separate serum from coagulate. Species, sex, sexual maturity, weight and standardized length measurements were recorded for each animal. We then collected replicate samples of urine as well as lung, liver, kidney, spleen, and tail tissue. All liquid and tissue samples were immediately transferred to a −80 °C freezer for storage until later use.

2.3. Metagenomic Sequencing

We utilized serum samples to characterize blood-borne viruses in 482 rodents, consisting of 160 Norway rats, 173 roof rats, and 149 house mice. Individual samples were combined into 110 pools on the basis of host species and trapping site (Table 1). Following Bergner et al. [27,46], each pool consisted of sera from two to seven individuals (Table 1). Serum samples used in the pools were first centrifuged at 5000 rpm for five minutes to remove cells and particulates. After centrifugation, 120 µL of supernatant from each individual was treated with nuclease enzymes to remove cell-free host nucleic acid, which was followed by nucleic acid extraction using QIAamp Viral RNA Mini Kits (Qiagen) as per the manufacturer's protocol. The nucleic acid extractions from each pool were tagged with a unique barcode variant of the A0 primer (5′-CGTCAAATCCCTCGGTCAG GNNNNNNN-3′) during cDNA and second–strand synthesis, which involved use of Superscript III reverse transcriptase (Thermo Fisher Scientific) and Klenow Exo- polymerase (New England Biolab), respectively. The tagged nucleic acid was amplified with the pool

specific barcoded primer for Illumina library preparation. The PCR product for each pool was size selected to a length of 200-500 base pairs using 1.3X of Axyprep beads (Axygen Scientific) as per the manufacturer's instruction except that elutions used 14 µL of buffer.

Table 1. The number of pools followed by the corresponding number of individuals used in assays of viral diversity and composition in each study area.

Study Area	*M. musculus*	*R. norvegicus*	*R. rattus*	Total
Bywater	0, 0	2, 6	4, 12	6, 18
French Quarter	0, 0	5, 25	1, 4	6, 29
Gentilly	5, 25	2, 10	5, 21	12, 56
Lakeshore	0, 0	0, 0	9, 28	9, 28
Lakeview	0, 0	0, 0	4, 23	4, 23
Lower 9th Ward	14, 71	14, 60	5, 21	33, 152
Natural Area	5, 19	0, 0	3, 17	8, 36
St. Bernard Parish	7, 32	0, 0	1, 4	8, 36
Underpass	0, 0	5, 26	0, 0	5, 26
Upper 9th Ward	0, 0	5, 26	5, 20	10, 46
Uptown	1, 2	2, 7	6, 23	9, 32
Total	32, 149	35, 160	47, 173	110, 482

PCR products were included in libraries at equimolar concentrations and subjected to dA tailing and Illumina adaptor ligation. Adaptor ligated libraries were amplified with Illumina I7 and I5 primers as follows: 98 °C for 30 s; 10 cycles of 98 °C for 15 s, 65 °C for 30 s, 72 °C for 30 s; 72 °C for 5 min; and an extended hold at 10 °C. Libraries were then purified with 0.7× of Axyprep beads (Axygen Scientific) and eluted in 30 µL of buffer. Library size was determined using a High Sensitivity DNA kit on an Agilent BioAnalyzer 2100 instrument (Agilent) and concentrations were measured using a KAPA Library Quantification Kit (Kapa Biosystems)**.** Libraries were sequenced on a HiSeq™ 4000 platform (Illumina) for 2 × 150 cycles at the Institute for Genomic Medicine, Nationwide Children's Hospital (Columbus, OH, USA). Consistent with manufacturer expectations, we estimated that the sequencing error rate—measured according to average % hopping reads—was 1.82% for Norway rat libraries, ranging from 0.82% to 3.94% across all pools. Comparable error rates were estimated for roof rat and house mice libraries. Error estimation was based on a 12 base pair barcode protocol allowing for a one base pair mismatch while binning.

2.4. Bioinformatics

FastQ files were demultiplexed based on each sample's pool-specific barcode present within 25 BP at the 5′ and 3′ ends, allowing for a one base pair mismatch in the barcode search using BBDUK (BBTools). The demultiplexed FastQ files were adapter trimmed using cutadapt v1.8.3, with quality reports generated using FastQC v0.11.5. To verify our pipeline, we also included 100–300 base pair long sequences of 30 different known viruses as positive controls in each quality filtered FastQ file. Demultiplexed and Q30-filtered FastQ files were mapped against each respective host species genome and the Phi X reference genome using Bowtie v2.3.3.1 to determine the host background and Phi X level percentage. All unaligned reads were then clustered to remove duplicate sequences using CD-HIT v4.6.5 software. The resulting unique reads were *de novo* assembled using MIRA v 4.0 assemblers. Contigs and unassembled reads were first analyzed using BLASTN [BLAST 2.7.1+], with an e-value cutoff of $1e^{-8}$ against the GenBank nucleotide database. Sequences that remained unclassified were then screened with BLASTX against the GenBank viral protein database [23,46]. All sequences were also analyzed using DIAMOND v0.9.13.114 with comparisons drawn to a nonredundant protein database. All sequences classified as a virus from DIAMOND were again processed using a BLASTX [BLAST 2.7.1+] protein alignment with an e-value cutoff of 0.01 against a nonredundant NCBI database for classification to the lowest taxonomic unit possible [23,46]. Final taxonomy reports were

generated by combining BLASTn and BLASTx virus classification entries for pool-wise comparisons [23,46].

2.5. Statistical Analyses

Prior studies [27,46] have demonstrated that low-to-moderately diverse viral assemblages can be confidently characterized (i.e., where detection plateaus with the community approaching or achieving saturation) at the sequencing depths that we attained in this study, allowing for representative comparisons of virome composition. We nonetheless accounted for potential differences due to variation in the number of individuals included in each pool by (1) standardizing values according to read count; and (2) rarefying values recovered for all pools relative to the pools with the smallest numbers of individuals [47], despite concerns that rarefaction involves discarding potentially informative data [48]. As comparisons revealed that the two approaches yielded qualitatively similar results, we have elected to present those based on rarefication. We first visualized rarified assemblage composition according to host species by plotting the first two principal components of $\log_2$ rarefied virus assemblages. We also visualized rarified assemblage composition from only the trapping sites for which we obtained data from more than one host species. We then conducted a PERMANOVA [49] with 999 permutations to determine the extent to which host species explains the sum of squared variance in composition. We conducted a second PERMANOVA utilizing only data from locations with data from more than one host species to determine whether variance was explained by species, trapping site and a species x trapping site interaction term.

We took several approaches to assess socioecological predictors of rarified assemblage composition. First, we utilized a linear regression model and post-hoc comparisons with Tukey's p-value corrections to determine whether virus richness differed among host species and whether virus richness differed according to site-level host species abundance as well as measures of vacancy and abandonment, such as flooding depth, the proportion of vacancy and unmaintained vegetation at a trapping site [8]. For this analysis, we standardized richness estimates by taking the natural log of rarefied richness divided by the total number of assayed animals of a given species. Separate models were constructed using different measures of abundance as follows: individual measures of average abundance for each host species across all trapping seasons; individual measures of total abundance for each host species across all trapping seasons; total abundance of all host species trapped at a site, with the analysis restricted to the subset of sites where rats and mice were trapped; and likewise, total abundance of all rats at a site, with the analysis restricted to the subset of sites where only rats were trapped. To further explore relationships between viral richness and rodent host species co-occurrence, we compared standardized viral richness of all individuals in a pool and the number of rodent hosts present at a site, focusing only on locations where we had trapping data available for rats and mice. We did so using an ANOVA of natural log transformed richness estimates that were standardized to account for differences in sample sizes among locations. We standardized richness estimates by dividing rarefied richness by the total number of animals of a given species from each site for which virus data was available.

We conducted separate Mantel tests for each species to determine whether viral assemblages exhibited greater dissimilarity with increasing geographic distance between trapping sites. Due to the patchiness in trapping site locations (Figure 1) [8], we restricted these analyses to data from specimens captured across contiguous study areas to limit the potential influence of outlier sites and landscape features (e.g., waterways) that can result in sharp discontinuities [50]. Accordingly, we examined spatial differentiation of viral assemblages in house mice from the Lower 9th Ward and St. Bernard Parish neighborhoods. For roof rats, we examined viral assemblages in animals from the Lakeview, Lakeshore and the Gentilly neighborhoods, and for Norway rats, we examined viral assemblages in animals from the French Quarter, Lower 9th and Upper 9th Ward neighborhoods (Figure 1). We plotted the correlation coefficients between the Bray–Curtis dissimilarity matrix of

rarefied viral assemblages and the geodesic distance matrix based on latitude and longitude coordinates representing the centroid of each trapping site, as well as scatterplots of the dissimilarity matrix relative to geodesic distance.

Unless otherwise noted, all statistical analyses were completed in R with the vegan, glmmTMB, ecodist, and multcomp packages [51–54].

3. Results

3.1. Viral Richness

Metagenomic sequencing of the 110 pools encompassing 482 specimens (Table 1) recovered a total of 443.78 M reads, yielding an average of 4.03 M reads per pool. We detected a total of 46 viruses across all pools (Table S1). Rarefaction removed singleton and very low frequency viruses from further consideration (Table 2), resulting in an inventory of 34 viruses including 19 detected in Norway rats, 14 detected in roof rats, and 13 detected in house mice (Table 2). Each pool contained an average of 4.2 (range, 1–9), 3.0 (range, 1–6), and 2.1 (range, 1–4) viruses in Norway rats, roof rats, and house mice, respectively (Table 2). Compiling across species-specific pools, the total richness of viruses at individual trapping sites averaged 4.25 viruses (range, 2–7) in Norway rats; 2.3 viruses (range, 1–3) in roof rats; and 2.5 viruses (range, 1–3) in house mice. Rarified measures of total virus richness in the study areas ranged from 1.6 viruses at sites in St. Bernard to 5.3 viruses at sites in the French Quarter. Differences in viral richness across study areas were statistically significant when considering all host species together (Kruskal–Wallis chi-squared = 25.637, df = 10, p-value = 0.004261), although there were no significant pairwise differences among neighborhoods (Kruskal–Wallis rank sum test with Wilcoxon pairwise comparisons, all $p > 0.05$). Likewise, there were no significant differences among neighborhoods when considering each host species separately (Kruskal–Wallis rank sum test with Wilcoxon pairwise comparisons, all $p > 0.05$; Figure 2).

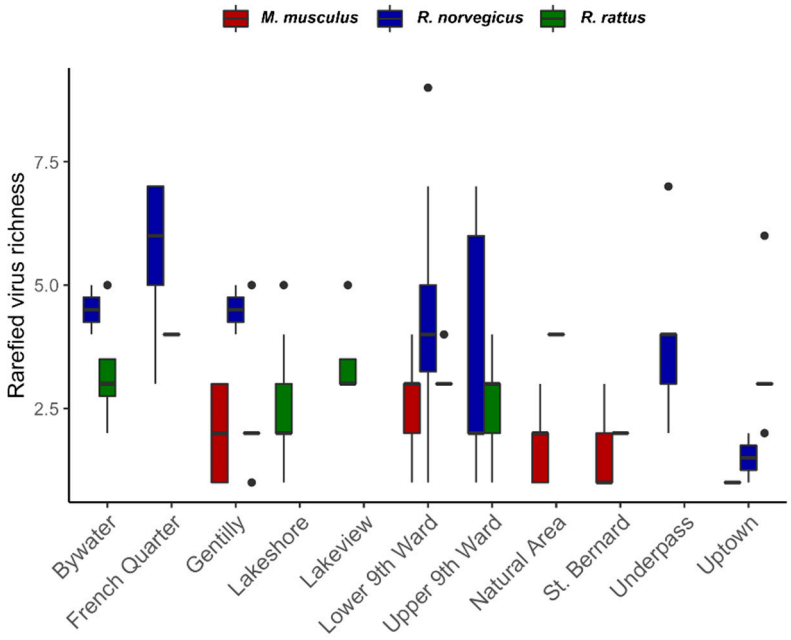

Figure 2. Standardized rarefied virus richness by species and study area.

Table 2. Post-rarefaction list of blood-borne viruses detected in *Mus musculus*, *Rattus norvegicus*, and *Rattus rattus* collected across New Orleans, presented as the number of positive pools, followed by the percentage of positive pools in parentheses. *n* = total number of pools.

Virus	*M. musculus* (*n* = 32)	*R. norvegicus* (*n* = 35)	*R. rattus* (*n* = 43)
Anelloviridae sp.	0 (0)	14 (40)	40 (93)
Beihai picobirna-like virus 8	3 (9)	0 (0)	0 (0)
H1 parvovirus	1 (3)	1 (3)	1 (2)
Lymphocytic choriomeningitis virus	2 (6)	0 (0)	0 (0)
Mouse kidney parvovirus	5 (16)	0 (0)	0 (0)
Mouse parvovirus 1	10 (31)	0 (0)	0 (0)
Murine adeno-associated virus (new)	6 (19)	0 (0)	0 (0)
Murine adeno-associated virus 1	1 (3)	0 (0)	0 (0)
Murine adenovirus 1	2 (6)	0 (0)	0 (0)
Murine bocavirus	1 (3)	0 (0)	0 (0)
Murine cytomegalovirus strain K181	10 (31)	0 (0)	0 (0)
Murine leukemia virus	21 (66)	0 (0)	0 (0)
Mus musculus polyomavirus 2	5 (16)	0 (0)	0 (0)
Rat adeno-associated virus	0 (0)	2 (6)	0 (0)
Rat bocavirus	0 (0)	1 (3)	1 (2)
Rat cytomegalovirus	0 (0)	1 (3)	2 (5)
Rat kobuvirus MM33	0 (0)	1 (3)	0 (0)
Rat minute virus	0 (0)	12 (34)	2 (5)
Rat minute virus 1b	0 (0)	3 (9)	0 (0)
Rat minute virus 1c	0 (0)	5 (14)	0 (0)
Rat minute virus 2a	0 (0)	1 (3)	0 (0)
Rat parvovirus 1a	0 (0)	2 (6)	0 (0)
Rat parvovirus NTU1	0 (0)	2 (6)	3 (7)
Rattus norvegicus papillomavirus 1	0 (0)	1 (3)	0 (0)
Rodent bocavirus	1 (3)	0 (0)	0 (0)
Rodent hepacivirus	0 (0)	15 (43)	2 (5)
Rodent pegivirus	0 (0)	27 (77)	0 (0)
Rodent torque teno virus 1	0 (0)	21 (60)	35 (81)
Rodent torque teno virus 2	0 (0)	26 (74)	10 (23)
Rodent torque teno virus 3	0 (0)	10 (29)	18 (42)
Torque teno mini virus 4	0 (0)	0 (0)	1 (2)
Torque teno mini virus 5	0 (0)	0 (0)	4 (9)
Torque teno virus	0 (0)	0 (0)	4 (9)
Torque teno virus-like mini virus	0 (0)	3 (9)	6 (14)

We detected a number of viruses known to infect commensal rodents (Table 2), including viruses that can be pathogenic to rodent hosts and humans. Torque teno viruses were among the most prevalent viruses found in the sampled individuals (Table 2 and Table S1). Mouse kidney parvovirus, which can compromise the health of rodent hosts, was detected in house mice. Murine leukemia virus, which can also be problematic to rodent hosts, was among the most prevalent viruses detected in house mice (Table 2 and Table S1). Notably, a new murine adeno-associated virus was discovered in house mice (Table 1 and Table S1), and a rodent hepacivirus was detected in a large proportion of Norway rats assayed for this study (Table 2 and Table S1). We also detected Lymphocytic choriomeningitis virus (LCMV)—known to be pathogenic in humans—at relatively low prevalence in house mice. It was recovered in 6% of the house mice pools (Table 2), which were composed of individuals collected from one trapping site in the Lower 9th Ward and from one trapping site in the Gentilly neighborhood.

3.2. Correlates of Viral Richness and Differentiation

We found that standardized viral richness significantly differed among host species, with Norway rats exhibiting greater richness than house mice (coef = 0.82, $p < 0.01$) but not roof rats. We also found that roof rats supported greater viral richness than house mice

(coef = 0.67, $p < 0.01$). Though locations with more host species exhibited relatively higher viral richness, the difference was not statistically significant (coef = 0.17292, $p = 0.11$). The richness of viruses detected in Norway rats was, however, higher at sites of co-occurrence with roof rats (coef = 5.32, $p = 0.029$; Figure S1), although the richness of viruses in roof rats did not differ according to co-occurrence with Norway rats (coef = 0.044, $p = 0.83$; Figure S1). We did not recover significant relationships between viral richness and total or average abundance of individual species (Figure S2). Notably, we did recover a significant relationship between viral richness and total abundance of all rodents at sites where we trapped rats and mice, (coef = 0.0004, $p = 0.045$). A similar but nonsignificant relationship was detected at sites where we only trapped rats (coef = 0.006, $p = 0.08$). Notably, we did not detect relationships between viral richness and vacancy (coef = 0.008, $p = 0.98$) or flooding depth (coef = −0.014, $p = 0.319$) at trapping sites.

Viral assemblage composition significantly differed by host species. Differences among host species were evident when viral assemblage composition was visualized for all trapping sites (Figure 3). When considering data from all rodents from all locations included in this study, we found that most of the variation in viral assemblage composition was explained by host species identity ($R^2 = 0.43$, $p = 0.001$). Viral assemblage composition also differed by study area when considering all species together ($R^2 = 0.08$, $p = 0.01$) and separately for house mice ($R^2 = 0.21$, $p = 0.035$) as well as for Norway rats ($R^2 = 0.28$, $p = 0.024$) but not for roof rats ($R^2 = 0.24$, $p = 0.23$). We additionally recovered a significant species x study area interaction for all species ($R^2 = 0.06$, $p = 0.043$). The distinctiveness of viral assemblage composition by species ($R^2 = 0.40$, $p = 0.001$) also was apparent at the subset of trapping sites for which we obtained data from more than one host species (Figure 4). Site ($R^2 = 0.15$, $p = 0.456$) and species x site interactions ($R^2 = 0.22$, $p = 0.371$) were not significant, however, when restricting analyses to trapping sites from which we had data from more than one species.

The extent of geographic variation in viral assemblage composition differed by host species. Mantel tests revealed that relationships between viral assemblage dissimilarity and spatial distance varied according to host species (Figure 5). We did not find a significant relationship between spatial distance and assemblage dissimilarity for house mice (all $p > 0.26$, mantel R = 0.04) and Norway rats (all $p > 0.39$, mantel R = −0.03; Figure 5). Roof rats collected from more proximate locations, however, exhibited more similar viral assemblages than those that were geographically farther apart, and those farther apart generally exhibited more different viral assemblages (mantel R = 0.25; Figure 5). Similarity of viral assemblages in roof rats emerged over distances of ≤50 m, corresponding to a significant positive correlation in the corresponding mantel correlogram, whereas dissimilarity emerged over distances of ≥6 km, corresponding to a significant negative correlation in the correlogram (Figure 5).

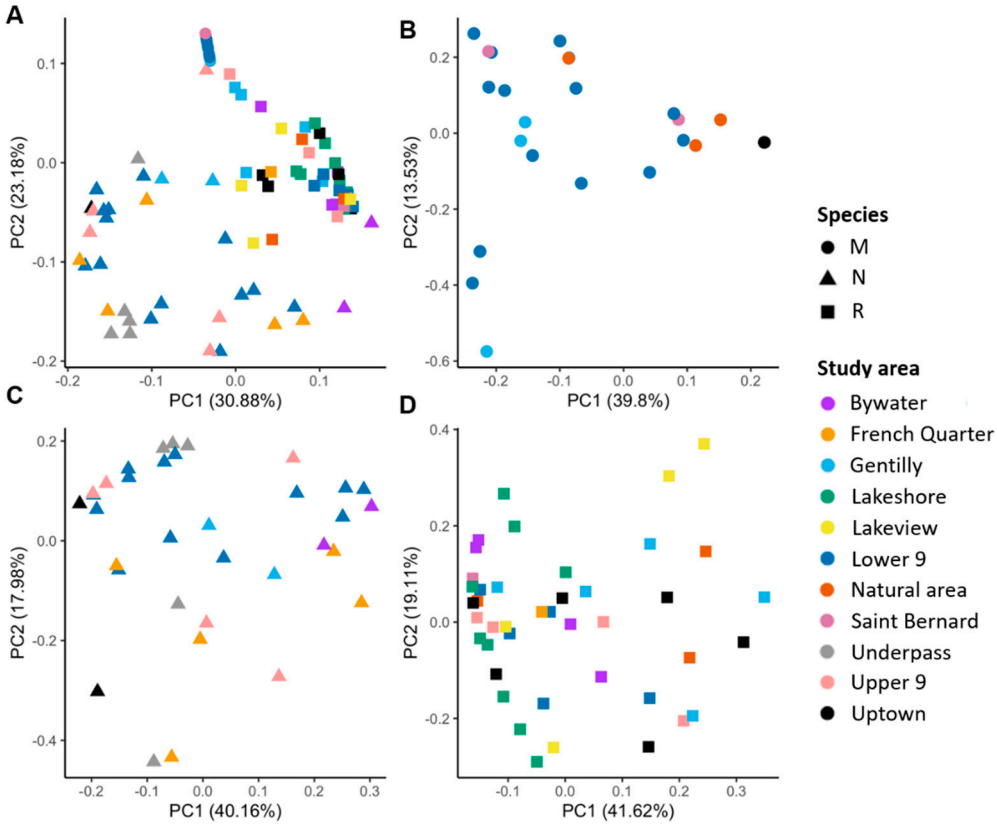

Figure 3. PCA plot of $\log_2$ transformed rarefied virus assemblages by species and study area. (**A**) All species combined; (**B**) *Mus musculus* (M); (**C**) *Rattus norvegicus* (N); (**D**) *Rattus rattus* (R).

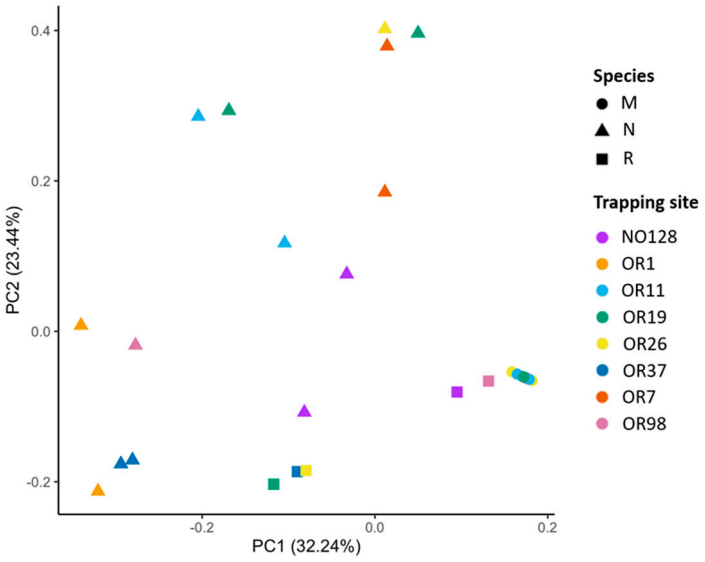

Figure 4. PCA plot of $\log_2$ transformed rarefied virus assemblages from each host species collected at trapping sites with data for more than rodent species (*Mus musculus* (M); *Rattus norvegicus* (N); *Rattus rattus* (R)).

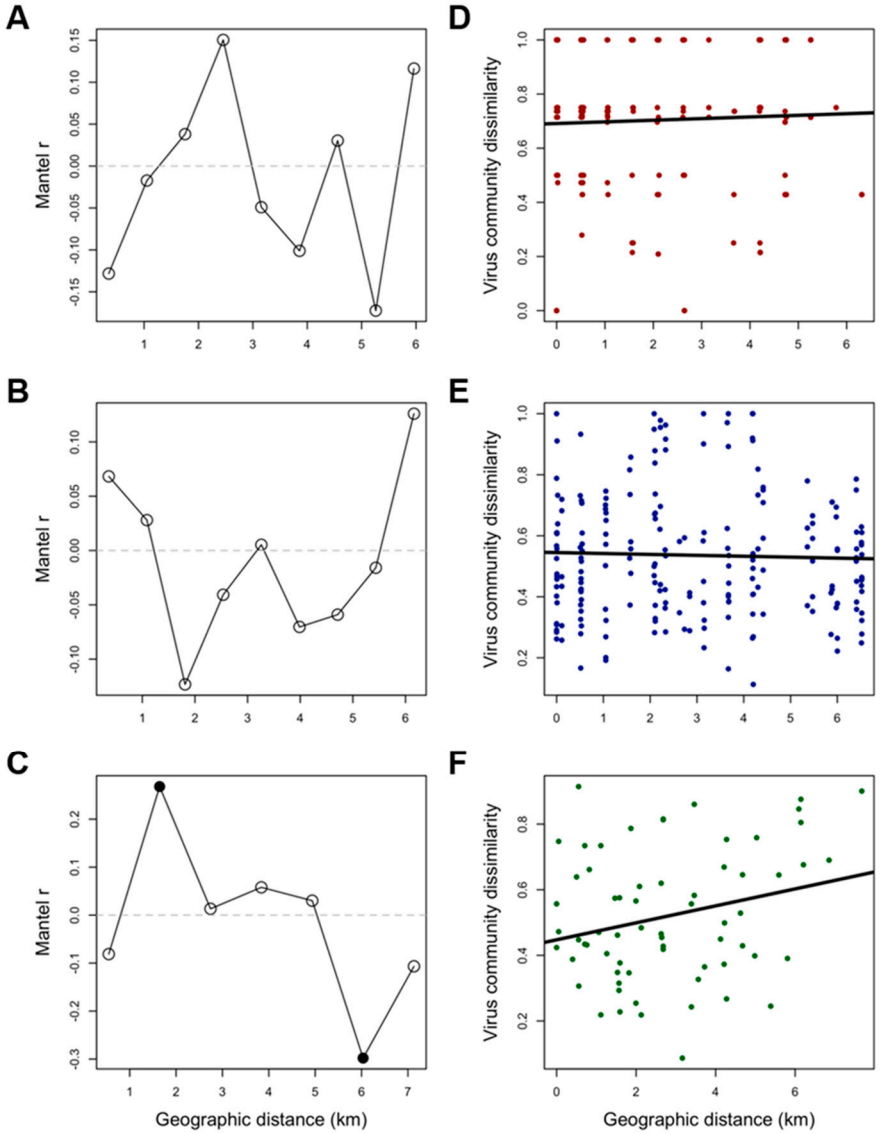

Figure 5. Mantel correlograms and scatterplots of virus assemblage dissimilarity by geographic distance for *Mus musculus* (**A**,**D**), *Rattus rattus* (**B**,**E**), and *Rattus norvegicus* (**C**,**F**) from the study sites identified in Figure 1. Filled circles (**A**–**C**) represent distances with significantly positive or negative correlation with community composition at a given spatial lag.

4. Discussion

In this study, we examined blood-borne viral assemblages within and among three cosmopolitan rodent hosts trapped across post-Katrina New Orleans in order to shed further light on how disasters can influence the risk of pathogen transmission to humans. Assays of serum can serve as a powerful lens for detecting viruses in rodents, including pathogens of concern including LCMV and Hantaviruses like the Seoul virus [55], which can cause hemorrhagic fever with renal syndrome in humans. We assessed the extent to which blood-borne viral assemblages reflect host identity, co-occurrence, and abundance as well as geographic distance and landscape features that structure rodent diversity and distributions in the city [8]. Our results indicate that rodent viral assemblages are primarily defined by host identity. We found significant differences in viral richness and little compositional overlap according to rodent host species, even when comparing viral assemblage composition in different species captured at the same trapping site. Consistent with this, we found that spatial variation in viral assemblage composition differed by host species. These results parallel evidence from studies of other rodent-associated

viral and symbiont communities, like gut microbiota, that highlight the importance of host identity [23,56]. Indications that viral assemblages reflect features of rodent host assemblages like host co-occurrence and overall abundance support the premise that cross-species transmission structures viral assemblages [27,31]. Notably, landscape features known to structure urban rodent assemblages do not appear to exert a strong influence on associated viral assemblages. Further comparisons of host-to-viral assemblage structure over time, as well as within and among cities, are nonetheless warranted as pathogen pool diversity and human activities are key risk factors in predictions of zoonotic disease outbreaks [57–59].

4.1. Patterns of Viral Richness

Consistent with profiles of viruses in commensal rodents elsewhere [20,22,23], we found that Norway rats harbor a more diverse viral assemblage relative to assemblages in roof rats and house mice. Differences among host species could reflect innate variation in tolerance to sustain infections [60], although it might also reflect ecological differences in infection risk attributable to host-specific habitat use, diet, or some combination thereof [27,61,62]. The observed differences in viral richness might, however, be a legacy of past events and conditions that have shaped host demography and distributions across the city [4,8]. For example, viral richness might differ according to invasion history because all three species have been introduced to New Orleans [63]. Introduced species tend to support depauperate symbiont communities compared to source or native range populations [64], which raises the possibility that differences in richness could reflect time since invasion, founding population size, the number of introductions, and provenance. Similarly, we posited that the richness of viral symbionts in extant populations might differ according to variation in the severity of flood-driven (Figure 1) population decline and the nature of demographic recovery following Hurricane Katrina (e.g., in situ expansion versus immigration from unflooded areas) [45,65]. Contrary to expectations, we did not detect evidence that viral richness differs according to flooding history (i.e., depth or severity of flooding) of the sites included in this study. Broadening comparisons to include additional sites across New Orleans as well as sites within other cities could shed further light on how macroecology and demography [12,30,31,37,45,63] influence viral richness in commensal rodent hosts.

4.2. Patterns of Viral Assemblage Structure

There was little compositional overlap according to host identity (Figure 3), even at sites harboring more than one host species (Figure 4). Prior studies similarly indicate that commensal rodent hosts carry distinct bacterial symbionts (e.g., Bartonella, Leptospira) across New Orleans. Some indications suggest, however, that cross-species transmission influences the structure of blood-borne viral assemblages in urban populations of commensal rodents. We found that Norway rats, for example, exhibit elevated viral richness at sites that also harbor roof rats. The reverse was not found, suggesting that the potential for transmission is asymmetric. Consistent with this, signatures of directional transmission have been found for bacterial symbionts among co-occurring rodent host species [12,19]. We also detected several viruses in multiple host species, which is consistent with trends indicating that viruses exhibit less host specificity than bacterial symbionts [31]. Several torque teno viruses (TTVs), for instance, were the most frequently detected viruses in both Norway rats and roof rats (Table 2 and Table S1). Prior surveys of other study areas have likewise detected TTVs in both species of commensal rats [23], and as in surveys of M. musculus in New York City and elsewhere [21,66], we did not detect TTVs in M. musculus assayed for this study. Parallels like this highlight the value of characterizing the prevalence of TTVs and other viruses carried by multiple hosts [31,66,67] to understand the relative influence of host specificity in governing viral assemblage structure. Greater understanding of the distribution and abundance of urban rodents e.g., [8] could offer additional insight into the potential for transmission among co-occurring host species.

The compositional distinctiveness of rodent viral assemblages (Figures 3 and 4) appears to partly reflect the heterogeneous distribution of relatively low frequency viral taxa. The known pathogen LCMV, for example, was found in just 6% of the M. musculus pools (Table 2), which included individuals collected from one site in the Lower 9th Ward and from one site in the Gentilly neighborhood. This pattern is similar to those found in prior studies of LCMV in other areas [68], including Baltimore where LCMV infections were found to be highly heterogeneous (range, 0–50%) among nearby households [16]. The distribution of LCMV in New Orleans thus appears to provide further support for the premise that geographic heterogeneity in pathogen infection may be the norm in urban rodent populations and assemblages, even on relatively small spatial scales e.g., [10–13]. Nonetheless, the occurrence of low-frequency viruses like LCMV (Table 2 and Table S1) could in part reflect shifts in rodent assemblage structure driven by Katrina-related flooding and post-Katrina differences in municipal landscape management (Figure 1) [8,43]. More insight about the distribution of low frequency viruses might thus be gained from further sampling across discrete socioecological transitions as well as assays based on individual specimens and greater sequencing depths [46–48].

Notably, our work revealed that signatures of spatial variation in viral assemblage structure differed by host species. Although studies of free-living bacteria suggest that distance decay is not a universal feature of microbial communities [69], we expected that blood-borne viral assemblages would exhibit distance-dependent structure like the pattern observed in the rodent host assemblage in New Orleans [8]. Yet signatures of spatial structure were only found for viral assemblages in roof rats (Figure 5). The observed differences might in part reflect host-specific variation in viral metacommunity dynamics [30,70]. It is also possible that the spatial scale of transmission differs for each host species, with transmission being more localized in roof rats than either Norway rats or house mice (Figure 5). This may be due to aggregate differences in the route(s) of virus transmission (e.g., environmental, direct contact, etc.) as well as differences in host dispersal, where hosts with lower dispersal ability exhibit more pronounced patterns of distance decay in pathogen community similarity [71]. If so, then our findings would suggest that roof rats are more dispersal limited than either Norway rats or house mice. This hypothesis could be tested through comparisons of direct measures of movement potential or indirect measures of dispersal such as population genomic variation. For example, comparisons of genomic variation suggest that white-footed mice are more dispersal limited than Norway rats in New York City [72,73]. It is also possible that viral assemblages in some, but not all, rodent hosts are saturated within the city, which would yield inconsistent patterns of spatial variation with little association with host dispersal ability [71].

4.3. Predictors of Viral Diversity

We found some indications that viral assemblages reflect features of rodent host assemblages that foster cross-species transmission. Some species-specific measures of viral richness differed according to host co-occurrence (Figure S1), for example, which is consistent with evidence that bacterial symbionts may be transmitted at sites where rodent hosts co-occur [12]. It is also consistent with observations that the richness of bacterial and other symbionts can scale with host richness [19,74–76] including evidence that the infection prevalence of particular groups of viruses (e.g., Hantaviruses) can decline with rodent host diversity [32,34]. While these parallels are intriguing, further work is warranted to clarify the extent to which rodent hosts and blood-borne viruses exhibit relationships akin to those observed in other host-symbiont systems. It would be prudent to conduct studies that aim to rectify elements of our study design that possibly limited detection of relationships. For example, our analyses are based on collections made years following Hurricane Katrina. We also were unable to evaluate the full complement of rodent diversity found across the study region [19]. Accordingly, examining relationships during the immediate aftermath of the disaster or comparing sites that capture a larger range of host species richness might have provided stronger evidence of scaling. While

this would be a worthwhile endeavor, it should be noted that host–symbiont relationships may have been disrupted by Katrina-related flooding and subsequent efforts to rebuild the city [43], as even well-established relationships can be diminished by anthropogenic activity [77].

We found that rodent-associated viral richness corresponds to some measures of host abundance but not landscape features known to shape rodent demography and assemblage structure in New Orleans [8,45]. As has been suggested for other vertebrate hosts that form colonies (e.g., vampire bats) [27], viral richness can potentially trend with host abundance if transmission is density dependent. Evidence that viral richness corresponds to total rodent host and rat host abundance suggests that this could be the case with blood-borne viruses in rodents, but it may be a stronger feature of other viral assemblages (e.g., faecal assemblages) that are more likely to be subject to density-dependent transmission (i.e., via direct contact) [27]. Likewise, other viral assemblages might align more closely with measures of disaster-driven abandonment across the city. It has been hypothesized that counter-urbanizing areas can support a larger number and more diverse complement of hosts and associated pathogen pools [4,9,44]. Although our findings do not support this hypothesis, other work has shown that rodents are more abundant and more diverse in areas of New Orleans that are burdened with greater disaster-driven abandonment [8,45]. A positive relationship also has been found between rodent diversity and Leptospira infection across New Orleans [8]. Further study to explore possible linkages between prevailing environmental conditions, hosts, and viral assemblages would thus be worthwhile. It would be particularly valuable to test whether life history (e.g., individual age and reproductive status, age composition of a population) mediates the influence of resource availability on viral assemblages [27,65,78]. It would also be prudent to draw comparisons among co-hosted assemblages (e.g., blood-borne versus fecal) [27], which could reveal other key contingencies like transmission route. This could shed further light on why the diversity of viral infections in rodents does not necessarily translate to greater risk of pathogen transmission to humans [76]. Disease risk might instead correspond more to the distribution and prevalence of particular constituents of concern that more closely align with socioecological conditions. Reconstructing chronologies, anchored by comparisons conducted during the immediate aftermath a disaster, would likely offer a stronger basis for differentiating between disturbance-driven and response-driven pressures on host–virus relationships. Accordingly, deconstructing observed patterns of host–virus relationships into constituent elements could help identify key risk factors associated with pathogens of concern, clarifying whether and how disaster-driven counter-urbanization poses a risk to resident communities [79,80].

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su13148034/s1, Figure S1: Standardized rarefied virus richness. Figure S2: Linear regressions of rarified viral richness. Table S1: Non-rarified list of blood-borne viruses.

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References

1. Small, C.; Nicholls, R.J. A global analysis of human settlement in coastal zones. *J. Coast Res.* **2003**, *19*, 584–599.
2. Elsner, J.B.; Kossin, J.P.; Jagger, T.H. The increasing intensity of the strongest tropical cyclones. *Nature* **2008**, *455*, 92–95. [CrossRef]
3. Leaning, J.; Guha-Sapir, D. Natural disasters, armed conflict, and public health. *N. Engl. J. Med.* **2013**, *369*, 1836–1842. [CrossRef]
4. Rael, R.C.; Peterson, A.C.; Ghersi, B.M.; Childs, J.; Blum, M.J. Disturbance, reassembly, and disease risk in socioecological systems. *Ecohealth* **2016**, *13*, 450–455. [CrossRef]
5. Ostfeld, R.S.; Holt, R.D. Are predators good for your health? Evaluating evidence for top-down regulation of zoonotic disease reservoirs. *Front. Ecol. Environ.* **2004**, *2*, 13–20. [CrossRef]
6. Watson, J.T.; Gayer, M.; Connolly, M.A. Epidemics after natural disasters. *Emerg. Infect. Dis.* **2007**, *13*, 1–5. [CrossRef] [PubMed]
7. Wilcox, B.A.; Gubler, D.J. Disease ecology and the global emergence of zoonotic pathogens. *Environ. Health Prev. Med.* **2005**, *10*, 263. [CrossRef]
8. Peterson, A.C.; Ghersi, B.M.; Campanella, R.; Riegel, C.; Lewis, J.A.; Blum, M.J. Rodent assemblage structure reflects socioecological mosaics of counter-urbanization across post-Hurricane Katrina New Orleans. *Landsc. Urban Plan.* **2020**, *195*, 103710. [CrossRef]
9. Eskew, E.A.; Olival, K.J. De-urbanization and zoonotic disease risk. *Ecohealth* **2018**, *15*, 707–712. [CrossRef] [PubMed]
10. Himsworth, C.G.; Parsons, K.L.; Jardine, C.; Patrick, D.M. Rats, cities, people, and pathogens: A systematic review and narrative synthesis of literature regarding the ecology of rat-associated zoonoses in urban centers. *Vector Borne Zoonotic Dis.* **2013**, *13*, 349–359. [CrossRef]
11. Himsworth, C.G.; Bidulka, J.; Parsons, K.L.; Feng, A.Y.T.; Tang, P.; Jardine, C.M.; Kerr, T.; Mak, S.; Robinson, J.; Patrick, D.M. Ecology of *Leptospira interrogans* in Norway rats (*Rattus norvegicus*) in an inner-city neighborhood of Vancouver, Canada. *PLoS Negl. Trop Dis.* **2013**, *7*, e2270. [CrossRef]
12. Peterson, A.C.; Ghersi, B.M.; Alda, F.; Firth, C.; Frye, M.J.; Bai, Y.; Osikowicz, L.M.; Riegel, C.; Lipkin, W.I.; Kosoy, M.Y.; et al. Rodent-borne *Bartonella* infection varies according to host species within and among cities. *Ecohealth* **2017**, *14*, 1–12. [CrossRef] [PubMed]
13. Rothenburger, J.L.; Himsworth, C.H.; Nemeth, N.M.; Pearl, D.L.; Jardine, C.M. Environmental factors and zoonotic pathogen ecology in urban exploiter species. *Ecohealth* **2017**, *14*, 630–641. [CrossRef]
14. Meerburg, B.G.; Singleton, G.R.; Kijlstra, A. Rodent-borne diseases and their risks for public health. *Crit. Rev. Microbiol.* **2009**, *35*, 221–270. [CrossRef]
15. Himsworth, C.G.; Bai, Y.; Kosoy, M.Y.; Wood, H.; DiBernardo, A.; Lindsay, R.; Bidulka, J.; Tang, P.; Jardine, C.; Patrick, D. An investigation of *Bartonella* spp., *Rickettsia typhi*, and Seoul hantavirus in rats (*Rattus* spp.) from an inner-city neighborhood of Vancouver, Canada: Is pathogen presence a reflection of global and local rat population structure? *Vector Borne Zoonotic Dis.* **2015**, *15*, 21–26. [CrossRef] [PubMed]
16. Childs, J.E.; Glass, G.E.; Korch, G.W.; Ksiazek, T.G.; Leduc, J.W. Lymphocytic choriomeningitis virus infection and house mouse (*Mus Musculus*) distribution in urban Baltimore. *Am. J. Trop. Med. Hyg.* **1992**, *47*, 27–31. [CrossRef]
17. Easterbrook, J.D.; Ka, J.B.; Vanasco, N.B.; Reeves, W.K.; Pu, R.H.; Kosoy, M.Y.; Glass, G.E.; Watson, J.; Klein, S.L. A survey of zoonotic pathogens carried by Norway rats in Baltimore, Maryland, USA. *J. Epidemiol. Infect.* **2007**, *135*, 1192–1199. [CrossRef]
18. Bharti, A.R.; Nally, J.E.; Ricaldi, J.N.; Matthias, M.A.; Diaz, M.M.; Lovett, M.A.; Levett, P.N.; Gilman, R.H.; Willig, M.R.; Gotuzzo, E.; et al. Leptospirosis: A zoonotic disease of global importance. *Lancet Infect. Dis.* **2003**, *3*, 757–771. [CrossRef]
19. Peterson, A.C.; Ghersi, B.M.; Riegel, C.; Wunder Jr, E.A.; Childs, J.E.; Blum, M.J. Amplification of pathogenic *Leptospira* infection with greater abundance and co-occurrence of rodent hosts across a counter-urbanizing landscape. *Mol. Ecol.* **2021**, *30*, 2145–2161. [CrossRef] [PubMed]
20. Firth, C.; Bhat, M.; Firth, M.A.; Williams, S.H.; Frye, M.J.; Simmonds, P.; Conte, J.M.; Ng, J.; Garcia, J.; Bhuva, N.P.; et al. Detection of zoonotic pathogens and characterization of novel viruses carried by commensal *Rattus norvegicus* in New York City. *MBio* **2014**, *5*, 1–16. [CrossRef] [PubMed]

21. Williams, S.H.; Che, X.; Garcia, J.A.; Klena, J.D.; Lee, B.; Muller, D.; Ulrich, W.; Corrigan, R.M.; Nichol, S.; Jain, K.; et al. Viral diversity of house mice in New York City. *MBio* **2018**, *9*, e01354-17. [CrossRef] [PubMed]
22. Williams, S.H.; Che, X.; Paulick, A.; Guo, C.; Lee, B.; Muller, D.; Uhlemann, A.-C.; Lowy, F.D.; Corrigan, R.M.; Lipkin, W.I. New York City house mice (*Mus musculus*) as potential reservoirs for pathogenic bacteria and antimicrobial resistance determinants. *MBio* **2018**, *9*, e00624-18. [CrossRef]
23. Wu, Z.; Lu, L.; Du, J.; Yang, L.; Ren, X.; Liu, B.; Jiang, J.; Yang, J.; Dong, J.; Sun, L.; et al. Comparative analysis of rodent and small mammal viromes to better understand the wildlife origin of emerging infectious diseases. *Microbiome* **2018**, *6*, 178. [CrossRef]
24. Morse, S.S.; Mazet, J.A.; Woolhouse, M.; Parrish, C.R.; Carroll, D.; Karesh, W.B.; Zambrana-Torrelio, C.; Lipkin, W.I.; Daszak, P. Prediction and prevention of the next pandemic zoonosis. *Lancet* **2012**, *380*, 1956–1965. [CrossRef]
25. Han, B.A.; Schmidt, J.P.; Bowden, S.E.; Drake, J.M. Rodent reservoirs of future zoonotic diseases. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 7039–7044. [CrossRef] [PubMed]
26. Olival, K.J.; Hosseini, P.R.; Zambrana-Torrelio, C.; Ross, N.; Bogich, T.L.; Daszak, P. Host and viral traits predict zoonotic spillover from mammals. *Nature* **2017**, *546*, 646–650. [CrossRef] [PubMed]
27. Bergner, L.M.; Orton, R.J.; Benavides, J.A.; Becker, D.J.; Tello, C.; Biek, R.; Streicker, D.G. Demographic and environmental drivers of metagenomic viral diversity in vampire bats. *Mol. Ecol.* **2020**, *29*, 26–39. [CrossRef]
28. Wille, M. Unravelling virus community ecology in bats through the integration of metagenomics and community ecology. *Mol. Ecol.* **2020**, *29*, 23–25. [CrossRef] [PubMed]
29. Phan, T.G.; Kapusinszky, B.; Wang, C.; Rose, R.K.; Lipton, H.L.; Delwart, E.L. The fecal viral flora of wild rodents. *PLoS Pathog.* **2011**, *7*, e1002218. [CrossRef] [PubMed]
30. Nieto-Rabiela, F.; Suzán, G.; Wiratsudakul, A.; Rico-Chávez, O. Viral metacommunities associated to bats and rodents at different spatial scales. *Community Ecol.* **2018**, *19*, 168–175. [CrossRef]
31. Shaw, L.P.; Wang, A.D.; Dylus, D.; Meier, M.; Pogacnik, G.; Dessimoz, C.; Balloux, F. The phylogenetic range of bacterial and viral pathogens of vertebrates. *Mol. Ecol.* **2020**, *29*, 3361–3379. [CrossRef] [PubMed]
32. Mills, J.N. Biodiversity loss and emerging infectious disease: An example from the rodent-borne hemorrhagic fevers. *Biodiversity* **2006**, *7*, 9–17. [CrossRef]
33. Dizney, L.J.; Ruedas, L.A. Increased host species diversity and decreased prevalence of sin nombre virus. *Emerg. Infect Dis.* **2009**, *15*, 1012–1018. [CrossRef]
34. Dearing, M.D.; Clay, C.; Lehmer, E.; Dizney, L. The roles of community diversity and contact rates on pathogen prevalence. *J. Mammal.* **2015**, *96*, 29–36. [CrossRef]
35. Krasnov, B.R.; Shenbrot, G.I.; Mouillot, D.; Khokhlova, I.S.; Poulin, R. Spatial variation in species diversity and composition of flea assemblages in small mammalian hosts: Geographical distance or faunal similarity? *J. Biogeogr.* **2005**, *32*, 633–644. [CrossRef]
36. Krasnov, B.R.; Mouillot, D.; Shenbrot, G.I.; Khokhlova, I.S.; Vinarski, M.V.; Korallo-Vinarskaya, N.P.; Poulin, R. Similarity in ectoparasite faunas of Palaearctic rodents as a function of host phylogenetic, geographic or environmental distances: Which matters the most? *Int. J. Parasitol.* **2010**, *40*, 807–817. [CrossRef] [PubMed]
37. Stephens, P.R.; Altizer, S.; Smith, K.F.; Alonso Aguirre, A.; Brown, J.H.; Budischak, S.A.; Byers, J.E.; Dallas, T.A.; Jonathan Davies, T.; Drake, J.M.; et al. The macroecology of infectious diseases: A new perspective on global-scale drivers of pathogen distributions and impacts. *Ecol. Lett.* **2016**, *19*, 1159–1171. [CrossRef]
38. Kendig, A.E.; Borer, E.T.; Mitchell, C.E.; Power, A.G.; Seabloom, E.W. Characteristics and drivers of plant virus community spatial patterns in US west coast grasslands. *Oikos* **2017**, *126*, 1281–1290. [CrossRef]
39. Robertson, C. Settlement Is Reached in Suit Over Katrina Grants. New York Times, 6 July 2011. Available online: https://www.nytimes.com/2011/07/07/us/07orleans.html (accessed on 29 July 2020).
40. Green, T.F.; Olshansky, R.B. Rebuilding housing in New Orleans: The road home program after the Hurricane Katrina disaster. *Hous. Policy Debate* **2012**, *22*, 75–99. [CrossRef]
41. Green, R.D.; Kouassi, M.; Mambo, B. Housing, race, and recovery from Hurricane Katrina. *Rev. Black Political. Econ.* **2013**, *40*, 145–163. [CrossRef]
42. Gotham, K.F. Reinforcing inequalities: The impact of the CDBG program on post-Katrina rebuilding. *Hous. Policy Debate* **2014**, *24*, 192–212. [CrossRef]
43. Lewis, J.A.; Zipperer, W.C.; Ernstson, H.; Bernik, B.; Hazen, R.; Elmqvist, T.; Blum, M.J. Socioecological disparities in New Orleans following Hurricane Katrina. *Ecosphere* **2017**, *8*, e01922. [CrossRef]
44. Gulachenski, A.; Ghersi, B.M.; Lesen, A.E.; Blum, M.J. Abandonment, ecological assembly and public health risks in counter-urbanizing cities. *Sustainability* **2016**, *8*, 491. [CrossRef]
45. Ghersi, B.M. Rat Demography and Rodent-Borne Pathogens across Post-Katrina New Orleans. Ph.D. Thesis, University of Tennessee, Knoxville, TN, USA, 2020.
46. Bergner, L.M.; Orton, R.J.; da Silva Filipe, A.; Shaw, A.E.; Becker, D.J.; Tello, C.; Biek, R.; Streicker, D.G. Using noninvasive metagenomics to characterize viral communities from wildlife. *Mol. Ecol. Resourc.* **2019**, *19*, 128–143. [CrossRef]
47. Weiss, S.; Xu, Z.Z.; Peddada, S.; Amir, A.; Bittinger, K.; Gonzalez, A.; Lozupone, C.; Zaneveld, J.R.; Vázquez-Baeza, Y.; Birmingham, A.; et al. Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome* **2017**, *5*, 27. [CrossRef] [PubMed]

48. McMurdie, P.J.; Holmes, S. Waste not, want not: Why rarefying microbiome data is inadmissible. *PLoS Comput. Biol.* **2014**, *10*, e1003531. [CrossRef]
49. Mcardle, B.H.; Anderson, M.J. Fitting multivariate models to community data: A comment on distance-based redundancy analysis. *Ecology* **2001**, *82*, 290–297. [CrossRef]
50. Combs, M.; Byers, K.A.; Ghersi, B.M.; Blum, M.J.; Caccone, A.; Costa, F.; Himsworth, C.G.; Richardson, J.L.; Munshi-South, J. Urban rat races: Spatial population genomics of brown rats (*Rattus norvegicus*) compared across multiple cities. *Proc. R. Soc. B Biol. Sci.* **2018**, *285*, 20180245. [CrossRef]
51. Oksanen, J.; Guillaume, F.B.; Friendly, M.; Kindt, R.; Legendre, P.; McGlinn, D.; Minchin, P.R.; O'Hara, R.B.; Simpson, G.L.; Solymos, P.; et al. Vegan: Community Ecology Package. R Package Version 2.4-5. 2017. Available online: https://CRAN.R-project.org/package=vegan (accessed on 15 August 2020).
52. Brooks, M.E.; Kristensen, K.; van Benthem, K.J.; Magnusson, A.; Berg, C.W.; Nielsen, A.; Skaug, H.J.; Maechler, M.; Bolker, B.M. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* **2017**, *9*, 378–400. [CrossRef]
53. Goslee, S.C.; Urban, D.L. The ecodist package for dissimilarity-based analysis of ecological data. *J. Stat. Softw.* **2007**, *22*, 1–19. [CrossRef]
54. Hothorn, T.; Bretz, F.; Westfall, P. Simultaneous inference in general parametric models. *Biom. J.* **2008**, *50*, 346–363. [CrossRef]
55. Childs, J.E.; Glass, G.E.; Ksiazek, T.G.; Rossi, C.A.; Oro, J.G.B.; Leduc, J.W. Human-rodent contact and infection with lymphocytic choriomeningitis and Seoul viruses in an inner-city population. *Am. J. Trop. Med. Hyg.* **1991**, *44*, 117–121. [CrossRef]
56. Knowles, S.; Eccles, R.; Baltrūnaitė, L. Species identity dominates over environment in shaping the microbiota of small mammals. *Ecol. Lett.* **2019**, *22*, 826–837. [CrossRef]
57. Patz, J.A.; Daszak, P.; Tabor, G.M.; Aguirre, A.A.; Pearl, M.; Epstein, J.; Wolfe, N.D.; Kilpatrick, A.M.; Foufopoulos, J.; Molyneux, D.; et al. Unhealthy landscapes: Policy recommendations on land use change and infectious disease emergence. *Environ. Health Perspect.* **2004**, *112*, 1092–1098. [CrossRef]
58. Jones, K.E.; Patel, N.G.; Levy, M.A.; Storeygard, A.; Balk, D.; Gittleman, J.L.; Daszak, P. Global trends in emerging infectious diseases. *Nature* **2008**, *451*, 990–993. [CrossRef] [PubMed]
59. Hosseini, P.R.; Mills, J.N.; Prieur-Richard, A.-H.; Ezenwa, V.O.; Bailly, X.; Rizzoli, A.; Suzán, G.; Vittecoq, M.; García-Peña, G.E.; Daszak, P.; et al. Does the impact of biodiversity differ between emerging and endemic pathogens? The need to separate the concepts of hazard and risk. *Philos. Trans. R Soc. B Biol. Sci.* **2017**, *372*, 20160129. [CrossRef]
60. Little, T.J.; Shuker, D.M.; Colegrave, N.; Day, T.; Graham, A.L. The coevolution of virulence: Tolerance in perspective. *PLoS Pathog.* **2010**, *6*, e1001006. [CrossRef] [PubMed]
61. Faust, C.L.; Dobson, A.P.; Gottdenker, N.; Bloomfield, L.S.P.; McCallum, H.I.; Gillespie, T.R.; Diuk-Wasser, M.; Plowright, R.K. Null expectations for disease dynamics in shrinking habitat: Dilution or amplification? *Philos. Trans. R. Soc. B* **2017**, *372*, 20160173. [CrossRef] [PubMed]
62. Mori, E.; Ferretti, F.; Fattorini, N. Alien war: Ectoparasite load, diet and temporal niche partitioning in a multi-species assembly of small rodents. *Biol. Invasions.* **2019**, *21*, 3305–3318. [CrossRef]
63. Puckett, E.E.; Park, J.; Combs, M.; Blum, M.J.; Bryant, J.E.; Caccone, A.; Costa, F.; Deinum, E.E.; Esther, A.; Himsworth, C.G.; et al. Global population divergence and admixture of the brown rat (*Rattus norvegicus*). *Proc. R. Soc. B Biol. Sci.* **2016**, *283*, 20161762. [CrossRef]
64. Torchin, M.E.; Lafferty, K.D.; Dobson, A.P.; McKenzie, V.J.; Kuris, A.M. Introduced species and their missing parasites. *Nature* **2003**, *421*, 628–630. [CrossRef]
65. van Dijk, J.G.B.; Hoye, B.J.; Verhagen, J.H.; Nolet, B.A.; Fouchier, R.A.M.; Klaassen, M. Juveniles and migrants as drivers for seasonal epizootics of avian influenza virus. *J. Anim. Ecol.* **2013**, *83*, 266–275. [CrossRef]
66. Nishiyama, S.; Dutia, B.M.; Stewart, J.P.; Meredith, A.L.; Shaw, D.J.; Simmonds, P.; Sharp, C.P. Identification of novel anelloviruses with broad diversity in UK rodents. *J. Gen. Virol.* **2014**, *95*, 1544–1553. [CrossRef] [PubMed]
67. Okamoto, H.; Takahashi, M.; Nishizawa, T.; Tawara, A.; Fukai, K.; Muramatsu, U.; Naito, Y.; Yoshikawa, A. Genomic characterization of TT viruses (TTVs) in pigs, cats and dogs and their relatedness with species-specific TTVs in primates and tupaias. *J. Gen. Virol.* **2002**, *83*, 1291–1297. [CrossRef] [PubMed]
68. Tagliapietra, V.; Rosà, R.; Hauffe, H.C.; Laakkonen, J.; Voutilainen, L.; Vapalahti, O.; Vaheri, A.; Henttonen, H.; Rizzoli, A. Spatial and temporal dynamics of lymphocytic choriomeningitis virus in wild rodents, northern Italy. *Emerg. Infect. Dis.* **2009**, *15*, 1019–1025. [CrossRef]
69. Fierer, N.; Jackson, R.B. The diversity and biogeography of soil bacterial communities. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 626–631. [CrossRef]
70. Mihaljevic, J.R. Linking metacommunity theory and symbiont evolutionary ecology. *Trends Ecol. Evol.* **2012**, *27*, 323–329. [CrossRef]
71. Poulin, R. The decay of similarity with geographical distance in parasite communities of vertebrate hosts. *J. Biogeogr.* **2003**, *30*, 1609–1615. [CrossRef]
72. Munshi-South, J.; Kharchenko, K. Rapid, pervasive genetic differentiation of urban white-footed mouse (*Peromyscus leucopus*) populations in New York City. *Mol. Ecol.* **2010**, *19*, 4242–4254. [CrossRef]

73. Combs, M.; Puckett, E.E.; Richardson, J.; Mims, D.; Munshi-South, J. Spatial population genomics of the brown rat (*Rattus norvegicus*) in New York City. *Mol. Ecol.* **2018**, *27*, 83–98. [CrossRef]
74. Hechinger, R.F.; Lafferty, K.D. Host diversity begets parasite diversity: Bird final hosts and trematodes in snail intermediate hosts. *Proc. R. Soc. B Biol. Sci.* **2005**, *272*, 1059–1066. [CrossRef]
75. Kamiya, T.; O'Dwyer, K.; Nakagawa, S.; Poulin, R. Host diversity drives parasite diversity: Meta-analytical insights into patterns and causal mechanisms. *Ecography* **2014**, *37*, 689–697. [CrossRef]
76. Johnson, P.T.J.; Ostfeld, R.S.; Keesing, F. Frontiers in research on biodiversity and disease. *Ecol. Lett.* **2015**, *18*, 1119–1133. [CrossRef] [PubMed]
77. Wood, C.L.; Zgliczynski, B.J.; Haupt, A.J.; Guerra, A.S.; Micheli, F.; Sandin, S.A. Human impacts decouple a fundamental ecological relationship—The positive association between host diversity and parasite diversity. *Glob. Chang. Biol.* **2018**, *24*, 3666–3679. [CrossRef] [PubMed]
78. Streicker, D.G.; Allgeier, J.E. Foraging choices of vampire bats in diverse landscapes: Potential implications for land-use change and disease transmission. *J. Appl. Ecol.* **2016**, *53*, 1280–1288. [CrossRef]
79. Reaser, J.K.; Witt, A.; Tabor, G.M.; Hudson, P.J.; Plowright, R.K. Ecological countermeasures for preventing zoonotic disease outbreaks: When ecological restoration is a human health imperative. *Restor. Ecol.* **2021**, *29*, e13357. [CrossRef]
80. Plowright, R.K.; Reaser, J.K.; Locke, H.; Woodley, S.J.; Patz, J.A.; Becker, D.J.; Oppler, G.; Hudson, P.J.; Tabor, G.M. Land use-induced spillover: A call to action to safeguard environmental, animal, and human health. *Lancet Planet. Health* **2021**, *5*, e237–e245. [CrossRef]