PLOS ONE

Our good neighbors: Understanding ecosystem services provided by insectivorous bats in Rwanda

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Abstract

Bats are prodigious consumers of agricultural and forest pests, and are, therefore, a natural asset for agricultural productivity, suppressing populations of such pests. This study provides baseline information of diet of 143 bats belonging to eight insectivorous bat species from agricultural areas of Rwanda while evaluating the effectiveness of bats as pest suppressors. Using DNA metabarcoding to analyze bat fecal pellets, 85 different insect species were detected, with 60% ($n = 65$), 64% ($n = 11$) and 78% ($n = 9$) found to be agricultural pests from eastern, northern and western regions, respectively. Given the high percentages of agricultural pests detected, we submit that Rwandan insectivorous bats have the capacity for biocontrol of agricultural pests. Rwandan bat populations should be protected and promoted since they may foster higher crop yields and sustainable livelihoods.

Introduction

Crop losses due to arthropod pests reduce production of food and cash crops globally [1], and this has become a greater concern due to climate change promoting insect pests [2]. Insectivorous bats prey on a variety of arthropods, many of which are considered major agricultural pests [3,4]. They can consume 30%–100% of their body weight in prey each night [3], and mounting evidence suggests that, through insect consumption, they contribute enormously to both forestry and agriculture [5–10]. In Europe, pest insects have been found to comprise a high percentage of prey diversity and to include pests of high economic impact [11–14]. Charbonnier et al. [13] found that in vineyards in France, bats increased hunting activity when a pest moth species was present. The service of pest suppression has been estimated to have a global value of billions of dollars to agriculture by decreasing insect crop damage and the need

to apply pesticides [4,15]. For instance, *Tadarida brasiliensis* bats have an estimated annual value of $741,000 USD as pest control agents on cotton fields in south-central Texas that have a regional value of about six million dollars, a saving of 15% to the industry [16]. This economic pest control value on cotton was based on the bats´ diet of 31% moths [17,18].

The foregoing examples are drawn from North American and European ecosystems because comparable information is largely unavailable for Africa. In Eswatini, *Chaerephon pumilus* and *Mops condylurus* were found selectively foraging over sugarcane plantations from a nearby roost [19], and fed on boring moths (*Eldana saccharina* and *Mythimna phaea*) and stink bugs (*Hemiptera*, *Pentatomidae*), which are major insect pests of sugarcane [20]. In macadamia plantations in South Africa, ecosystem services of insectivorous bats were estimated at $59–139 per hectare through the suppression of stink-bug pest species [21,22]. Finally, in Madagascar, bats in agricultural areas were found to prey on six known pest species [6].

Rwanda is home to >35 insectivorous bat species [23,24]. Although bat density in households was high in eastern Rwanda (42% of 574 assessed houses), very little is known about the potentially important role they play for agriculture. Agriculture represents the second most important contribution to Rwanda´s gross domestic product at 26% [25]. Yet, like in many African countries, bats are shrouded in mystery, misconception, and misinformation, and their ecological roles remain largely unknown. Consequently, they are often feared and persecuted. Much of the recent persecution bats are facing across Rwanda is due to a fear that bats are the source of the virus that causes COVID-19, even though this risk is null [26,27]. The importance of bats especially to agriculture can be demonstrated to communities via outreach supported by research. Studies to determine the economic value of bats is one way to promote bat conservation and to increase the interest of people in protecting them. Increasing local bat populations by decreasing persecution and through artificial roost programs may be an effective and less environmentally destructive means of suppressing insect pests than increasing chemical pesticide use, which may ultimately harm other organisms like insectivorous spiders and birds through poisoning [28,29].

To contribute toward the goal of ultimately showing local communities the ecological services of bats and demonstrating their economic value to policymakers, we collected baseline data on the diet of insectivorous bat species residing in abandoned buildings, residential houses, and caves in the main agricultural areas of Rwanda. We aimed to determine the range of insect pests consumed by bats and therefore assess the effectiveness of these bats as pest suppressors. We hypothesized that a proportion of these insects would be agricultural pests.

Materials and methods

Study sites

The study sites were in the eastern part of Rwanda at four main agricultural areas of Kayonza, Nyagatare, Bugesera, and Rwamagana Districts with rice, maize, and tomatoes as the main food crops (Fig 1, Table 1). Eastern regions of Rwanda are affected by prolonged drought while the northern and western regions experience abundant rainfall that at times cause flooding, soil erosion and landslides. The eastern Districts and Bugarama site in the west of Rwanda have low rainfall and hot temperatures. The field activities took place between April 2018 and February 2019. We sampled insectivorous bats across six sites from residential houses, abandoned buildings, and caves. For comparative purposes, additional opportunistic samples were collected from Bugarama (and Musanze Districts, in western and northern Rwanda, respectively).
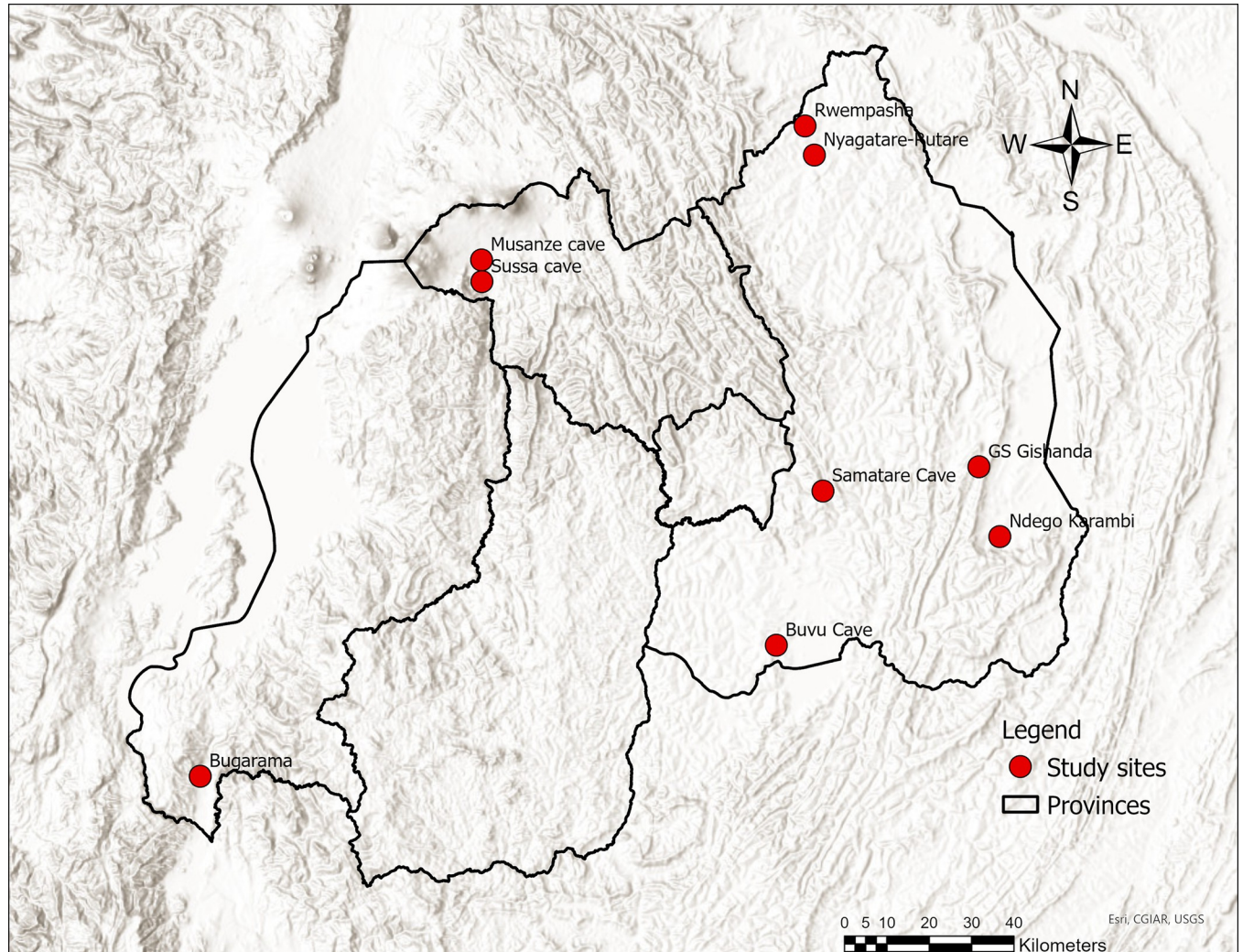
Fig 1. Map of Rwanda, with study sites (ESRI, CGIAR, USGS). Layers include ESRI World Topographic Map [30], Province Boundaries [31], and study sites (generated by the authors).

Field methods

This study was approved by the Rwanda Development Board, Tourism, and Conservation Department (#RDB-T&C/V.U./18) and the Institutional Animal Care and Use Committee at Northern Arizona University (Protocols 07-006-R2, 14–008, and 15–006). All genetic sampling and sequencing complied with relevant guidelines and regulations, and no bats suffered injury or mortality as part of this study. We selected three molossid bat species that are widespread across sub-Saharan Africa and are known to be avid insectivores that consume multiple agricultural pests [20,21,32,33]. Two species, *Chaerephon pumilus* and *Mops condylurus*, are synanthropic, often occupying structures built by humans in large numbers. The third, *Otomops martiensseni*, is fairly ubiquitous and common in caves, forming colonies of up to thousands and is known to consume agricultural pests [21,34].

Bats were captured at roosts in caves or human buildings using mist nets (9 × 2.5 m or 12 × 2.5 m, denier 75/2, mesh 16 × 16 mm, five shelves; Ecotone, Inc., Poland) or hand nets at emergence and return to their roosts. Mist nets were set outside roosts while hand nets were

Table 1. Sites included in this study, their location in relation to the main agricultural areas/crops, bat species sampled, and estimated bat numbers at the roost sites.

District	Site	Crop	Sampled bat species	Estimated bat numbers
Kayonza	Ndego house	Tomato plantation	*Chaerephon pumilus*	4,000
			Mops condylurus	
	Gishanda Primary School	Bramin marshland and Rwinkwavu rice	*Chaerephon pumilus*	400
			Scotophilus dinganii	
Nyagatare	Rutare cell	Nyagatare rice	*Chaerephon pumilus*	100
			Mops condylurus	
	Rwempasha conference hall		*Chaerephon pumilus*	1,000
Bugesera	Buvu cave	Maize	*Miniopterus minor*	unknown
			Hipposideros ruber	
Rwamagana	Samatare Cave	Maize and banana	*Otomops martiensseni*	300
			Nycteris spp.	
			Afronycteris nana	
Rusizi	Bugarama house	Bugarama rice	*Mops condylurus*	3,000
			Chaerephon pumilus	
Musanze	Musanze Sussa Cave	Maize	*Otomops martiensseni*	unknown
			Hipposideros ruber	

https://doi.org/10.1371/journal.pone.0287536.t001

used in caves, tunnels, and dwellings to capture bats at roosts. Nets remained open until 0300. Captured bats were placed in clean individual cloth bags (to prevent cross-contamination) and processed a few meters from the capture sites. We measured forearm length of each captured bat with a digital caliper to the nearest 0.1 mm and weight of the bat with Pesola scale to the nearest 0.1 g. Small wing biopsies were also taken for laboratory DNA confirmation of the bat species. Bat identifications and nomenclature followed Patterson & Webala [23].

We collected fecal samples of all captured bats; these accumulated as bats were held in individual cloth bags for an hour on their return from foraging bouts. Additionally, we collected fecal samples from other species that were opportunistically included in the study as a comparison, such as *Miniopterus minor*, *Afronycteris nana*, and *Scotophilus dinganii*, among others. All bat handling techniques and sampling were carried out according to the standards established by the American Society of Mammalogists [35]. We collected approximately 200 fecal pellets from the same bat species into 15 ml sterile conicals, which were then filled with 7.5ml of RNAlater. Biopsy punches for individual bats were collected in 1.5 ml cryovials. To contribute to the reference library for the genetic identification of prey, we collected insects at the study sites at the same time and place as the bats were foraging [36]. We collected insects from tomato (Ndego area), maize (Bugarama area), and rice fields (Nyagatare and Bugarama areas) using UV scorpion light traps. A UV light torch was placed on a string one meter above a white UV-fluorescent cloth to attract insects. We then collected insects with tweezers, each into its own cryovial containing RNAlater. The cryovials with bat fecal samples, wing biopsies, and insects were stored in a dry shipper from the field and then transferred to -80˚ C freezers until transported to Northern Arizona University for DNA analysis.

Genetic identification of bat diets

DNA extraction. We subsampled fecal pellets from RNAlater into 1.5 mL vials and extracted DNA using the QiaAmp Fast DNA Stool Mini Kit (Qiagen, Valencia, CA, USA) following the human DNA analysis protocol. Samples were lysed for 30 min and DNA eluted at 100 μL. We extracted DNA from site-collected insects using the DNeasy Blood and Tissue Kit

(Qiagen, Valencia, CA, USA) following the Animal Tissue protocol. Depending on the size of the specimen, a whole specimen (body length < 7 mm) or a whole leg was initially homogenized with one 5/23" stainless steel grinding ball (OPS Diagnostics) for 40 sec. (6 m/sec) using a FastPrep-24™ 5G Sample Preparation System (MP Biomedicals).

PCR amplification and sequencing. We PCR amplified cytochrome c oxidase I (COI) to 1) identify arthropod taxa consumed by the bat species, 2) confirm bat field identification, and 3) identify insects collected from the same localities as the bat species. For goals 1 and 2, we used the ANML primer set (185 bp insert) [37]. For goal 2, we used the Species from Feces (SFF; 202 bp insert) primer set [38,39]. Both primer sets were modified to include 5' universal tails for indexing [40]. Non-template controls (NTCs) were included in all PCR reactions and mock communities were included for Illumina sequencing. The insect mock community included cloned sequences of the ANML target of 24 known arthropod taxa [37,41]. A bat specific mock community included four North American bat species: *Myotis lucifugus*, *Eptesicus fuscus*, *Euderma maculatum*, and *Idionycteris phyllotis*. PCR for both SFF and ANML were run in 15 μL reaction volumes with 3 μL of genomic DNA template. Reactions included 8.46 μL of PCR grade water, 1.5 μL 10X Mg-free PCR buffer (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), 1.5 mM MgCl2, 0.2 mM each dNTP, 0.2 μM each primer, 0.16 ug/μL bovine serum albumin (Ambion Ultrapure BSA), and 0.03 U/μL PlatinumTaq DNA polymerase (Invitrogen, Thermo Fisher Scientific). Cycling conditions included an initial denaturation of 94˚C 5 min, 5 cycles of 94˚C for 1 min, 45˚C for 1.5 min, and 72˚C for 1 min, then 35 cycles of 94˚C for 1 min, annealing for 1.5 min (60˚C for SFF and 50˚C for ANML), and 72˚C for 1 min, with a final extension cycle of 72˚C for 5 min. To minimize tag-jumping [42], we used a custom indexing scheme [40] whereby forward and reverse reads were tagged (via PCR) with 8 nucleotide-long indices, extended from the universal tail in a second PCR. An index was used only once per sample. The indexing PCR was run in 25 μL reaction volumes with 2 μL amplicon template, 12.5μ2X Kapa HiFi HotStart ReadyMix (Roche Sequencing, Wilmington, MA, USA), 8.5 μL PCR-grade water, and 1 μL each index primer (starting concentration: 10 uM). Cycling conditions were run at 98˚C for 2 min, followed by 8 cycles of 98˚C for 30 s, 60˚C for 20 s, and 72˚C for 5 min, and then a final extension step of 72˚C for 5 min. We sequenced libraries with Illumina MiSeq V3 600 cycle kits in two sequencing runs, each with 30% PhiX and loaded with 5 μL (3.5 pM) of the pooled libraries. ANML amplicon from site-collected insects was prepared for bi-directional Sanger sequencing using the BigDye Terminator v3.1 kit (Applied Biosystems, Foster City, CA, USA) and sequenced on an ABI3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). We discarded priming regions, verified quality, and edited base calls using Sequencher 5.3 software (http://www.gencodes.com).

Bioinformatics. We processed reads for each marker separately. Reads were separated by marker with primers and read-through removed using cutadapt v.2.1 [43]. We used Qiime2 v2020.2 [44] and custom Tidyverse [45] scripts with R statistical software v3.5.1 [46] for all further bioinformatics analysis. Based on quality scores, ANML reads were truncated to 175 bases and SFF reads to 202 bases. We filtered reads by quality, joined paired-ends, denoised, dereplicated into amplicon sequencing variants (ASVs), and filtered chimeras using DADA2 [47]. We further filtered the ASVs of ANML amplicon to exclude bat species [37,41]. ASVs were excluded based on the Vsearch global alignment algorithm [48] if an ASV matched at 95% identity to the bat reference library [49]. We retained any sample that contained > 4000 reads [41]. NTCs did not amplify and so instead of determining a relative abundance threshold using NTCs, we instead used the mock community method [41], indicating that we should exclude ASVs observed in fewer than 0.15% of reads per sample. The ANML and SFF datasets were classified with marker-specific reference libraries [39,41]. ASVs from the ANML dataset were classified with the Vsearch classifier in Qiime2 (classify-consensus-vsearch) at 97%

identity and 89% query coverage. We performed the same classification of the ANML dataset against a reference library of only the site-collected insect sequences under the same parameters. This allowed us to identify whether site-collected insects could be detected in the diet. ASVs from the SFF dataset were classified using the Naïve-Bayes classifier [50] at a 90% confidence threshold. Sequence variants were also cross-referenced using the BOLD identification tool.

We evaluated dietary richness using the ANML ASVs rarified to a minimum read depth of 10,000 reads. Any samples with fewer reads were not included in this analysis. Using base functions in R, we calculated the mean richness per sample and 95% confidence intervals with 10,000 bootstrap iterations. We estimated dietary breadth with a species accumulation curve using the specaccum function (method = "exact") in R package vegan (Dixon 2003). Sanger sequences derived from the site-collected insects were clustered at 98% identity in Vsearch and identified using both Vsearch and the BOLD identification tool [51] to reach consensus. Ambiguous assignments within or among classifiers were collapsed to the lower taxonomic level. The relative proportion of arthropod species in the diet is reported as a percentage of the total number of species detected.

Results

Bat fecal sample collection

We obtained fecal samples from 143 bats comprising eight species belonging to five families. Species included *Chaerephon pumilus* (little free-tailed bat; family Molossidae; n = 33), *Mops condylurus* (Angolan free-tailed bat; family Molossidae; n = 23), *Scotophilus dinganii* (African yellow bat; family Vespertilionidae; n = 1), *Miniopterus minor* (least long-fingered bat; family Miniopteridae; n = 20), *Hipposideros ruber* (Noack's roundleaf bat; family Hipposideridae; n = 7), *Otomops martiensseni* (large-eared free-tailed bat; family Molossidae; n = 19), *Afronycteris nana* (banana serotine; family Vespertilionidae; n = 10), and *Nycteris spp* (slit-faced bats; family Nycteridae; n = 4).

Genetic validation of host species and identification of prey

To validate bat species (SFF marker), we retained 1,030,718 reads for 19/23 samples (mean = 49081.81 ± 37935.62 SD). Two samples failed to amplify and two failed to meet the read minimum threshold, producing only 273 and 1,170 reads. For the ANML marker, we retained 707,652 paired-end reads (mean = 33,697.71 ± 28731.41 SD reads/sample) among 21/23 individuals (324 ASVs). Two of the samples failed to meet the read minimum threshold, producing only 11 and 90 reads. We recovered 25 unique Sanger sequences for the 26 site-collected insects because two specimens shared the same sequence. No negative controls amplified. Genetic identifications also resolved species identifications, and allowed us to determine which pooled samples contained feces from more than one bat species due to mis-identification in the field. One individual field identified to the genus *Nycteris* was genetically identified to a taxonomically unresolved *Nycteris* species known to occur in Sudan (*Nycteris* sp. DMFR-2017).

A taxonomic analysis of arthropod (ANML) sequences revealed that all potential diet items were of the class Insecta. This included 7 orders, 32 families, 40 genera, and 22 species. No negative controls amplified. Although some of the samples revealed a mixture of bat species for the SFF analysis, we still incorporated dietary information from these samples as pooled data to help address our main study question (whether local bat assemblages consumed pests). We found that 24% (n = 25) of unique Sanger sequences generated from site collected arthropods were able to be classified to either genus or species in either Vsearch or the BOLD

Table 2. Insect specimens that were collected from the study sites and sequenced, along with indication of whether they were identified in the diet of the bat species in this study.

Specimen	Taxonomic classification (Vsearch + BOLD Identification Tool)	Detected	No. ASVs	Bat species
I001_2018	Insecta;Lepidoptera			
I002_2018	Insecta;Lepidoptera;Noctuidae			
I002_2019	Insecta;Lepidoptera			
I003_2018	Insecta;Diptera;Culicidae;Mansonia;*Mansonia sp.*			
I004_2018	Insecta;Lepidoptera; Noctuidae			
I006_2018	Insecta;Diptera;Muscidae			
I006_2019	Insecta;Lepidoptera			
I007_2018	Insecta;Lepidoptera			
I008_2018	Insecta;Diptera;Drosophilidae;Scaptomyza;*Scaptomyza sp.*			
I005_2018, I009_2018	**Insecta;Diptera**	✓	1	*Chaerephon pumilus*
I010_2018	Insecta;Lepidoptera			
I010_2019	Insecta			
I011_2019	Insecta;Lepidoptera;Nolidae			
I012_2019	Insecta;Lepidoptera;Pyralidae;Emmalocera;*Emmalocera sp.*			
I013_2018	Insecta			
I013_2019	Insecta;Lepidoptera			
I014_2018	**Insecta;Lepidoptera**	✓	1	*Hipposideros ruber*
I015_2018	Insecta			
I016_2019	Insecta;Lepidoptera			
I017_2018	Insecta;Coleoptera;Staphylinidae;Paederus;*Paederus sp.*			
I018_2018	Insecta;Lepidoptera;Erebidae;Aroa;*Aroa discalis*			
I018_2019	**Insecta;Hemiptera;Cicadellidae;Cofana;*Cofana spectra***	✓	1	*Mops condylurus*
I019_2019	**Insecta;Diptera;Culicidae; *Culex* or *Lutzia***	✓	1	*Chaerephon sp.*
I020_2019	Insecta;Diptera			
IO15_2019	**Insecta;Lepidoptera**	✓	1	*Mops condylurus, Chaerephon pumilus*

identification tool (Table 2). Five sequence variants from the fecal data aligned to sequences of five of the site-collected arthropods. However, only two could be classified to a lower taxonomic level than order.

We retained a total of 247 ASVs in the ANML dataset among 18 samples after re-sampling for even read depth of 10,000 reads. The remaining three samples produced fewer than 10,000 reads for ANML and were therefore excluded from analysis of diversity. Although a small number of species were classified, we detected a bootstrapped mean of 15.6 ± 3.3 (95% CI) ASVs (observed SD = 7.37) per sample (minimum = 5, maximum = 28), suggesting that on average we detected 13–19 arthropod taxa per fecal sample. The species accumulation curve (Fig 2) did not approach a clear asymptote, which indicates that the taxonomic richness observed among the individuals was likely a small fraction of the true dietary diversity of the species in this study.

Arthropod species consumed by insectivorous bats in Rwanda

The diet of insectivorous bats sampled in eastern Rwanda marked 76.4% of insect prey (n = 85) and comprised all orders found in this study except Odonata. Conversely, insect prey of Musanze cave and Bugarama houses constituted 10.5% and 12.9% of the diet of bats in this study, respectively. Among seven orders of insects that were identified in this study, Lepidoptera (Moths) constituted 58.5%, followed by Diptera (14.6%) and Hemiptera (9.8%). The least
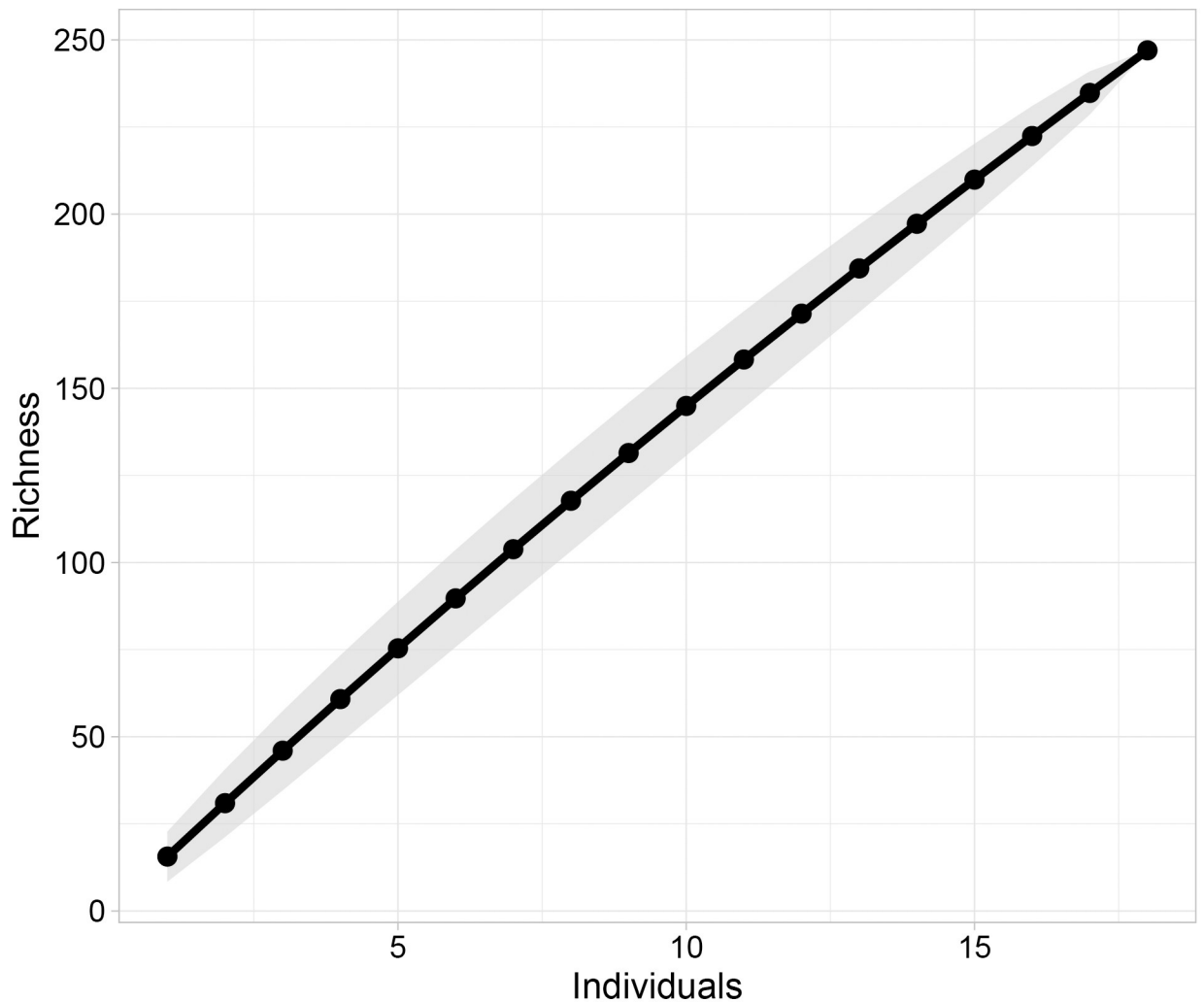
Fig 2. Species accumulation curve for arthropods detected in bat diets. The grey shaded region indicates standard error of the estimate.

https://doi.org/10.1371/journal.pone.0287536.g002

common orders were Blattodea (4.9%), Coleoptera (4.9%), Orthoptera (4.9%), and Odonata (2.4%) (Fig 3, S1 Table). *Chaerephon pumilus* (n = 33) preyed on all insect orders identified in this study except Odonata. Fecal samples of *C. pumilus* collected in eastern Rwanda consisted of Diptera (40%), Lepidoptera (33.3%), Hemiptera (20%) and Blattodea (6.6%), while the diet of *C. pumilus* from Bugarama area consisted of Diptera (44.4%) (*Culex* and *Drosophila*), Lepidoptera (33.3%), Hemiptera (11.1%) and Odonata (11.1%). *Mops condylurus* (n = 22) consumed only three orders of insects including Lepidoptera (58.3%), Diptera (33.3%) and Hemiptera (8.3%). Fecal pellets of *Hipposideros ruber* (n = 7) and *Miniopterus minor* (n = 20) consisted 100% of Lepidoptera. The diet of *Afronycteris nana* (n = 10) consisted of four insect orders dominated by Diptera and Lepidoptera, marking 37.5% of its total prey for each as well as Hemiptera and Coleoptera (12.5%) per each, respectively. *Nycteris* spp. (n = 1) was found to consume one species *Conocephalus* (Orthoptera) and this was the only species for which we did not find any moth (Lepidoptera) DNA in fecal pellets. *Otomops martiensseni* (n = 19) consumed two orders: Lepidoptera (66.6%) and Diptera (33.3%). *Scotophilus dinganii* (n = 1) consumed Lepidoptera (40%), Coleoptera, Orthoptera and Blattodea comprising 20% of its total
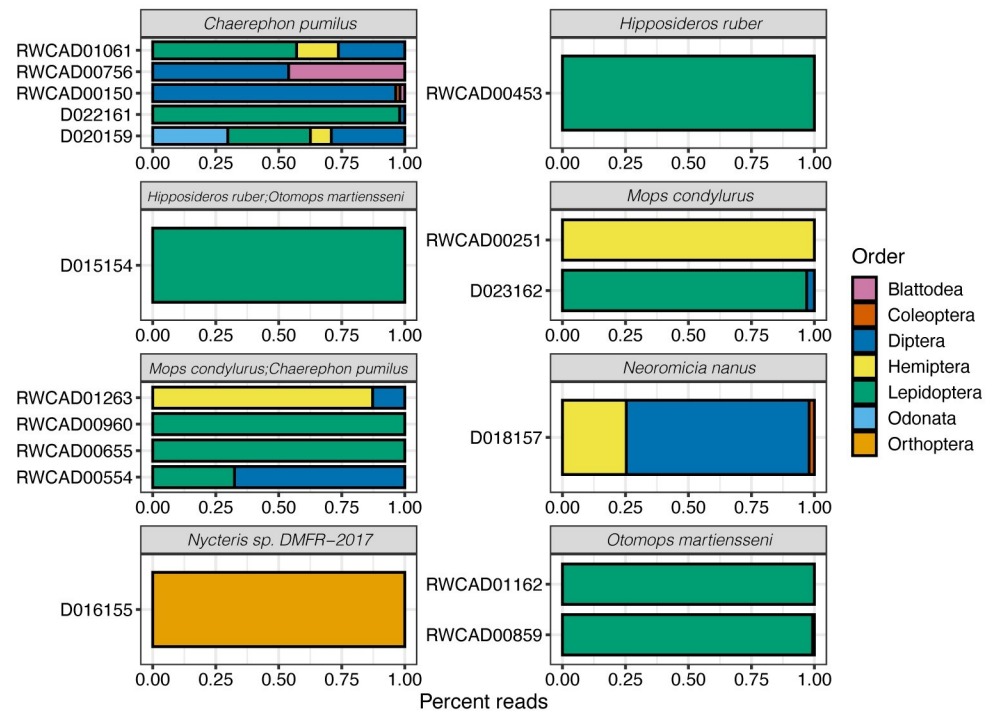
Fig 3. Sample barplots with aggregated read proportions for insect orders identified in the sample pools. Only shown are samples with both genetically supported bat species and dietary information (n = 17/23).

https://doi.org/10.1371/journal.pone.0287536.g003

diet each. In Bugarama area, *Chaerephon pumilus* (n = 33) was found to consume 10.5% of insect prey of this study, dominated by Diptera (44.4%) (*Culex* and *Drosophila*) and were also represented by Lepidoptera (33.3%), Hemiptera (11.1%) and Odonata (11.1%). *Otomops martiensseni* (n = 19) samples from Musanze yielded 12.9% of the prey of this study. These bats consumed almost exclusively Lepidoptera (93%) and some Diptera (7%).

Pest arthropods in the diet of bats

Sixty percent (n = 65) of insect prey species that were consumed by bats in eastern Rwanda were found to be pests of major agricultural importance. Of the prey species from Musanze caves (northern Rwanda) and in Bugarama (southwestern Rwanda), 63.6% (*n* = 11) and 78% (*n* = 9) of insects were also pest species, respectively. The majority (83.3%) of the pests identified in this study are affiliated with damage to maize, rice, fruits and vegetables (Table 3). In addition, a high number of prey taxa which can potentially become nuisance pests associated with anthropogenic aquatic habitats (e.g., sewage treatment facilities) were found in the diet. These taxa included Dipterans from the family Psychodidae (drain flies) and Chironomidae (Midges) (Table 3). We also found potential disease vectors such as moquitoes (Culicidae), which can transmit various human and livestock diseases. Lepidoptera formed the largest component (56.6%) of the total diet pests (*n* = 30) with Erebidae to be the family most represented (35.4%) among lepidopterans. Geometridae and Crambidae were also common, occurring in 14.7% and 11.7% of the total number of insect prey, respectively. Noctuidae, Sphingidae, and Tortricidae made up 8.8% of the lepidopteran pests each. The least represented families of moth pests were Cosmopterigidae and Nolidae occurring in 5.9% of the total number of pellets per each. Diptera was the second common order made up (23.3%) of the prey pests, with Drosophilidae to be the highest represented (60%), followed by Chironomidae

Table 3. Prey of human concern detected in the diet of insectivorous bats from Rwanda, as determined by DNA metabarcoding of fecal samples (n = 143) that were pooled into 21 successfully sequenced samples. A bat species with an asterisk (*) indicates a species only identified in the field. "AND/OR" indicates that the pest was identified in a sample that was mixed with multiple bat species.

Insect pest							
Order	Family	Taxon	Common name	Affiliation	Category	Status & Distribution (Province, this study)	Bat species that consumed pests
Hemiptera	Cicadellidae	*Cofana spectra*	White leafhopper	Rice & Maize	Minor in India but major in West Africa	Widespread in West Africa and in India (East)	*Mops condylurus*
Lepidoptera	Crambidae	*Duponchelia fovealis*	European pepper moth	strawberries	Major agricultural pest	Worldwide but mostly abundant in Turkey (East)	*Miniopterus minor**
Lepidoptera	Erebidae	*Anomis flava*	Cotton looper moth	Cotton	Major agricultural pest	Reported to be severe in Cote d ,Ivoire (East)	*Miniopterus minor**
Lepidoptera	Crambidae	*Herpetogramma licarsisalis*	Tropical grass webworms	Pastures	Nuisance pest	Widely distributed, even in Africa (East)	*Hipposideros ruber*
Lepidoptera	Erebidae	*Sphingomorpha chlorea*	Sundowner moth, Banana hawk	Fruits	Minor agricultural pest	Widespread in Southeast Asia and Africa (North)	*Otomops martiensseni* AND/OR *Hipposideros ruber*
Lepidoptera	Sphingidae	*Daphnis nerii*	Oleander hawk-moth	Plant and forest	Nuisance pest	Minor importance and worldwide distribution (North)	*Otomops martiensseni*
Lepidoptera	Tortricidae	*Thaumatotibia leucotreta*	False codling moth	About 70 plants, including crops	Major agricultural pest	Major importance in tropical Africa, Rwanda (East)	*Chaerephon pumilus*
Hemiptera	Miridae	*Taylorilygus apicalis*	Brocken backed bug	Hardwood trees, coniferous nurseries	Major agricultural pest	Widely distributed in USA, South Africa, and Guatemala (Kigali)	*Afronycteris nanus*
Diptera	Drosophilidae	*Zaprionus indianus*	African big fly	Fruits (Oranges, Peaches and Figs)	Major agricultural pest	Widely distributed in Panama and USA (West)	*Chaerephon pumilus**
Lepidoptera	Tortricidae	*Cydia sennae*	-	Fruits	Minor agricultural pest	Known to occur on native fruits of Kenya (East)	*Mops condylurus*
Diptera	Drosophilidae	*Drosophila* sp.	Fruit fly	Berries, cherries, grapes, tree fruits	Major fruit pest	Originated in Asia but currently widely distributed (West)	*Chaerephon pumilus*
Diptera	Culicidae	*Culex* sp.	Common house mosquitoes	Rift Valley fever virus and West Nile virus	Nuisance pest, disease vector	Widespread in East Africa and known to occur in Rwanda (West)	*Chaerephon pumilus*
Lepidoptera	Crambidae	*Parapoynx* sp.	Rice caseworm	Rice	Major agricultural pest	Worldwide (East)	*Miniopterus minor**
Lepidoptera	Nolidae	*Earias cupreoviridis*	Cotton green moth	Cotton	Major agricultural pest	Widely distributed in Africa (East)	*Hipposideros ruber*
Diptera	Chironomidae	*Kiefferulus brevibucca*	Midge	Aquatic habitats	Major agricultural pest	Widely distributed in Africa (East)	*Chaerephon pumilus* AND/OR *Mops condylurus*
Orthoptera	Tettigoniidae	*Conocephalus conocephalus*	African meadow katydid	Rice, sugarcane, forests, fruit orchards	Major agricultural pest	Widely distributed in Asia and Africa (East)	*Nycteris* sp.

(Continued)

Table 3. (Continued)

Insect pest							Bat species that consumed pests
Order	Family	Taxon	Common name	Affiliation	Category	Status & Distribution (Province, this study)	
Lepidoptera	Noctuidae	*Chrysodeixis* sp.	Golden twin-spot moth	Many fruits, vegetables, ornamental crops	Major agricultural pest	Widely distributed in tropical and subtropical regions (East)	*Hipposideros ruber* AND/OR *Otomops martiensseni*
Lepidoptera	Erebidae	*Eudocima* sp.	Fruit piercing moth	Banana, citrus, fig, guava, mango, stonefruit, persimmon, and ripening papaya.	Major and sporadic	East coast of Australia (North)	*Otomops martiensseni*
Hemiptera	Delphacidae	*Perkinsiella* sp.	Sugarcane planthopper	Sugarcane	Major agricultural pest	Mostly tropical Asia, Australia, parts of Africa and the Middle East (East)	*Chaerephon pumilus*
Diptera	Psychodidae	*Psychoda alternata*	Trickling filter fly or drain fly	Aquatic habitats	Nuisance pest	Originating in North America, has spread around the world (Kigali)	*Afronycteris nanus*
Hemiptera	Rhyparochromidae	*Rhyparochromidae* sp.	Seed bug	Strawberries	Major agricultural pest	Worldwide (East)	*Chaerephon pumilus*
Lepidoptera	Nolidae	*Nolidae* sp.	Tuft moth	Cotton	Major agricultural pest	Worldwide (Kigali)	*Scotophilus* sp.*
Lepidoptera	Geometridae	*Geometridae* sp.	Measuring worm moth, looper, cankerworm inchworm	Apple and tea	Major agricultural pest	Worldwide (Kigali)	*Scotophilus* sp.* AND/OR *Chaerephon pumilus*
Hemiptera	Delphacidae	*Delphacidae* sp.	Delphacid planthopper	Rice	Major agricultural pest	Worldwide (East)	*Chaerephon pumilus*
Lepidoptera	Noctuidae	*Noctuidae* sp.	Owlet moth, cutworm, or armyworm	More than 80 species of plants	Major agricultural pest	Worldwide (North)	*Miniopterus minor** AND/OR *Otomops martiensseni*
Lepidoptera	Erebidae	*Erebidae* sp.	Underwing, litter moth, tiger, lichen, wasp moth, tussock moth, arctic woolly bear moth, and piercing moth	Forest and tea	Major agricultural pest	Found on all continents except Antarctica (North)	All mollosids
Lepidoptera	Cosmopterigidae	*Cosmopterigidae* sp.	Cosmet moth	Flowers	Nuisance pest	Widely distributed in Australia and pacific region (East)	*Chaerephon pumilus*
Hemiptera	Cicadellidae	*Cicadellidae* sp.	Leafhopper	Maize, potatoes, rice	Major agriculture pest	Worldwide (West)	*Chaerephon pumilus*

https://doi.org/10.1371/journal.pone.0287536.t003

(40%), Psychodidae 20%, and Culicide 20%). Hemiptera (16.6%) was mainly represented by Delphaciidae 40% (an example of *Perkinsiella*), Miridae 20% (*Taylorilygus apicalis)*, Cicadellidae (20%; with *Cofana spectra* accounting all with this family), and Rhyparochromidae 20% (FM). Orthoptera (3.3%) was the least represented order among the pests found in the diet, accounting for only Tettigoniidae (*Conocephalus conocephalus).*

Discussion

This study used DNA metabarcoding to investigate the diet of insectivorous bats, through which we highlighted their potential ecosystem services associated with pest consumption, especially documenting ingestion of major agricultural pests and mosquitoes. The diet of eight insectivorous bat species comprised two main insect orders, Lepidoptera and Diptera. The presence of mosquitoes (Culicidae) underscores the likelihood of insectivorous bats playing a role in the suppression of certain mosquito species. Clarifying the role of bats in agricultural pest and mosquito vector suppression will likely lead to benefits for both bat conservation and public health. For instance, erecting bat houses to attract bats in farms so that they feed on insects could reduce the use of pesticides, as has been documented in southern Spain [52,53].

The large difference in percentages of insect prey found across the regions could be explained by a sample size effect and/or the different types of habitat sampled. In the east of Rwanda we sampled a larger number of bats in caves, abandoned houses, and human habitations, while in the north and west we had small sample sizes of limited habitat types (16 *Otomops martiensseni* from Musanze cave in the north and 22 *Chaerephon pumilus* from in Bugarama houses in the west).

Insect diet of eight bat species

Our results are broadly consistent with a study in Cameroon which reported that microbats consumed mainly Lepidoptera, Coleoptera, and Diptera [54] and in Swaziland where two free-tailed bat species, *Chaerephon pumilus* and *Mops condylurus*, also mainly consumed Lepidoptera and Diptera [20]. In this study, bats of the same species living in different regions consumed the same groups of insects, such as *Otomops martiensseni* from Samatare cave (eastern Rwanda) and Musanze cave (northern Rwanda) as well as *Chaerephon pumilus* sampled from the east and west of Rwanda (Bugarama area). Likewise, *C. pumilus* from both areas (east and west of Rwanda) appeared to have consumed the same prey, and prey consisted of similar taxa as in Kenya and South Africa where the most important dietary items were dipterans [33], coleopterans, and hemipterans [55]. The species has also been reported to consume large cockroaches [32,56]. Researchers have highlighted that many insectivorous bats are opportunistic predators [57], choosing particular insect families from different taxa available [58,59], often switching their predatory activity in relation to prey abundance [60]. The diet of bats in our study is in line with what would be expected given that most of the moths are active at night.

The diet of *Mops condylurus* comprised Lepidoptera, Diptera, and Hemiptera. This is consistent with the study by Bohmann et al. [20] who reported the diet of *M. condylurus* to consist mainly of Lepidoptera (46.7%) and Diptera (26.7%), and that the species also consumed a wider range of insect species than *M. condylurus*. We found a similar result: the diet of *C. pumilus* consisted of eight insect orders while the diet of *M. condylurus* consisted of three.

Our results demonstrate that *Hipposideros ruber* and *Miniopterus minor* are moth specialist feeders, as their diet was composed of 100% Lepidoptera. This is consistent with a study that found that *H. ruber* tended to increase foraging around midnight to coincide with moth species such as those of the family Geometridae that have a very late activity peak [61], and other studies that found *H. ruber* to feed predominantly on Lepidoptera [62,63]. In this study we found that *M. minor* fed on five families of Lepidoptera, which is the first information on the diet of the species in Africa.

The diet of *Afronycteris nana* consisted mainly of Diptera and Lepidoptera and to a lesser extent Hemiptera and Coleoptera. These results are consistent with previous studies that found that this species preyed upon Lepidoptera, Coleoptera, and Diptera [64,65]. In South Africa, *A. nana* fed upon Lepidoptera, Diptera, Coleoptera, Hemiptera, Trichoptera, and

Hymenoptera [66]. In the present study, the diet of *Otomops martiensseni* consisted mainly of Lepidoptera with a few dipterans. *O. martiensseni* from Musanze cave, northern Rwanda, consumed Lepidoptera and Diptera and *O. martiensseni* from Samatare cave (eastern Rwanda) consumed mainly Lepidoptera and some Diptera. Our results are consistent with those of another study of 40 droppings collected from *O. martiensseni* in Rwanda, with diet items consisting almost exclusively (97% by volume) of moths [67].

Our single individual *Scotophilus dinganii* consumed mainly Lepidoptera, Coleoptera, Orthoptera and Blattodea, unlike a study in Kenya which reported that the species consumed almost exclusively small beetles [68]. Others have reported that the diet of *S. dinganii* comprises mainly medium-sized Coleoptera, but also may include Hemiptera, Hymenoptera, Isoptera, and Diptera [55,69]. The diet of *Nycteris* sp. in this study included the genus *Conocephalus* (Orthoptera); this was the only species for which we did not detect Lepidoptera in fecal pellets. In Zambia, the diet of *Nycteris macrotis* included Orthoptera, Coleoptera, Isoptera and Diptera [70]. These results point to generalist predator diets for the two bat species within the context of available prey. As generalists, their population dynamics are linked to a wide range of prey [71], with the consumption of a predator per unit time as a function of prey availability [72]. If so, then the differences between our study and other studies could be attributed to prey switching according to the optimal foraging theory [73].

Pest arthropods in the diet of bats

Globally, insectivorous bats have been reported to regulate insect pests in agricultural systems by decreasing insect crop damage and increasing yield [4,15,16,74]. However, few studies have examined the diversity of agricultural pests and other insects consumed. Our results highlighted that the majority of the total pests are of major agricultural importance, affiliated with damage to maize, rice, fruits, and vegetables. Analysis of the diet also showed nuisance pests associated with anthropogenic aquatic habitats as well as mosquitoes. Among agricultural pests, Lepidoptera was the most frequently reported, which is consistent with a study indicating that most African insectivorous bats feed mainly on Lepidoptera and Coleoptera [55]. Vreysen et al. [75] reported Lepidoptera to be a key insect pest that requires control to avoid losses in many crop systems of temperate, subtropical, and tropical regions of the world. Erebidae was the most common Lepidopteran family found in this study and has been reported to be a pest of several crops as well as forest trees and many ornamental shrubs [76]. Fruit-piercing moths (*Eudocima* sp.) from the family Erebidae are important pests for fruit crops throughout Africa and Southeast Asia [77]. We also found several Geometridae (14.7%) and Crambidae (11.7%). Members of Geometridae are known to cause serious damage to tea plantations (5 to 55% yield loss) [78]. *Duponchelia fovealis* is one of the Crambidae species identified in this study and was reported to cause serious damage to Strawberries in Portugal, France, Italy and Turkey [79–81]. There are records of its occurrence in different regions of Europe, North America, Asia and Africa [79,82,83].

Noctuidae, Sphingidae and Tortricidae were the next most common Lepidopteran families. Noctuidae is one of the top families of concern in agriculture areas. Their larvae are typically known as "cutworms" or "armyworms" due to enormous swarms that destroy crops, orchards, and gardens every year worldwide. In the diet, we found *Noctuid* pest species of major agricultural importance such as *Earias cupreoviridis*, which damaged cotton fields in Tanzania and Uganda [84]. *Anomis flava* is another *Noctuid* pest that we detected in this study and has been reported to damage cotton in Australia, the Philippines, India, and Madagascar [85–87]. *Sphingomorpha chlorea* (a widespread noctuid) was found to cause considerable damage to fruits in Southeast Asia and Africa [88]. Many species of *Chrysodeixis* spp such as *Chrysodeixis*

chalcites and *Chrysodeixis eriosoma* feed on a wide variety of fruit and vegetables [89]. *Daphnis nerii* (Sphingidae) reported damage to plants, trees, and shrubs from various families [90]. Another species found in the bat diets was *Thaumatotibia leucotreta* (false codling moth; Tortricidae), reported to damage more than 70 plants and crops in tropical Africa including Rwanda [91]. In South Africa, *T. leucotreta* was reported as one of the most damaging and economically important citrus pests causing the loss of crops equated to more than ZAR 100 Million annually to the South African Citrus Industry [92]. Rice planthoppers (Homoptera: Delphacidae) and leafhoppers (Homoptera: Cicadelidae) reported in this study are important rice pests in rice granary areas [93].

Our results revealed that Dipterans were predominant in bat diets, and 60% were *Drosophila* species. Culicidae (mosquito family) constituted 20% of the Dipteran pests; this suggests that bats are potentially important in the suppression of disease vectors in the Bugarama area and the eastern province of Rwanda. *Chaerephon pumilus* from the two regions fed on prey associated with human habitats such as *Culex* mosquitoes that can carry diseases like the West Nile virus (WNV) [94]. Indeed, the eastern province of Rwanda had the highest seroreactivity of WNV [95]. The area reported a high number of mosquito vectors of this virus, which is transmitted by the bite of Culex mosquitoes where humans and horses are incidental hosts. We also found drain flies (e.g., *Psychoda alternate*) and midges (*Kiefferulus brevibucca*) which are considered nuisance pests when their populations are abundant in residential areas [96]. This justifies the role of insectivorous bats as predators and the ecosystem services they provide expanding beyond their contribution to agricultural-related services.

Conclusions

This study illustrates the capability for pest suppression and corroborates other studies that show strong ecosystem services provided by bats. It is likely that reference libraries involving African arthropods are depauperate, resulting in the underrepresentation of genus and species-level of prey insects identified in bat fecal samples. In the future, we suggest adding to the reference libraries by collecting and sequencing a large number of insects from the study sites. We also suggest investigating how composition of pest species in bat diets changes over different seasons. Finally, we recommend further research to demonstrate the economic value of bats in order to promote bat conservation and increase the interest of people in protecting bats. This may in turn increase bat population numbers and provide additional ecosystem services.

Supporting information

S1 Table. Genetic identifications of bat and insect species in pooled guano samples from Rwanda.
(XLSX)

Acknowledgments

Author Contributions

References

1. Oerke E-C. Crop losses to pests. The Journal of Agricultural Science. 2005; 144:31–43.

2. Deutsch CA, Tewksbury JJ, Tigchelaar M, Battisti DS, Merrill SC, Huey RB, et al. Increase in crop losses to insect pests in a warming climate. Science. 2018; 361(6405):916–9. https://doi.org/10.1126/science.aat3466 PMID: 30166490

3. Kunz TH, de Torrez EB, Bauer D, Lobova T, Fleming TH. Ecosystem services provided by bats. In: Ostfeld RS, Schlesinger WH, editors. Year in Ecology and Conservation Biology. Annals of the New York Academy of Sciences. 1223. Oxford: Blackwell Science Publ; 2011. p. 1–38.

4. Maine JJ, Boyles JG. Bats initiate vital agroecological interactions in corn. Proceedings of the National Academy of Sciences. 2015; 112(40):12438–43. https://doi.org/10.1073/pnas.1505413112 PMID: 26371304

5. Baroja U, Garin I, Aihartza J, Arrizabalaga-Escudero A, Vallejo N, Aldasoro M, et al. Pest consumption in a vineyard system by the lesser horseshoe bat (*Rhinolophus hipposideros*). PLoS One. 2019; 14(7).

6. Kemp J, Lopez-Baucells A, Rocha R, Wangensteen OS, Andriatafika Z, Nair A, et al. Bats as potential suppressors of multiple agricultural pests: A case study from Madagascar. Agriculture Ecosystems & Environment. 2019; 269:88–96.

7. Long BL, Kurta A, Clemans DL. Analysis of DNA from Feces to Identify Prey of Big Brown Bats (*Eptesicus fuscus*) Caught in Apple Orchards. The American Midland Naturalist. 2013; 170(2):287–97, 11.

8. Maslo B, Mau RL, Kerwin K, McDonough R, McHale E, Foster JT. Bats provide a critical ecosystem service by consuming a large diversity of agricultural pest insects. Agriculture Ecosystems & Environment. 2022;324.

9. Maslo B, Valentin R, Leu K, Kerwin K, Hamilton GC, Bevan A, et al. Chirosurveillance: The use of native bats to detect invasive agricultural pests. PLoS One. 2017; 12(3). https://doi.org/10.1371/journal.pone.0173321 PMID: 28355216

10. da Silva LP, Oliveira D, Mata VA. Can bats help paper industry? An evaluation of eucalypt insect-related predation by bats. Mammalian Biology. 2022.

11. Ancillotto L, Falanga A, Agostinetto G, Tommasi N, Garonna AP, de Benedetta F, et al. Predator-prey traits and foraging habitat shape the diet of a common insectivorous bat. Acta Oecologica. 2023; 118:103890.

12. Blažek J, Konečný A, Bartonička T. Bat aggregational response to pest caterpillar emergence. Scientific Reports. 2021; 11(1):13634. https://doi.org/10.1038/s41598-021-93104-z PMID: 34211071

13. Charbonnier Y, Papura D, Touzot O, Rhouy N, Sentenac G, Rusch A. Pest control services provided by bats in vineyard landscapes. Agriculture, Ecosystems & Environment. 2021; 306:107207.

14. Garin I, Aihartza J, Goiti U, Arrizabalaga-Escudero A, Nogueras J, Ibáñez C. Bats from different foraging guilds prey upon the pine processionary moth. PeerJ. 2019; 7:e7169. https://doi.org/10.7717/peerj.7169 PMID: 31316870

15. Boyles JG, Cryan PM, McCracken GF, Kunz TH. Economic Importance of Bats in Agriculture. Science. 2011; 332(6025):41–2.

16. Cleveland CJ, Betke M, Federico P, Frank JD, Hallam TG, Horn J, et al. Economic value of the pest control service provided by Brazilian free-tailed bats in south-central Texas. Frontiers in Ecology and the Environment. 2006; 4(5):238–43.

17. Lee Y-F, McCracken GF. Foraging activity and food resource use of Brazilian free-tailed bats, *Tadarida brasiliensis* (Molossidae). Écoscience. 2002; 9(3):306–13.

18. Lee Y-F, McCracken GF. Dietary Variation of Brazilian Free-Tailed Bats Links to Migratory Populations of Pest Insects. Journal of Mammalogy. 2005; 86(1):67–76.

19. Lehmkuhl Noer C, Dabelsteen T, Bohmann K, Monadjem A. Molossid bats in an African agro-ecosystem select sugarcane fields as foraging habitat. African Zoology. 2012; 47(1):1–11.

20. Bohmann K, Monadjem A, Lehmkuhl Noer C, Rasmussen M, Zeale MR, Clare E, et al. Molecular diet analysis of two african free-tailed bats (molossidae) using high throughput sequencing. PLoS One. 2011; 6(6):e21441. https://doi.org/10.1371/journal.pone.0021441 PMID: 21731749

21. Taylor PJ, Grass I, Alberts AJ, Joubert E, Tscharntke T. Economic value of bat predation services–a review and new estimates from macadamia orchards. Ecosystem Services. 2018; 30:372–81.

22. Weier SM, Moodley Y, Fraser MF, Linden VMG, Grass I, Tscharntke T, et al. Insect pest consumption by bats in macadamia orchards established by molecular diet analyses. Global Ecology and Conservation. 2019; 18.

23. Patterson B, Webala P. Keys to the Bats (Mammalia: Chiroptera) of East Africa. Fieldiana: Life and Earth Sciences. 2012; 6:1–63.

24. Zarek SC. A Checklist of the Mammals of Rwanda. Journal of East African Natural History. 2022; 111 (1):1–17.

25. Worldbank. Rwanda: World Development Indicators database 2020 [Available from: https://databank.worldbank.org/views/reports/reportwidget.aspx?Report_Name=CountryProfile&Id=b450fd57&tbar=y&dd=y&inf=n&zm=n&country=RWA).

26. Galindo-González J, Medellín R. Los murciélagos y la COVID-19, una injusta historia. CIENCIA ergo sum. 2021; 28.

27. Rocha R, Aziz SA, Brook CE, Carvalho WD, Cooper-Bohannon R, Frick WF, et al. Bat conservation and zoonotic disease risk: a research agenda to prevent misguided persecution in the aftermath of COVID-19. Animal Conservation. 2021; 24(3):303–7.

28. Pocock MJO, Jennings N. Testing biotic indicator taxa: the sensitivity of insectivorous mammals and their prey to the intensification of lowland agriculture. Journal of Applied Ecology. 2008; 45(1):151–60.

29. Wickramasinghe LP, Harris S, Jones G, Vaughan N. Bat Activity and Species Richness on Organic and Conventional Farms: Impact of Agricultural Intensification. Journal of Applied Ecology. 2003; 40 (6):984–93.

30. ESRI Rwanda, World Topographic Map. https://basemaps.arcgis.com/arcgis/rest/services/World_Basemap_v2/VectorTileServer. Accessed 2 April 2023.

31. NISR, Province Boundaries. https://nsdi-rla.hub.arcgis.com/datasets/fccdd8a0e91649b398a24e75053b4a2f/explore?location=-1.943462%2C29.880500%2C9.60. Accessed 2 April 2023.

32. Aspetsberger F, Brandsen D, Jacobs DS. Geographic variation in the morphology, echolocation and diet of the little free-tailed bat, *Chaerephon pumilus* (Molossidae). African Zoology. 2003; 38(2):245–54.

33. Clark FL, Smart AC. The diet of Tadarida (Chaerephon) pumila (Cretzschmar), Molossidae, at Lake Naivasha, Kenya. Journal of African Zoology. 1991.; 105:493–6.

34. Andriafidison D, Kofoky A, Mbohoahy T, Racey PA, Jenkins RK. Diet, reproduction and roosting habits of the Madagascar free-tailed bat, *Otomops madagascariensis* Dorst, 1953 (Chiroptera: Molossidae). Acta Chiropt. 2007; 9:445–50.

35. Sikes RS, Mammalogists ACaUCotASo. Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. Journal of Mammalogy. 2016; 97(3):663–88.

36. Kunz TH, Kurta A. Capture methods and holding devices. In: Kunz TH, editor. Ecological and Behavioral Methods for the Study of Bats. Washington, D. C.: Smithsonian Institution Press; 1988. p. 1–28.

37. Jusino MA, Banik MT, Palmer JM, Wray AK, Xiao L, Pelton E, et al. An improved method for utilizing high-throughput amplicon sequencing to determine the diets of insectivorous animals. Mol Ecol Resour. 2019; 19(1):176–90. https://doi.org/10.1111/1755-0998.12951 PMID: 30281913

38. Walker FM, Tobin A, Simmons NB, Sobek CJ, Sanchez DE, Chambers CL, et al. A fecal sequel: Testing the limits of a genetic assay for bat species identification. PLoS One. 2019; 14(11):e0224969. https://doi.org/10.1371/journal.pone.0224969 PMID: 31725795

39. Walker FM, Williamson CHD, Sanchez DE, Sobek CJ, Chambers CL. Species From Feces: Order-wide identification of Chiroptera from guano and other non-invasive genetic samples. PLoS One. 2016; 11 (9):e0162342. https://doi.org/10.1371/journal.pone.0162342 PMID: 27654850

40. Colman RE, Schupp JM, Hicks ND, Smith DE, Buchhagen JL, Valafar F, et al. Detection of low-level mixed-population drug resistance in *Mycobacterium tuberculosis* using high fidelity amplicon sequencing. PLoS One. 2015; 10(5):e0126626.

41. O'Rourke DR, Bokulich NA, Jusino MA, MacManes MD, Foster JT. A total crapshoot? Evaluating bioinformatic decisions in animal diet metabarcoding analyses. Ecol Evol. 2020; 10(18):9721–39. https://doi.org/10.1002/ece3.6594 PMID: 33005342

42. Schnell IB, Bohmann K, Gilbert MT. Tag jumps illuminated—reducing sequence-to-sample misidentifications in metabarcoding studies. Mol Ecol Resour. 2015; 15(6):1289–303. https://doi.org/10.1111/1755-0998.12402 PMID: 25740652

43. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnetJournal. 2011; 17(1):10–2.

44. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet C, Al-Ghalith GA, et al. Reproducible, interactive, scalable, and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019; 8:852–7.

45. Wickham H, Mara Averick M, Jennifer Bryan J, Chang W, McGowan LDA, François R, et al. Welcome to the Tidyverse. Journal of Open Source Software. 2019; 4(43):1686.

46. Team RC. R: A language and environment for statistical computing 2022 [Available from: https://www.R-project.org/.

47. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods. 2016; 13(7):581–+. https://doi.org/10.1038/nmeth.3869 PMID: 27214047

48. Rognes T, Flouri T, Nichols B, Quince C, Mahe F. VSEARCH: a versatile open source tool for metagenomics. Peerj. 2016; 4:22. https://doi.org/10.7717/peerj.2584 PMID: 27781170

49. O'Rourke DR, Mangan MT, Mangan KE, Bokulich NA, MacManes MD, Foster JT. Lord of the Diptera (and Moths and a Spider): Molecular Diet Analyses and Foraging Ecology of Indiana Bats in Illinois. Frontiers in Ecology and Evolution. 2021; 9.

50. Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, et al. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome. 2018; 6(1):90. https://doi.org/10.1186/s40168-018-0470-z PMID: 29773078

51. Ratnasingham S, Hebert PDN. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes. 2007; 7:355–64.

52. Puig-Montserrat X, Flaquer C, Gómez-Aguilera N, Burgas A, Mas M, Tuneu C, et al. Bats actively prey on mosquitoes and other deleterious insects in rice paddies: Potential impact on human health and agriculture. Pest Manag Sci. 2020; 76(11):3759–69. https://doi.org/10.1002/ps.5925 PMID: 32431084

53. Puig-Montserrat X, Torre I, López-Baucells A, Guerrieri E, Monti MM, Ràfols-García R, et al. Pest control service provided by bats in Mediterranean rice paddies: linking agroecosystems structure to ecological functions. Mammalian Biology. 2015; 80(3):237–45.

54. Mbeng DW, Bakwo FE-M, Atagana PJ, Joseph LT. The Diet Composition of Four Vesper Bats (Chiroptera: Vespertilionidae) from the Centre Region of Cameroon (Central Africa). International Journal of Natural Resource Ecology and Management. 2019; 4(6):153–63.

55. Aldridge HDJN Rautenbach IL. Morphology, Echolocation and Resource Partitioning in Insectivorous Bats. Journal of Animal Ecology. 1987; 56(3):763–78.

56. Marshall AJ, Corbet PS. The breeding biology of equatorial vertebrates: reproduction of the bat *Chaerephon hindei* Thomas at latitude 0 26 N. Proceedings of the Zoological Society of London. 1959; 132:607–16.

57. Heim O, Lorenz L, Kramer-Schadt S, Jung K, Voigt CC, Eccard JA. Landscape and scale-dependent spatial niches of bats foraging above intensively used arable fields. Ecological Processes. 2017; 6 (1):24.

58. McCracken GF, Westbrook JK, Brown VA, Eldridge M, Federico P, Kunz TH. Bats track and exploit changes in insect pest populations. PLoS One. 2012; 7(8):e43839. https://doi.org/10.1371/journal. pone.0043839 PMID: 22952782

59. Murray SW, Kurta A. Spatial and temporal variation in diet. In: Kurta A, Kennedy J, editors. The Indiana bat: Biology and management of an endangered species. Austin, Texas, USA: Bat Conservation International; 2002. p. 182–92.

60. Gonsalves L, Law B, Webb C, Monamy V. Foraging Ranges of Insectivorous Bats Shift Relative to Changes in Mosquito Abundance. PLoS One. 2013; 8(5):e64081. https://doi.org/10.1371/journal.pone. 0064081 PMID: 23667699

61. Nkrumah EE, Vallo P, Klose SM, Ripperger SP, Badu EK, Gloza-Rausch F, et al. Foraging Behavior and Habitat Selection of Noack's Round-Leaf Bat (*Hipposideros aff*. ruber) and Conservation Implications. Tropical Conservation Science. 2016; 9(4):1940082916680428.

62. Brehm G, Pitkin LM, Hilt N, Fiedler K. Montane Andean Rain Forests Are a Global Diversity Hotspot of Geometrid Moths. Journal of Biogeography. 2005; 32(9):1621–7.

63. Bell GP, Fenton MB. The Use of Doppler-Shifted Echoes as a Flutter Detection and Clutter Rejection System: The Echolocation and Feeding Behavior of *Hipposideros ruber* (Chiroptera: Hipposideridae). Behavioral Ecology and Sociobiology. 1984; 15(2):109–14.

64. Fenton MB, Boyle NGH, Harrison TM, Oxley DJ. Activity Patterns, Habitat Use, and Prey Selection by Some African Insectivorous Bats. Biotropica. 1977; 9:73.

65. Fenton MB, Thomas DW. Dry-Season Overlap in Activity Patterns, Habitat Use, and Prey Selection by Sympatric African Insectivorous Bats. Biotropica. 1980; 12(2):81–90.

66. Naidoo S, Mackey RL, Schoeman MC. Foraging ecology of insectivorous bats (Chiroptera) at a polluted and an unpolluted river in an urban landscape. Durban Museum Novitates. 2011; 34:21–8.

67. Rydell J, Yalden DW. The diets of two high-flying bats from Africa. Journal of Zoology. 1997; 242(1):69–76.

68. Whitaker JO. Food habit analysis of insectivorous bats In: Kunz TH, editor. Ecological and behavioural methods for the study of bats. Washington: Smithsonian Press; 1978. p. 171–89.

69. Jacobs DS, Geeta NE, Schoeman MC, Matthee CA. Cryptic Species in an Insectivorous Bat, *Scotophilus dinganii*. Journal of Mammalogy. 2006; 87(1):161–70.

70. Findley JS, Black H. Morphological and Dietary Structuring of a Zambian Insectivorous Bat Community. Ecology. 1983; 64(4):625–30.

71. Asseburg C, Harwood J, Matthiopoulos J, Smout S. The functional response of generalist predators and its implications for the monitoring of marine ecosystems. In: Camphuysen CJ, Boyd IL, Wanless S, editors. Top Predators in Marine Ecosystems: Their Role in Monitoring and Management Cambridge: Cambridge University Press; 2006. p. 262–74.

72. GASCOIGNE JC, LIPCIUS RN. Allee effects driven by predation. Journal of Applied Ecology. 2004; 41 (5):801–10.

73. van Leeuwen E, Brännström Å, Jansen VAA, Dieckmann U, Rossberg AG. A generalized functional response for predators that switch between multiple prey species. Journal of Theoretical Biology. 2013; 328:89–98. https://doi.org/10.1016/j.jtbi.2013.02.003 PMID: 23422235

74. Naylor RL, Ehrlich PR. Natural pest control services and agriculture. In: Daily GC, editor. Nature's services: Societal dependence on natural ecosystems. Washington, DC: Island Press; 1997.

75. Vreysen MJB, Klassen W, Carpenter JE. Overview of Technological Advances Toward Greater Efficiency and Efficacy in Sterile Insect-Inherited Sterility Programs Against Moth Pests. Florida Entomologist. 2016; 99(sp1):1–12.

76. Bayramoglu Z, Nalcacioglu R, Demirbag Z, Demir I. Characterization of a Betabaculovirus from the fall webworm, *Hyphantria cunea* Drury. (Lepidoptera: Erebidae), in Turkey. Biocontrol Science and Technology. 2018; 28(12):1178–90.

77. Leong SCT, Kueh RJH. Seasonal Abundance and Suppression of Fruit-Piercing Moth *Eudocima phalonia* (L.) in a Citrus Orchard in Sarawak. The Scientific World JOURNAL. 2011; 11:753484.

78. Hazarika LK, Bhuyan M, Hazarika BN. Insect Pests of Tea and Their Management. Annual Review of Entomology. 2009; 54(1):267–84. https://doi.org/10.1146/annurev.ento.53.103106.093359 PMID: 19067632

79. Bonsignore CP, Vacante V, editors. *Duponchelia fovealis* (Zeller). Una nuova emergenza per la fragola2010.

80. Franco MC, Baptista MC. *Duponchelia fovealis* Zeller—nova praga em Portugal. Frutas, legumes e flores 2010; 110:34–5.

81. Efil L, Özgür O, Efil F. A new pest, *Duponchelia fovealis* Zeller, on strawberries in Turkey–damage, distribution and parazitoid. 2014; 328:328–34.

82. Epstein ME. New Pyraloidea in California. California; 2004.

83. Brambila J, Stocks I. The European pepper moth, *Duponchelia fovealis* Zeller (Lepidoptera: Crambidae), a Mediterranean pest moth discovered in central Florida. 2010.

84. Reed WT. Populations and host-plant preferences of *Earias* spp. (Lepidoptera, Noctuidae) in East Africa. Bulletin of Entomological Research. 1974; 64:33–44.

85. Bishop AL, Blood PRB, Day RE, Evenson JP. The distribution of cotton looper (*Anomis flava* Fabr.) larvae and larval damage on cotton and its relationship to the photosynthetic potential of cotton leaves at the attack sites. Australian Journal of Agricultural Research. 1978; 29:319–25.

86. Ferino MP, Calora FB, Magallona ED. Population dynamics and economic threshold level of the cotton semi-looper, *Anomis flava flava* (Fabr.) (Noctuidae, Lepidoptera). The Philippine Entomologist. 1982; 5:401–46.

87. Kuklinski F. The cotton pests in Madagascar, with special emphasis on the cotton aphid *Aphis gossypii* (Hom.: Aphididae) and its natural enemies: Hannover University; 2000.

88. Gupta A, Smetacek P. A new larval host record for *Sphingomorpha chlorea* (Cramer) (Insecta: Lepidoptera: Noctuidae) from Karnataka, India. Journal of Threatened Taxa. 2011; 3:1553–4.

89. Murillo H, Hunt D, Vanlaerhoven S. First records of *Chrysodeixis chalcites* (Lepidoptera: Noctuidae: Plusiinae) for east-central Canada. The Canadian Entomologist. 2013; 145:338–42.

90. HOSTS A database of the world's lepidopteran host plants [Internet]. Natural History Museum, London. 2008. Available from: http://www.nhm.ac.uk/research-curation/research/projects/hostplants/.

91. EPPO. Pest risk analysis for *Thaumatotibia leucotreta*. Paris; 2013.

92. Moore SD. The development and evaluation of *Cryptophlebia leucotreta granulovirus* (CrleGV) as a biological control agent for the management of false codling moth, *Cryptophlebia leucotreta*, on citrus: Rhodes University; 2002.

93. Mansor M, Idris A. Temporal Population Abundance of Leafhopper (Homoptera: Cicadelidae) and Planthopper (Homoptera: Delphacidae) as Affected by Temperature, Humidity and Rice Growth Stages. 2014.

94. Mixão V, Bravo Barriga D, Parreira R, Novo MT, Sousa CA, Frontera E, et al. Comparative morphological and molecular analysis confirms the presence of the West Nile virus mosquito vector, Culex univittatus, in the Iberian Peninsula. Parasit Vectors. 2016; 9(1):601. https://doi.org/10.1186/s13071-016-1877-7 PMID: 27884174

95. Seruyange E, Ljungberg K, Muvunyi CM, Gahutu JB, Katare S, Nyamusore J, et al. Seroreactivity to Chikungunya and West Nile Viruses in Rwandan Blood Donors. Vector borne and zoonotic diseases (Larchmont, NY). 2019; 19(10):731–40. https://doi.org/10.1089/vbz.2018.2393 PMID: 31246538

96. Robinson WH. Urban Insects and Arachnids: A Handbook of Urban Entomology. Cambridge: Cambridge University Press; 2005.