Agriculture, Ecosystems and Environment 269 (2019) 88–96

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Bats as potential suppressors of multiple agricultural pests: A case study from Madagascar

James Kemp[a,*], Adrià López-Baucells[a,b,c], Ricardo Rocha[c,d], Owen S. Wangensteen[e], Zo Andriatafika[c,f], Abhilash Nair[c], Mar Cabeza[c]

[a] Center for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Ciências da Universidade de Lisboa, 1749-016, Lisboa, Portugal
[b] Granollers Museum of Natural Sciences, 08402, Granollers, Catalonia, Spain
[c] Global Change and Conservation Lab, Helsinki Institute of Sustainability Science, Faculty of Biological and Environmental Science, University of Helsinki, FI-00014, Helsinki, Finland
[d] Conservation Science Group, Department of Zoology, University of Cambridge, Cambridge, CB2 3EJ, UK
[e] Norwegian College of Fishery Science, University of Tromsø The Arctic University of Norway, Norway
[f] Institute of Science and Technics of the Environment (ISTE), University of Fianarantsoa, BP 1264, Fianarantsoa, Madagascar

ARTICLE INFO

Keywords:
Chiroptera
Acoustics
Agriculture
Rice
Ecosystem services
Crop pests
Pest control
Metabarcoding

ABSTRACT

The conversion of natural habitats to agriculture is one of the main drivers of biotic change. Madagascar is no exception and land-use change, mostly driven by slash-and-burn agriculture, is impacting the island's exceptional biodiversity. Although most species are negatively affected by agricultural expansion, some, such as synanthropic bats, are capable of exploring newly available resources and benefit from man-made agricultural ecosystems. As bats are known predators of agricultural pests it seems possible that Malagasy bats may be preferentially foraging within agricultural areas and therefore provide important pest suppression services. To investigate the potential role of bats as pest suppressors, we conducted acoustic surveys of insectivorous bats in and around Ranomafana National Park, Madagascar, during November and December 2015. We surveyed five landcover types: irrigated rice, hillside rice, secondary vegetation, forest fragment and continuous forest. 9569 bat passes from a regional assemblage of 19 species were recorded. In parallel, we collected faeces from the six most common bat species to detect insect pest species in their diet using DNA metabarcoding. Total bat activity was higher over rice fields when compared to forest and bats belonging to the open space and edge space sonotypes were the most benefited by the conversion of forest to hillside and irrigated rice. Two economically important rice pests were detected in the faecal samples collected - the paddy swarming armyworm *Spodoptera mauritia* was detected in *Mops leucogaster* samples while the grass webworm *Herpetogramma licarsisalis* was detected from *Mormopterus jugularis* and *Miniopterus majori* samples. Other crops pests detected included the sugarcane cicada *Yanga guttulata*, the macadamia nut-borer *Thaumatotibia batrachopa* and the sober tabby *Ericeia inangulata* (a pest of citrus fruits). Samples from all bat species also contained reads from important insect disease vectors. In light of our results we argue that Malagasy insectivorous bats have the potential to suppress agricultural pests. It is important to retain and maximise Malagasy bat populations as they may contribute to higher agricultural yields and promote sustainable livelihoods.

1. Introduction

The pervasive conversion of forests for food production is a conspicuous symbol of the Anthropocene (Malhi, 2017). Large swaths of forest have already been cleared for agriculture and the encroachment of natural ecosystems is due to continue as human populations and food demand continue to rise (Giam, 2017). Madagascar holds a unique ensemble of ecosystems and wildlife that is almost unmatched in its biological uniqueness (Goodman and Benstead, 2005). However, despite its high level of endemism and species diversity, Madagascar's forests continue to face one of the highest rates of conversion in the world with approximately 1% of the island's forest cover being cleared each year (Rasolofoson et al., 2015; Eklund et al., 2016; Vieilledent et al., 2018). While most Malagasy biodiversity is adversely affected by agriculture-driven habitat modification, some 'winner' species benefit from habitat modification and increase their abundance in agricultural

---

[*] Corresponding author.
E-mail address: jamesrussellkemp@gmail.com (J. Kemp).

https://doi.org/10.1016/j.agee.2018.09.027
Received 19 June 2018; Received in revised form 28 August 2018; Accepted 4 September 2018
Available online 01 October 2018
0167-8809/ © 2018 Elsevier B.V. All rights reserved.

areas. Several of these are insectivorous birds (Martin et al., 2012; Rocha et al., 2015) and bats (López-Baucells et al., 2017b) that through the suppression of agricultural pests can provide valuable ecosystem services to local populations (Karp and Daily, 2014; Maas et al., 2016).

Rice (*Oryza* spp.) is one of the most important staple food crops worldwide (Muthayya et al., 2014). It is the main crop cultivated by Malagasy subsistence farmers (Kari and Korhonen-Kurki, 2013) throughout the island, and as in numerous other high-biodiversity regions across the tropics, much of the ongoing deforestation is due to agricultural expansion for rice production (McConnell et al., 2004; Neudert et al., 2017). Such a high dependency on rice creates problems when yields are affected by climatic events or pest outbreaks (Harvey et al., 2014). Insect rice pests are known to cause severe damage to rice crop yields (Oerke, 2006). Rice crop losses are predominantly caused by Lepidopteran stem borers found across several families such as the Noctuidae, Pyralidae, Tortricidae or Geometridae (Nwilene et al., 2013).

Modern day farming techniques incorporate Integrated Pest Management (IPM) to control pest populations (Stenberg, 2017). However, many small-holder farmers in sub-Saharan Africa are unable to access IPM techniques due to lack of financial capital or expertise (Parsa et al., 2014). A sustainable and low cost method to aid pest control and reduce crop losses is through biological control (Bommarco et al., 2013; Naranjo et al., 2015). Biological control, as part of a wider application of integrated pest management practices, can involve insectivorous bats, and has already been proven effective for pecan and rice farms in the USA and Catalonia (Brown et al., 2015; Puig-Montserrat et al., 2015). Multiple lines of evidence support that aerial hawking insectivorous bats provide valuable agricultural pest control services in both temperate and tropical regions (Boyles et al., 2011; Karp and Daily, 2014; Wanger et al., 2014; Brown et al., 2015; Russo et al., 2018). For instance, in the Mediterranean the soprano pipistrelle *Pipistrellus pygmaeus* was found to suppress rice borer moth *Chilo suppressalis* populations through opportunistic foraging (Puig-Montserrat et al., 2015). However, to date most research on tropical bat predation services has focussed on coffee and cacao agroecosystems (Maas et al., 2016), with limited research targeting rice (Wanger et al., 2014). One notable exception comes from Thailand where it was estimated that predation of white backed planthoppers *Sogathella furcifera* by wrinkle-lipped bats *Tadarida plicata* prevents rice crop losses valued at > 1.2 million USD (or > 26,000 rice meals) each year (Wanger et al., 2014).

Numerous bat species (particularly of the Molossidae and Vespertilionidae families) are known to coexist synanthropically by exploring newly available resources. These bat families have been shown to feed on pests (Brown et al., 2015) and to select crops as preferred foraging areas especially during insect pest outbreaks (Lehmkuhl Noer et al., 2012; Taylor et al., 2013a; Davidai et al., 2015). In fact, bats tend to select foraging areas based upon the resources available (Ancillotto et al., 2017), which makes them excellent pest suppressors during seasonal insect pest outbreaks.

Large colonies of molossid bats roost in buildings across Madagascar (Razafindrakoto et al., 2010; López-Baucells et al., 2017b). However, any potential predation services provided by these colonies are yet to be explored. Forty-two species of insectivorous bats occur in Madagascar, with several species occurring more frequently in anthropogenic landscapes as opposed to forest habitats (Randrianandrianina et al., 2006; Rakotoarivelo et al., 2007). In general, most studies have focused on the dry western region (Goodman et al., 2005; Kofoky et al., 2006; Bambini et al., 2010; Racey et al., 2010; Fernández-Llamazares et al., 2018) as opposed to the humid eastern zone (Randrianandrianina et al., 2006) and only a few studies have tackled habitat selection while none have addressed the potential pest suppressor role in agricultural areas.

The DNA metabarcoding of bat faecal pellets can offer valuable insights into the dietary preferences of bats and their potential role as pest suppressors (Bohmann et al., 2014; Swift et al., 2018). Recent diet analyses of multiple bat species have detected a wide range of arthropods in bat populations (Galan et al., 2018) including several economically important pest species (Taylor et al., 2017).

Here, we combine bioacoustics and DNA metabarcoding to investigate if Malagasy insectivorous bats are foraging within the island's agricultural matrix and if they are consuming important pest species. Specifically, we address the following questions:

i) How does total bat activity, species (or species-group) activity and assemblage composition change across a rice-dominated agroecosystem landscape? We hypothesise that due to higher insect availability some bats will be more active over rice fields compared to forested sites. We also predict a clear shift in assemblage composition from open to closed landcover types.
ii) Which species (or species-groups) are more common within the agricultural matrix? We predict that synanthropic molossids will be particularly abundant in rice fields but we still anticipate some forest associated species to forage outside the forest border.
iii) Are bats roosting within the agricultural matrix predating on agricultural insect pests? We expect bats to predate mainly on moths and beetles and we predict that several of these will be agricultural pests of rice and other crops.

2. Methods

2.1. Study area

Fieldwork was conducted primarily in the peripheral zone surrounding the Ranomafana National Park (RNP) (21°16′S, 47°20′E). The peripheral zone comprises over 160 villages with a population in excess of 50,000 in an area of approximately 500 km^2 (Kari and Korhonen-Kurki, 2013). Agricultural communities in the region, like many throughout Madagascar, cultivate rice through slash-and-burn agriculture (*tavy*) and irrigated paddies (Peters, 1998; Brooks et al., 2009). The RNP is located between the central highlands and the eastern lowlands and is of particular ecological and economic interest due to its high biodiversity and watershed protection role.

2.2. Bat surveys

Bats were surveyed from November to December 2015 in 54 sites in and around RNP (Fig. 1). Sites were clustered around seven villages (Kelilalina, Tsaratanana, Mangevo, Andriamamovoka, Amboasary, Mandriandry and Tolongoina) and were classified into five landcover categories: irrigated rice fields ($n = 12$), hillside rice fields ($n = 8$), secondary vegetation i.e. fallow agricultural land of mixed successional vegetation ($n = 11$), forest fragment ($n = 9$) and continuous forest in RNP ($n = 15$) (for landcover images and description see Supplementary materials Fig. A.1.). Bat activity was recorded using SongMeter SM2BAT + and SM3 autonomous bat detectors (Wildlife Acoustics, Concord, MA, USA). Detectors were secured to a tree at approximately 1.5 m with external SMX-II omni-directional microphones (Wildlife Acoustics, Concord, MA, USA). Detectors were set to record calls continuously from 18:00 until 06:00 for three consecutive nights at each locality. Bat activity was sampled for 1,956 h across a total of 147 detector-nights of sampling effort. Detectors were set with a 384 kHz sample rate, 12 kHz digital high pass filter, 18 dB trigger level, microphone bias off, and 36 dB gain. We used a 1.0 s trigger window minimum to capture calls prior to the initial trigger.

2.3. Bioacoustic analysis

Recordings were manually classified using Kaleidoscope software version 3.1.7 (Wildlife Acoustics, Concord, MA, USA). We defined a bat pass as a recording of five seconds maximum with at least two pulses with more than 20 dB of difference between the background noise and bat call (Appel et al., 2017) Call sequences were manually identified to

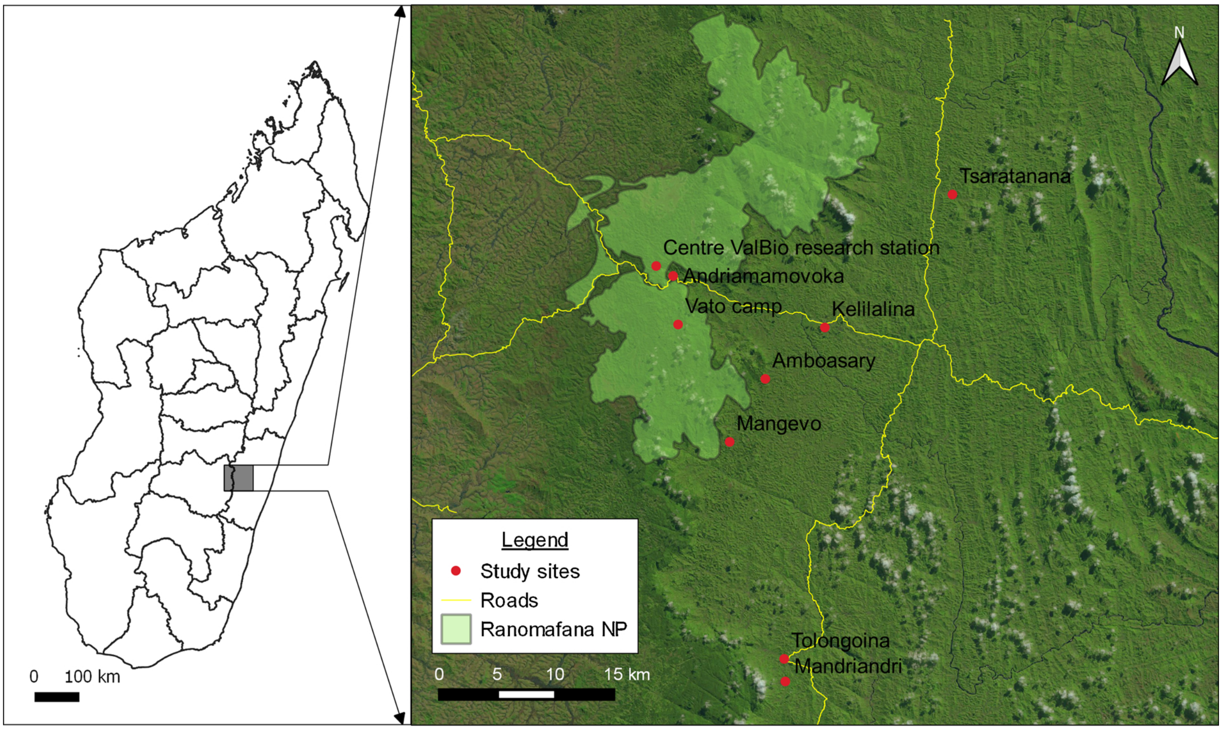

Fig. 1. Map of sampling sites within and surrounding the Ranomafana National Park, Madagascar.

Table 1
List of species known to occur in the region incorporating Ranomafana National Park with sonotypes created from mean peak frequency ranges from the existing literature.

Sonotype	Family	Species	Mean peak frequency ranges (kHz)	Call shape	IUCN category
H.commersoni	Hipposideridae	*Hipposideros commersoni* [1]	61.6–76.5	FM-CF-FM	NT
M.goudoti	Vespertilionidae	*Myotis goudoti* [1]	55.3–72.1	FM	LC
M.gle/maj	Miniopteridae	*Miniopterus gleni* [1, 2], *Miniopterus majori* [1, 2]	42.9–50.3	FM-QCF	LC; LC
M.manavi	Miniopteridae	*Miniopterus manavi* [1, 2]	58.3–61.5	FM-QCF	LC
M.aurita	Myzopodidae	*Myzopoda aurita* [3]	14	FM-CF	LC
O.mad/T.ful	Molossidae	*Otomops madagascariensis* [3] *Tadarida fulminans* [3]	13.0–20.0	CF	LC; LC
P.atrata	Emballonuridae	*Paraemballonura atrata* [1]	50.0–54.3	FM-CF	LC
S.rob/M.gle	Vespertilionidae/ Minioperidae	*Miniopterus gleni* [1, 2], *Scotophilus robustus* [1]	38.4–42.8	FM-QCF	LC; LC
VMi1	Vespertilionidae/ Miniopteridae	*Miniopterus gleni* [1, 2], *Miniopterus manavi* [1, 2], *Miniopterus majori* [1, 2], *Miniopterus soroculus* [2], *Neoromicia matroka* [4], *Pipistrellus hesperidus* [4], *Pipistrellus raceyi* [4]	50.4–58.2	FM-QCF	LC; LC; LC; LC; LC; LC; DD
S.rob/M.jug	Molossidae; Vespertilionidae	*Mormopterus jugularis* [5], *Scotophilus robustus* [1]	30.6–38.3	FM-QCF	LC; LC
Mo1	Molossidae; Emballonuridae	*Chaerephon atsinanana* [5], *Mops leucostigma* [5], *Mormopterus jugularis* [5], *Taphozous mauritianus* [6]	21.0–30.5	FM-QCF/ CF	NA; LC; LC; LC

Sources: Kofoky et al., 2009, 2. Ramasindrazana et al., 2011, 3. Russ and Bennett, 2001, 4. Goodman et al., 2015, 5. Release calls, 6. Fenton et al., 1980.

species level or left as mixed species groups, or sonotypes, where it was not possible to clearly assign a call to a particular species (Torrent et al., 2018). Call sequences were also identified as feeding buzzes (specific echolocation sequence that a bat uses as it pursues and subsequently catches its prey). We used the frequency of maximum energy or peak frequency (kHz), the start and ending frequencies (kHz), the duration (ms) and the call shape to identify or group species from the existing literature and our own release calls (Fenton et al., 1980; Russ and Bennett, 2001; Kofoky et al., 2009; Goodman et al., 2011, 2015). Our analysis included a total of 11 sonotypes from the families Emballonuridae, Hipposideridae, Molossidae, Miniopteridae, Myzopodidae and Vespertilionidae. Five sonotypes were classified to species level and the remaining six into sonotype groups (Table 1).

2.4. Faecal sample collection

Mist-nets were used to capture bats at roosts in five villages in the RNP area (for sampling details see López-Baucells et al., 2017a,b). Three caves were inspected and surveyed with mist-netting outside of the emergence point (for *Miniopterus* spp. and *Myotis goudoti*). Bats were measured, weighed and identified using keys (Russ and Bennett, 2001; Goodman, 2011). Bat capture and handling was conducted following guidelines approved by the American Society of Mammalogists (Sikes et al., 2011). We kept the bats in cloth bags for an hour until defecation occurred. Faecal pellets were labelled and stored in 2 ml tubes in 95% ethanol and stored in a cool dry space. Of the 322 bats caught, we collected faecal samples from 150 bats. Fifty-eight faecal samples from six species (*Chaerephon atsinanana*, *Mops leucostigma*, *Mormopterus* *jugularis*, *Myotis goudoti*, *Miniopterus manavi*, *Miniopterus majori*) were used for the diet analysis.

2.5. DNA extraction and PCR amplification

The DNA was extracted from the faecal samples using the Norgen Stool Kit following instructions provided by the manufacturers (Norgen Biotek Corp.). Amplification of DNA from the faeces was achieved using the Leray-XT PCR primer set (Wangensteen et al., 2018b), a highly degenerated primer set targeting a 313-bp fragment of the mitochondrial cytochrome c. oxidase subunit I (COI) region. The mlCOIintF-XT primer (5′-GGWACWRGWTGRACWITITAYCCYCC-3′) was used as forward primer. This modified version (Wangensteen et al., 2018b) of the mlCOIintF primer (Leray et al., 2013) included two extra degenerate bases (equimolar mixtures of two different bases at a given position) and two inosine nucleotides to enhance its eukaryotic universality. The reverse primer was jgHCO2198 (5′-TAIACYTCIGGRTGICCRAARAAYCA-3′;(Geller et al., 2013)). The Leray fragment has already been successfully applied to the characterisation of both marine fish gut contents (Leray et al., 2013), marine invertebrates (Siegenthaler et al., 2018) and terrestrial arthropods (Macías-Hernández et al., 2018). A single-PCR step using primers with attached eight-base oligo-tags (Coissac et al., 2012) was used to label different samples in a multiplexed library; moreover a variable number (2, 3 or 4) of fully degenerate positions (Ns) was added at the beginning of each primer, in order to increase variability of the amplicon sequences (Guardiola et al., 2015). The PCR mix recipe included 10 µl AmpliTaq gold 360 master mix (Applied Biosystems), 3.2 µg Bovine Serum Albumin (Thermo-Scientific), 1 µl of each of the 5 µM forward and reverse tagged-primers, 5.84 µl water and 2 µl extracted DNA template (~ 5 ng µl-1). The PCR profile included an initial denaturing step of 95 °C for 10 min, 35 cycles of 94 °C for 1 min, 45 °C for 1 min and 72 °C for 1 min and a final extension step of 72 °C for 5 min. After a quality check of all amplicons by electrophoresis, the tagged PCR products (including a negative control) were pooled into a multiplexed sample pool and purified using MinElute columns (Qiagen). An Illumina library was subsequently built from these pools, using the NextFlex PCR-free library preparation kit (BIOO Scientific). The library was sequenced on an Illumina MiSeq platform using v3 chemistry (2 × 250 bp paired-ends), as part of a multiplexed run including ten other unrelated libraries.

2.6. Bioinformatic analyses

Bioinformatic analyses were performed using the OBITools metabarcoding software suite (Boyer et al., 2016). Read quality assessment was performed with FastQC and only paired-end reads with phred quality score > 40 was retained. Demultiplexing and primer removal were achieved using ngsfilter. Obigrep was applied to select all aligned reads with a length between 303–323 bp and without ambiguous bases. Obiuniq was used to dereplicate the reads and the uchime-denovo algorithm (Edgar et al., 2011) implemented in VSEARCH (Rognes et al., 2016) was used to remove chimeric sequences. Amplicon clustering was performed using the SWARM 2.0 algorithm (Mahé et al., 2015) with a distance value of $d = 13$, which offers a conservative solution to the high variability of the COI gene (Siegenthaler et al., 2018). Taxonomic assignment of the representative sequences for each molecular operational taxonomic unit (MOTU) was performed using the ecotag algorithm (Boyer et al., 2016), using a local reference database (Wangensteen et al., 2018b) containing filtered COI sequences retrieved from the BOLD database (Ratnasingham and Hebert, 2007) and the EMBL repository (Kulikova et al., 2004). This algorithm uses a phylogenetic approach to assign sequences to the most reliable monophyletic unit, so that sequences are assigned to different taxonomic ranks, depending on the density of the reference database. The data was refined by removing contaminations of marine origin (originated by tag-switching from other multiplexed libraries in the sequencing run). A minimum abundance filter of 5 total reads was used to avoid false positives and low frequency noise (De Barba et al., 2014; Wangensteen and Turon, 2017). This pipeline, with little variations, has been previously used for analysing metabarcoding data for the same COI marker in a variety of systems (e.g. Wangensteen and Turon, 2017; Macías-Hernández et al., 2018; Siegenthaler et al., 2018; Wangensteen et al., 2018a, b). The resulting data has been deposited on Mendeley Data ([dataset] Kemp et al., 2018)

2.7. Statistical analysis

Bat activity was defined as the total number of bat passes per night from all species as well as for each sonotype (Torrent et al., 2018). As appropriate for count data, negative binomial or Poisson generalised linear mixed models (GLMMs) with a log link function were used to model the relationship between bat activity and landcover type (continuous forest, forest fragments, secondary vegetation, hillside rice and irrigated rice) (Burnham and Anderson, 2003). Species with less than 300 recordings were not used in the analysis due to a lack of model convergence. Since preliminary analyses suggested that the count data were overdispersed, we accounted for this overdispersion by using a Poisson or negative binomial regression in *glmer* or *glmmADMB* and adding a random intercept of "Site" nested within "Location" (Bates, 2010).

Numbers of bat passes were positively correlated with feeding buzzes (Table A.1). We therefore only used the larger bat passes dataset for modelling as a proxy for feeding activity (Torrent et al., 2018). Moran's *I* test showed that there is no residual spatial autocorrelation between sites (Table A.2). The difference in assemblage structure between landcover types was analysed using the analysis of dissimilarity test *adonis*. It was visualised through a non-metric multidimensional scaling (NMDS), based on a Bray-Curtis dissimilarity matrix, using sonotype activity data per site. We analysed and presented the data using R statistical software 3.4.1 (R Development Core Team, 2017) with the packages: tidyverse (Wickham, 2016), lme4 (Bates et al., 2014), glmmADMB (Skaug et al., 2015) and vegan (Oksanen et al., 2013).

The relative abundance of MOTU reads for prey items (excluding predator reads and normalized to 10,000 total prey reads per sample) was calculated for all prey MOTUs. The relative abundances per faecal sample for all prey MOTUs were then averaged per bat species. We then grouped the MOTU sequences by arthropod orders and highlighted the pest and disease transmitting insect species, alongside any species or genera that we suspected to have a potential pest status.

3. Results

3.1. Bat activity

We recorded a total of 9569 bat passes, of which 1643 (17%) were identified to species level (*Hipposideros commersoni*, *M. manavi, M. goudoti, Myzopoda aurita*, *Paraemballonura atrata*), 2261 (24%) were identified to sonotypes of two species (*Miniopterus gleni/M. majori*, *Scotophilus robustus/M. gleni*, *S. robustus/M. jugularis, Otomops madagascariensis/Tadarida fulminans*), and 5665 (60%) were attributed to sonotypes Molossidae 1 (Mo1: *C. atsinanana, M. leucostigma, M. jugularis, Taphozous mauritianus)* and Vespertilionidae/Miniopteridae 1 (VMi1: *M. gleni, M. majori, M. manavi, Miniopterus soroculus, Neoromicia matroka, Pipistrellus hesperidus, Pipistrellus raceyi*). In total 1013 feeding buzzes were recorded, with Mo1 accounting for 389 (38%) of feeding buzzes, VMi1 for 334 (33%) and *P. atrata* for 102 (10%).

Bat activity was highest in hillside rice with a mean of 197 passes/night and more than double that of the next landcover type with more bat activity - irrigated rice at 89 passes/night (Table 2). Overall bat activity in both types of rice field, hillside and irrigated, was higher than activity levels in continuous forest (Table A.3). According to pairwise comparisons (Table A.4), total bat activity over hillside rice

Table 2
Mean bat passes (± SD) per night per sonotype across each landcover type. Significant differences to continuous forest from generalised linear mixed models highlighted in bold.

Sonotype	Landcover type					
	Continuous forest	Forest fragment	Secondary vegetation	Irrigated rice	Hillside rice	Total
Hipposideros commersoni	0	0.04 (± 0.2)	0	0.03 (± 0.2)	1.09 (± 3)	0.18 (± 1)
Myzopoda aurita	0	0	0.04 (± 0.2)	0.13 (± 0.6)	0.22 (± 0.5)	0.07 (± 0.3)
Myotis goudoti	4.21 (± 10)	14.65 (± 30)	2.16 (± 4)	**9 (± 23)**	**4.22 (± 4)**	6.69 (± 18)
Miniopterus gleni/M.majori	0.05 (± 0.2)	**0.58 (± 1)**	**2.56 (± 4)**	**7.8 (± 11)**	**15.65 (± 15)**	4.59 (± 10)
Miniopterus manavi	0	2.85 (± 8)	0.04 (± 0.2)	0	0.04 (+0.2)	0.52 (± 3)
Mo1	0.12 (± 0.5)	**5.5 (± 11)**	**20.08 (± 38)**	**10.67 (± 15)**	**98.7 (± 131)**	22.04 (± 64)
Otomops madagascariensis/Tadarida fulminans	0.07 (± 0.5)	0.92 (± 2)	0.52 (± 1)	0.17 (± 1)	**23.61 (± 100)**	4 (± 40)
Paraemballonura atrata	5.4 (± 29)	0.04 (± 0.2)	1.96 (± 7)	6.87 (± 22)	2.48 (± 6)	3.71 (± 19)
Scotophilus robustus/Miniopterus gleni	0	0	5.08 (± 8)	3.00 (± 5)	23.74 (± 26)	5.19 (± 14)
Scotophilus robustus/Mormopterus jugularis	0	0	1.52 (± 3)	1.93 (± 3)	6.04 (± 8)	1.60 (± 4)
VMi1	2.84 (± 7)	7.69 (± 15)	5.84 (± 8)	**48.9 (± 100)**	**21.3 (± 17)**	16.50 (± 49)
Total	12.72 (± 36)	32.27 (± 55)	39.80 (± 53)	**88.50 ± (127)**	**197.09 (± 228)**	65.1 (± 128)

was higher than in continuous forest ($p < 0.01$) and forest fragments ($p < 0.05$) whereas activity in irrigated rice was only higher than continuous forest ($p < 0.01$).

In hillside and irrigated rice, Mo1, VMi1, *M. goudoti* and *M. gleni/M. majori,* had significantly higher activity compared to continuous forest while *O. madagascariensis/T. fulminans* was higher in hillside rice compared to continuous forest. In continuous forest and forest fragments, *P. atrata* and *M. goudoti* had the highest mean bat passes/night, respectively (Fig. 2).

3.2. Assemblage composition

Assemblage composition varied between landcover type (adonis: $r^2 = 0.253$; $p = 0.001$). This was corroborated by the NMDS ordination which revealed distinct patterns of dissimilarities in assemblage composition between the five landcover classes (Fig. 3). The NMDS had a final stress value of 0.12 conveying a good representation of the data along the represented dimensions.

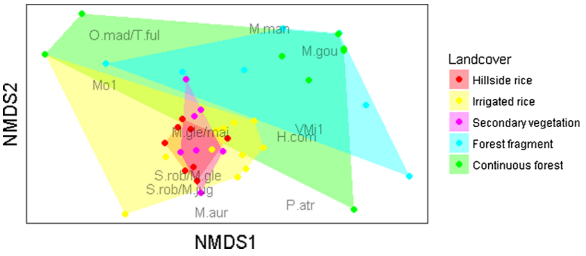

Fig. 3. NMDS plot showing community assemblage of sonotypes (in text) relative to sampling sites (coloured dots – corresponding to landcover type). See Table 1 for sonotype abbreviations.

3.3. Presence of insect pests in faecal samples (DNA metabarcoding)

We obtained a total number of 655,205 MOTU reads from all samples. 43.5% (285,978) of the reads were attributed to bats while 5.3% (34,599) of the reads were assigned to arthropods. Overall, when looking at the insect orders found in the faecal samples, the highest average relative abundance of MOTU reads found were of Coleoptera,

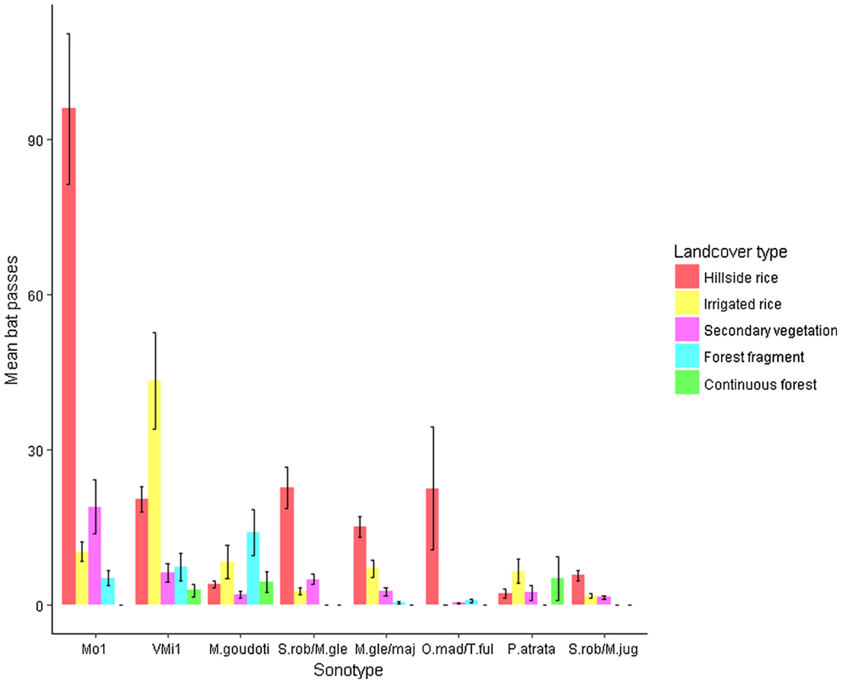

Fig. 2. Mean bat activity per night per sonotype (> 300 passes) for each landcover type, with standard errors. See Table 1 for sonotype abbreviations.

Table 3
Average relative abundance of MOTU reads per 10,000 reads for six bat species (number of samples in brackets) grouped by arthropod order. See Table A.5 for insect pest and disease vector species and genera.

Order name	*C. atsinanana* (12)	*M. leucogaster* (10)	*M. jugularis* (9)	*M. goudoti* (9)	*M. majori* (6)	*M. manavi* (12)	Average
Araneae	0	0	0	27.34	0	0	4.56
Astigmata	0	0	0.3	0	1.68	2.54	0.76
Blattodea	2.7	33.16	134.95	19.62	307.45	325.05	137.15
Coleoptera	1095.78	1708.65	1845.63	891.49	112.43	672.35	1054.39
Dermaptera	0	0	19.36	0	0	0	3.23
Diptera	64.56	94.34	834.68	163.94	137.09	208.7	250.55
Ephemeroptera	625.26	17.09	1053.68	67.67	76.4	44.52	314.1
Hemiptera	1.42	17.23	18.86	30.32	1933.65	661.81	443.88
Hymenoptera	0.05	1.3	0.1	352.68	104.67	708.29	194.51
Lepidoptera	138.94	63.06	414.78	324.2	2351.33	846.68	689.83
Mesostigmata	0	0.8	0	1.28	0	26.12	4.7
Neuroptera	0	0	3.96	0	0	0.69	0.78
Odonata	0.32	0	0	0	0	0	0.05
Orthoptera	3.23	2.68	3.35	0	0	0	1.54
Sarcoptiformes	0.66	14.46	2.57	72.57	1.8	8.05	16.69
Siphonaptera	0.31	0	0	0	0	0	0.05
Symphypleona	0	0	0	1.74	0	0	0.29
Trichoptera	3.05	0.02	0	3.51	10.22	0	2.8
Trombidiformes	0.5	0.33	0	0.2	185.73	2.67	31.57
Total	1936.78	1953.11	4332.25	1956.53	5222.45	3507.48	

Lepidoptera, Ephemeroptera, Diptera and Hemiptera (Table 3). All the bats species sampled fed on, at least, 11–13 orders of arthropods.

In 58 bat faecal samples we found six known pest species, seven insect vectors of human diseases and 17 potential pest taxa (Table A.5). Of the known agricultural pests found in the faecal samples, two economically important rice pest species were found – the paddy swarming caterpillar *Spodoptera mauritia* in *M. leucogaster* and the grass webworm *Herpetogramma licarsisalis* in *M. jugularis* and *M. majori*. Other crops pests detected were: the black twig borer *Xyleborus ferrugineus* a pest of coffee; the sugarcane cicada *Yanga guttulata;* the macadamia nut-borer *Thaumatotibia batrachopa* and the sober tabby *Ericeia inangulata* a pest of citrus fruits. Potential pest species and genera, from the order Lepidoptera, were found in all bat species. In particular: *Mythimna* sp. – a genus containing the rice armyworm *Mythimna unipuncta*; *Emmalocera* sp. – a genus containing a sugarcane root borer *Emmalocera depressella*; and *Cydia choleropa* – a sister species of the codling moth *Cydia pomonella* a pest of apples and pears.

4. Discussion

Large colonies of, predominantly, molossid, vespertillionid and miniopterid bats, were found to be preferentially selecting the rice fields surrounding the RNP. Six species of bats were shown to have fed upon economically important insect pests such as the paddy swarming caterpillar (*Spodoptera mauritia*) and the Grass webworm (*Herpetogramma licarsisalis*). In agreement with Puig-montserrat et al. (2011) and Wanger et al. (2014) insectivorous bats, particularly molossids, are likely to be preferentially selecting rice fields for foraging and feeding upon rice crops pests and other economically important insects.

4.1. Bat activity across landcover types

The highest overall mean activity was found in hillside rice followed by irrigated rice and secondary vegetation (Table 2). Hillside rice has markedly lower yields compared to lowland irrigated rice. Water and nutrient run-off impact the growth of upland rice. A lack of water and nutrient retention in the rice crop makes it more susceptible to insect pest infestations. This may be one reason why we recorded the highest activity in hillside rice. However, it is also possible that there was an altitudinal detection bias as hillside rice and secondary vegetation sites were on open hillsides with little vegetation and facing large valleys (Collins and Jones, 2009). Both sites, however, were found at similar altitudes and had markedly different results (Tables 2 and A.3). Irrigated rice sites, on the other hand, are found at the bottom of valleys. Despite the possible altitudinal bias, activity within irrigated rice was the second highest of the landcover types (Tables 2 and A.3). Intensive rice agriculture harbours high densities of insect pests which provide an excellent resource for insectivorous bats. Insects form swarms, especially tympanic moths (Noctuidae, Crambidae and Pyralidae), during mating and emergence, which bats are able to opportunistically prey upon (McCracken et al., 2012; Malmqvist et al., 2018).

The open space group, Mo1, was the most active overall and over hillside rice (Fig. 2, Table 2). This suggests that open-space aerial hawkers are important agents of pest suppression in the rice-dominated landscape surrounding the RNP and potentially throughout much of Madagascar's agroecosystems. Further research and action is required to improve the knowledge of bats dietary preferences, both temporally and spatially. The reputation of these bats among local communities needs to be improved, especially as they form large colonies in public buildings (López-Baucells et al., 2017b).

The results conform to the notion that molossids (in addition to *Taphozous mauritianus*), which are open-space aerial hawkers, commute and forage at higher altitudes than other families (Lee and McCracken, 2002; McCracken et al., 2008; Taylor et al., 2013b). Open space foragers have a high wing loading ratio (fast flight; low manoeuvrability) which suggests that they do not use cluttered sites and this explains their low detection in forested areas (Schnitzler and Kalko, 2001).

The sonotype VMi1, comprised of three vespertilionids and three miniopterids, was found to be the most active in irrigated rice fields and the second most active overall (Fig. 2, Table 2). The species in this sonotype forage as edge-space aerial-hawkers (Verboom and Huitema, 1997; Taylor et al., 2013b). The mosaic of vegetation and fruit trees, rivers and streams, paths, terraces and anthropomorphic structures within the vicinity of the rice fields may provide this group with the required heterogeneity or "edge" habitat to forage (Monck-Whipp et al., 2018). This is important for the contextualization of our results as edge-space foragers are known to predate upon insect pests within agroecosystems (Taylor et al., 2013a,b; Brown et al., 2015; Puig-Montserrat et al., 2015)

The edge-clutter species, *Myotis goudoti* and *Paraemballonura atrata*, were previously captured in forest in the same region as our study (Goodman et al., 2014). We recorded both species at relatively high levels in irrigated rice, forest fragments and continuous forest sites.

Although post hoc tests showed no significant differences, this activity shows that these species are selecting lowland irrigated rice and forest for foraging. The NMDS plots show that both species are strongly associated with forest sites (Fig. 3). NMDS axis 2 shows *P. atrata* is more associated with rice fields than *M. goudoti*. The fact that these edge-clutter species, *P. atrata* and *M. goudoti*, switch between open and closed sites highlights the importance of retaining forest nearby for roost provision and maintaining bat populations. The paucity of available roosts for bats in rice dominated landscapes is certainly an issue and one that requires addressing as a sustainable solution to crop losses. Installing bat houses and improving landscape heterogeneity are ways to address the lack of suitable roosts available (Flaquer et al., 2006; Lindell et al., 2018; Monck-Whipp et al., 2018).

Additionally, we recorded two charismatic, endemic and difficult to catch species - *Myzopoda aurita* and *Hipposideros commersoni*. The eastern sucker-footed bat *Myzopoda aurita* was recorded in hillside and irrigated rice and in secondary vegetation (Table 2). This species roosts in the furled-up leaves of the traveller's palm *Ravenala madagascariensis* which can grow in open areas of vegetation or forest. Commersoni's horseshoe bat *Hipposideros commersoni* is the largest insectivorous bat in Madagascar, listed as Near Threatened (Andriafidison et al., 2008), and it was mainly recorded in hillside rice (Table 2). The echolocation of *Hipposideros commersoni* (high duty cycle echolocation) is extremely efficient for hunting in cluttered spaces. The bat may be roosting in the remnant forests and foraging in the adjacent hillside rice. The rarity of both species might limit their predation services but their high association with forest habitat qualifies them as good indicator taxa for the evaluation of habitat disturbance.

As expected, from the NMDS plots, the assemblage composition in the landscape shows that there was a turnover of species and sonotypes (Fig. 3). One can see a gradient from irrigated rice to continuous forest, from left to right. The open space foragers (Mo1 and *O. madagascariensis/T. fulminans*) occupy the left side of NMDS axis 1 while the clutter and edge-space foragers (*M. goudoti* and *P. atrata*) occupy the right side of the plot, illustrating the foraging preferences of the aforementioned guilds (Schnitzler and Kalko, 2001). Hillside rice and secondary vegetation almost entirely overlap which illustrates the similarity of these sites in terms of species assemblage.

4.2. Diet analysis and implications of bat foraging behaviour

The DNA metabarcoding results illustrate that insectivorous bats feed on a wide range of prey including a number of economically important insect pest species that affect a range of crops in addition to insect disease vectors (see Supplementary materials Table A.5.). The results of this study, therefore, show the potential role of insectivorous bats in supressing economically important insects in agricultural landscapes.

We found that the sonotypes that were preferentially selecting rice fields were also the most important contributors to pest suppression in rice fields. For example, *M. leucogaster* and *M. jugularis* from the Mo1 sonotype were found to have fed upon the rice pests *Spodoptera mauritia* and *Herpetogramma licarsisalis*, respectively (Table A.5). Greater pest suppression leads to greater yields and less reliance upon slash and burn agriculture, or *tavy* (Styger et al., 2007). This form of agriculture is environmentally damaging and encroaches upon forests when fallow lands are no longer fertile. Forest fragments still offer valuable refuges for certain species, yet insectivorous bats generally prefer rice fields for foraging. By identifying the most active sonotypes and how they change across different land-uses we can begin to understand the level of pest suppression that bats provide to agricultural landscapes.

It is important to note that the fieldwork only spanned a short amount of time (approximately three days per locality). The research therefore does not reflect the seasonal and spatial variation of bat diets nor do the results intentionally follow peaks in insect populations. Additionally, although we have identified bat predation on predatory arthropods that can potentially contribute to the suppression of agricultural pests (e.g. spiders - order Araneae - were identified in the diet of *M. goudoti*; Table 3) we did not explore the effects of intra-guild predation on herbivorous arthropods. Since most Malagasy bats are predominantly aerial feeders we anticipated that bat predation on non-flying arthropods would be limited. We suggest that future research should try to investigate the effects of intra-guild predation and any potential cascading effects on the abundance of agricultural pests and on rice yield. Furthermore, despite the fact that our study focussed on a rice-dominated agroecosystem, it is important to note that the pests of other crops found in bat faeces illustrates the global potential of bats as pest suppressors. Further research quantifying the role of bats as pest suppressors in Madagascar is urgently needed as they: receive little protection from Malagasy legislation; fall under game species regulations i.e. they are not actively protected; many are data deficient; and there is little appreciation of their role in ecosystem services (Racey et al., 2010).

5. Conclusions

Deforestation and habitat loss due to agricultural expansion are the primary driver of biodiversity loss in Madagascar. The need for agricultural expansion to compensate for crop losses is exacerbated by climatic extremes and insect pest outbreaks. We found that Malagasy insectivorous bats have the potential to suppress these outbreaks as they predate upon insect pests. Therefore, retaining and maximising bat populations across the island's agricultural landscapes can contribute to higher agricultural yields and help promote sustainable livelihoods. Provision of artificial roosts such as bat-boxes (Puig-Montserrat et al., 2015; López-Baucells et al., 2017a) and increased landscape heterogeneity is an important consideration for agricultural and conservation planning, specifically for open and edge- foragers. Since some cave-dwelling bat species (i.e. *Miniopterus manavi, Miniopterus majori,* and *Myotis goudoti*) were also predating on insect pests, appropriate conservation legislation and cave protection initiatives (i.e. regulation of the harvesting of guano and cave tourism) are essential to keep their populations stable. Further research and action is required to improve the knowledge of bat dietary preferences, following pest outbreaks both temporally and spatially, while improving the reputation of bats among local communities.

Acknowledgements

We thank Georges Razafindrakoto, Eric Marcel Temba, Kristian Forbes and Tafita Tojosoa Rakotoarimanana for fieldwork and logistical assistance, and MICET and Centre ValBio staff for their hospitality and logistical support. We also thank the Department of Biosciences, University of Helsinki for supporting the RESPECT field course. Madagascar National Parks and the “Ministere de l'Environnement de l'ecologie et des forests” (Département de Biologie Animale, DBA) provided the research permits to capture bat specimens within the study area (293/15/MEEMF/SG/DGF/DAPT/SCBT). This work was supported by the Portuguese Foundation for Science and Technology under Grant PD/BD/52597/2014 for ALB, SFRH/BD/80488/2011 for RR and PD/BD/114363/2016 to JK. MC is financially supported by the Academy of Finland (grant #257686).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agee.2018.09.027.

References

Ancillotto, L., Ariano, A., Nardone, V., Budinski, I., Rydell, J., Russo, D., 2017. Effects of free-ranging cattle and landscape complexity on bat foraging: implications for bat conservation and livestock management. Agric. Ecosyst. Environ. 241, 54–61.

Andriafidison, D., Cardiff, S.G., Goodman, S.M., Hutson, A.M., Jenkins, R.K.B., Kofoky, A.F., Racey, P.A., Ranivo, J., Ratrimomanarivo, F.H., Razafimanahaka, H.J., 2008. Hipposideros commersoni. In: IUCN (Ed.), The IUCN Red List of Threatened Species 2008, https://doi.org/10.2305/IUCN.UK.2008.RLTS.T10120A3168011.en.

Appel, G., López-Baucells, A., Magnusson, W.E., Bobrowiec, P.E.D., 2017. Aerial insectivorous bat activity in relation to moonlight intensity. Mamm. Biol. 85, 37–46. https://doi.org/10.1016/j.mambio.2016.11.005.

Bambini, L., Kofoky, A., Mbohoahy, T., Ralisata, M., Manjoazy, T., Hosken, D.J., Jenkins, R.K., 2010. Do bats need trees? Habitat use of two Malagasy Hipposiderid bats Triaenops furculus and T. menamena in the Dry Southwest. Hystrix 22. https://doi.org/10.4404/Hystrix-22.1-4467.

Bates, D.M., 2010. lme4: Mixed-Effects Modeling with R. Springer, New York.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2014. lme4: Linear Mixed-effects Models Using Eigen and S4. R Package Version 1.

Bohmann, K., Evans, A., Gilbert, M.T.P., Carvalho, G.R., Creer, S., Knapp, M., Douglas, W.Y., De Bruyn, M., 2014. Environmental DNA for wildlife biology and biodiversity monitoring. Trends Ecol. Evol. 29, 358–367. https://doi.org/10.1016/j.tree.2014.04.003.

Bommarco, R., Kleijn, D., Potts, S.G., 2013. Ecological intensification: harnessing ecosystem services for food security. Trends Ecol. Evol. 28, 230–238. https://doi.org/10.1016/j.tree.2012.10.012.

Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., Coissac, E., 2016. Obitools: a unix-inspired software package for DNA metabarcoding. Mol. Ecol. Resour. 16, 176–182. https://doi.org/10.1111/1755-0998.12428.

Boyles, J.G., Cryan, P.M., McCracken, G.F., Kunz, T.H., 2011. Economic importance of bats in agriculture. Science 332, 41–42.

Brooks, C.P., Holmes, C., Kramer, K., Barnett, B., Keitt, T.H., 2009. The role of demography and markets in determining deforestation rates near Ranomafana National Park, Madagascar. PLoS One 4, e5783. https://doi.org/10.1371/journal.pone.0005783.

Brown, V.A., de Torrez, E.B., McCracken, G.F., 2015. Crop pests eaten by bats in organic pecan orchards. Crop Prot. 67, 66–71. https://doi.org/10.1016/j.cropro.2014.09.011.

Burnham, K., Anderson, D., 2003. Information and likelihood theory: a basis for model selection and inference. In: Burnham, K.P., Anderson, D.R. (Eds.), Model Selection and Multimodel Inference: a Practical Information-Theoretic Approach. Springer Science & Business Media pp. 49–97.

Coissac, E., Riaz, T., Puillandre, N., 2012. Bioinformatic challenges for DNA metabarcoding of plants and animals. Mol. Ecol. 21, 1834–1847. https://doi.org/10.1111/j.1365-294X.2012.05550.x.

Collins, J., Jones, G., 2009. Differences in bat activity in relation to bat detector height: implications for bat surveys at proposed windfarm sites. Acta Chiropt. 11, 343–350. https://doi.org/10.3161/150811009X485576.

Davidai, N., Westbrook, J.K., Lessard, J.-P., Hallam, T.G., McCracken, G.F., 2015. The importance of natural habitats to Brazilian free-tailed bats in intensive agricultural landscapes in the Winter Garden region of Texas, United States. Biol. Conserv. 190, 107–114. https://doi.org/10.1016/j.biocon.2015.05.015.

De Barba, M., Miquel, C., Boyer, F., Mercier, C., Rioux, D., Coissac, E., Taberlet, P., 2014. DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: application to omnivorous diet. Mol. Ecol. Resour. 14, 306–323. https://doi.org/10.1111/1755-0998.12188.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200. https://doi.org/10.1093/bioinformatics/btr381.

Eklund, J., Blanchet, F.G., Nyman, J., Rocha, R., Virtanen, T., Cabeza, M., 2016. Contrasting spatial and temporal trends of protected area effectiveness in mitigating deforestation in Madagascar. Biol. Conserv. 203, 290–297. https://doi.org/10.1016/j.biocon.2016.09.033.

Fenton, M., Bell, G., Thomas, D., 1980. Echolocation and feeding behaviour of *Taphozous mauritianus* (Chiroptera: emballonuridae). Can. J. Zool. 58, 1774–1777. https://doi.org/10.1139/z80-244.

Fernández-Llamazares, Á., López-Baucells, A., Rocha, R., Andriamitandrina, S.F., Andriatafika, Z.E., Burgas, D., Temba, E.M., Torrent, L., Cabeza, M., 2018. Are sacred caves still safe havens for the endemic bats of Madagascar? Oryx 52, 271–275. https://doi.org/10.1017/S0030605317001648.

Flaquer, C., Torre, I., Ruiz-Jarillo, R., 2006. The value of bat-boxes in the conservation of *Pipistrellus pygmaeus* in wetland rice paddies. Biol. Conserv. 128, 223–230. https://doi.org/10.1016/j.biocon.2005.09.030.

Galan, M., Pons, J.B., Tournayre, O., Pierre, E., Leuchtmann, M., Pontier, D., Charbonnel, N., 2018. Metabarcoding for the parallel identification of several hundred predators and their preys: application to bat species diet analysis. Mol. Ecol. Resour. 18, 474–489. https://doi.org/10.1111/1755-0998.12749.

Geller, J., Meyer, C., Parker, M., Hawk, H., 2013. Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. Mol. Ecol. Resour. 13, 851–861. https://doi.org/10.1111/1755-0998.12138.

Giam, X., 2017. Global biodiversity loss from tropical deforestation. Proc. Natl. Acad. Sci. U. S. A. 114, 5775–5777. https://doi.org/10.1073/pnas.1706264114.

Goodman, S., 2011. Les Chauves-Souris de Madagascar. Association Vahatra, Antananarivo.

Goodman, S.M., Benstead, J.P., 2005. Updated estimates of biotic diversity and endemism for Madagascar. Oryx 39, 73–77. https://doi.org/10.1017/S0030605305000128.

Goodman, S.M., Andriafidison, D., Andrianaivoarivelo, R., Cardiff, S.G., Ifticene, E., Jenkins, R.K., Kofoky, A., Mbohoahy, T., Rakotondravony, D., Ranivo, J., 2005. The distribution and conservation of bats in the dry regions of Madagascar. Anim. Conserv. 8, 153–165. https://doi.org/10.1017/S136794300500199X.

Goodman, S.M., Ramasindrazana, B., Maminirina, C.P., Schoeman, M.C., Appleton, B., 2011. Morphological, bioacoustical, and genetic variation in Miniopterus bats from eastern Madagascar, with the description of a new species. Zootaxa 2880, 1–19. https://doi.org/10.1111/zoj.12223.

Goodman, S.M., Rasoanoro, M., Ralisata, M., Ramasindrazana, B., 2014. The bats of the Kianjavato-Vatovavy region, lowland eastern central Madagascar. Malagasy Nat. 8, 89–102.

Goodman, S.M., Rakotondramanana, C.F., Ramasindrazana, B., Kearney, T., Monadjem, A., Schoeman, M.C., Taylor, P.J., Naughton, K., Appleton, B., 2015. An integrative approach to characterize Malagasy bats of the subfamily Vespertilioninae gray, 1821, with the description of a new species of Hypsugo. Zool. J. Linn. Soc. 173, 988–1018. https://doi.org/10.1111/zoj.12223.

Guardiola, M., Uriz, M.J., Taberlet, P., Coissac, E., Wangensteen, O.S., Turon, X., 2015. Deep-sea, deep-sequencing: metabarcoding extracellular DNA from sediments of marine canyons. PLoS One 10, e0139633. https://doi.org/10.1371/journal.pone.0139633.

Harvey, C.A., Rakotobe, Z.L., Rao, N.S., Dave, R., Razafimahatratra, H., Rabarijohn, R.H., Rajaofara, H., MacKinnon, J.L., 2014. Extreme vulnerability of smallholder farmers to agricultural risks and climate change in Madagascar. Philos. Trans. R. Soc. B: Biol. Sci. 369, 20130089. https://doi.org/10.1098/rstb.2013.0089.

Kari, S., Korhonen-Kurki, K., 2013. Framing local outcomes of biodiversity conservation through ecosystem services: a case study from Ranomafana. Madagascar. Ecosyst. Serv. 3, e32–e39. https://doi.org/10.1016/j.ecoser.2012.12.003.

Karp, D.S., Daily, G.C., 2014. Cascading effects of insectivorous birds and bats in tropical coffee plantations. Ecology 95, 1065–1074. https://doi.org/10.1890/13-1012.1.

Kemp, J., López-Baucells, A., Rocha, R., Wangensteen, O.S., Andriatafika, Z., Nair, A., Cabeza, M., 2018. Metabarcoding Results From the Research Article: "Bats as Potential Suppressors of Multiple Agricultural Pests: a Case Study from Madagascar". Mendeley Data. https://doi.org/10.17632/x43j7mv6tp.1.

Kofoky, A., Andriafidison, D., Ratrimomanarivo, F., Razafimanahaka, H.J., Rakotondravony, D., Racey, P.A., Jenkins, R.K., 2006. Habitat use, roost selection and conservation of bats in Tsingy de Bemaraha National Park, Madagascar. Vertebrate Conservation and Biodiversity. Springer pp. 213–227.

Kofoky, A.F., Randrianandrianina, F., Russ, J., Raharinantenaina, I., Cardiff, S.G., Jenkins, R.K., Racey, P.A., 2009. Forest bats of Madagascar: results of acoustic surveys. Acta Chiropt. 11, 375–392. https://doi.org/10.3161/150811009X485602.

Kulikova, T., Aldebert, P., Althorpe, N., Baker, W., Bates, K., Browne, P., van den Broek, A., Cochrane, G., Duggan, K., Eberhardt, R., 2004. The EMBL nucleotide sequence database. Nucleic Acids Res. 32, D27–D30. https://doi.org/10.1093/nar/gki098.

Lee, Y.-F., McCracken, G.F., 2002. Foraging activity and food resource use of Brazilian free-tailed bats, *Tadarida brasiliensis* (Molossidae). Ecoscience 9, 306–313. https://doi.org/10.1080/11956860.2002.11682717.

Leray, M., Yang, J.Y., Meyer, C.P., Mills, S.C., Agudelo, N., Ranwez, V., Boehm, J.T., Machida, R.J., 2013. A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. Front. Zool. 10, 34. https://doi.org/10.1186/1742-9994-10-34.

Lindell, C., Eaton, R.A., Howard, P.H., Roels, S.M., Shave, M.E., 2018. Enhancing agricultural landscapes to increase crop pest reduction by vertebrates. Agric. Ecosyst. Environ. 257, 1–11. https://doi.org/10.1016/j.agee.2018.01.028.

López-Baucells, A., Puig-Montserrat, X., Torre, I., Freixas, L., Mas, M., Arrizabalaga, A., Flaquer, C., 2017a. Bat boxes in urban non-native forests: a popular practice that should be reconsidered. Urban Ecosyst. 20, 217–225. https://doi.org/10.1007/s11252-016-0582-9.

López-Baucells, A., Rocha, R., Andriatafika, Z., Tojosoa, T., Kemp, J., Forbes, K., Cabeza, M., 2017b. Roost selection by synanthropic bats in rural Madagascar: what makes non-traditional structures so tempting? Hystrix 28, 28–35. https://doi.org/10.4404/hystrix-28.1-12046.

Maas, B., Karp, D.S., Bumrungsri, S., Darras, K., Gonthier, D., Huang, J.C.C., Lindell, C.A., Maine, J.J., Mestre, L., Michel, N.L., 2016. Bird and bat predation services in tropical forests and agroforestry landscapes. Biol. Rev. 91, 1081–1101.

Macías-Hernández, N., Athey, K., Tonzo, V., Wangensteen, O.S., Arnedo, M., Harwood, J.D., 2018. Molecular gut content analysis of different spider body parts. PLoS One 13, e0196589. https://doi.org/10.1371/journal.pone.0196589.

Mahé, F., Rognes, T., Quince, C., de Vargas, C., Dunthorn, M., 2015. Swarm v2: highly-scalable and high-resolution amplicon clustering. PeerJ 3, e1420. https://doi.org/10.7717/peerj.1420.

Malhi, Y., 2017. The concept of the anthropocene. Annu. Rev. Environ. Resour. 42, 77–104. https://doi.org/10.1146/annurev-environ-102016-060854.

Malmqvist, E., Jansson, S., Zhu, S., Li, W., Svanberg, K., Svanberg, S., Rydell, J., Song, Z., Bood, J., Brydegaard, M., 2018. The bat–bird–bug battle: daily flight activity of insects and their predators over a rice field revealed by high-resolution Scheimpflug Lidar. R. Soc. Open Sci. 5, 172303. https://doi.org/10.1098/rsos.172303.

Martin, E.A., Viano, M., Ratsimisetra, L., Laloë, F., Carrière, S.M., 2012. Maintenance of bird functional diversity in a traditional agroecosystem of Madagascar. Agric. Ecosyst. Environ. 149, 1–9. https://doi.org/10.1016/j.agee.2011.12.005.

McConnell, W.J., Sweeney, S.P., Mulley, B., 2004. Physical and social access to land: spatio-temporal patterns of agricultural expansion in Madagascar. Agric. Ecosyst. Environ. 101, 171–184. https://doi.org/10.1016/j.agee.2003.09.012.

McCracken, G.F., Gillam, E.H., Westbrook, J.K., Lee, Y.-F., Jensen, M.L., Balsley, B.B., 2008. Brazilian free-tailed bats (*Tadarida brasiliensis*: molossidae, Chiroptera) at high altitude: links to migratory insect populations. Integr. Comp. Biol. 48, 107–118. https://doi.org/10.1093/icb/icn033.

McCracken, G.F., Westbrook, J.K., Brown, V.A., Eldridge, M., Federico, P., Kunz, T.H., 2012. Bats track and exploit changes in insect pest populations. PLoS One 7, e43839.

https://doi.org/10.1371/journal.pone.0043839.

Monck-Whipp, L., Martin, A.E., Francis, C.M., Fahrig, L., 2018. Farmland heterogeneity benefits bats in agricultural landscapes. Agric. Ecosyst. Environ. 253, 131–139. https://doi.org/10.1016/j.agee.2017.11.001.

Muthayya, S., Sugimoto, J.D., Montgomery, S., Maberly, G.F., 2014. An overview of global rice production, supply, trade, and consumption. Ann. N. Y. Acad. Sci. 1324, 7–14. https://doi.org/10.1111/nyas.12540.

Naranjo, S.E., Ellsworth, P.C., Frisvold, G.B., 2015. Economic value of biological control in integrated pest management of managed plant systems. Annu. Rev. Entomol. 60. https://doi.org/10.1146/annurev-ento-010814-021005.

Neudert, R., Ganzhorn, J.U., Wätzold, F., 2017. Global benefits and local costs–the dilemma of tropical forest conservation: a review of the situation in Madagascar. Environ. Conserv. 44, 82–96. https://doi.org/10.1017/S0376892916000552.

Lehmkuhl Noer, C., Dabelsteen, T., Bohmann, K., Monadjem, A., 2012. Molossid bats in an African agro-ecosystem select sugarcane fields as foraging habitat. Afr. Zool. 47, 1–11. https://doi.org/10.3377/004.047.0120.

Nwilene, F.E., Nacro, S., Tamò, M., Menozzi, P., Heinrichs, E.A., Hamadoun, A., Dakouo, D., Adda, C., Togola, A., 2013. Managing insect pests of rice in Africa. In: Wopereis, Marco C.S., Johnson, David E., Ahmadi, Nourollah, Tollens, Eric, Jalloh, A. (Eds.), Realizing Africa's Rice Promise. CABI p. 229.

Oerke, E.C., 2006. Crop losses to pests. J. Agric. Sci. 144, 31–43. https://doi.org/10.1017/S0021859605005708.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. Package 'vegan'. Community Ecology Package, Version 2.

Parsa, S., Morse, S., Bonifacio, A., Chancellor, T.C., Condori, B., Crespo-Pérez, V., Hobbs, S.L., Kroschel, J., Ba, M.N., Rebaudo, F., 2014. Obstacles to integrated pest management adoption in developing countries. Proc. Natl. Acad. Sci. U. S. A. 111, 3889–3894. https://doi.org/10.1073/pnas.1312693111.

Peters, J., 1998. Transforming the integrated conservation and development project (ICDP) approach: observations from the Ranomafana National Park Project, Madagascar. J. Agric. Environ. Ethics 11, 17–47. https://doi.org/10.1023/A:1007796628731.

Puig-Montserrat, X., Torre, I., López-Baucells, A., Guerrieri, E., Monti, M.M., Ràfols-García, R., Ferrer, X., Gisbert, D., Flaquer, C., 2015. Pest control service provided by bats in Mediterranean rice paddies: linking agroecosystems structure to ecological functions. Mamm. Biol. 80, 237–245. https://doi.org/10.1016/j.mambio.2015.03.008.

R Development Core Team, 2017. R: a Language and Environment for Statistical Computing. ISBN 3–900051–07–0. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Racey, P.A., Goodman, S.M., Jenkins, R.K., 2010. The ecology and conservation of Malagasy bats. In: Fleming, T., Racey, P. (Eds.), Islands Bats: Evolution, Ecology, and Conservation. University of Chicago Press pp. 369–404.

Rakotoarivelo, A.A., Ranaivoson, N., Ramilijaona, O.R., Kofoky, A.F., Racey, P.A., Jenkins, R.K., 2007. Seasonal food habits of five sympatric forest microchiropterans in Western Madagascar. J. Mammal. 88, 959–966. https://doi.org/10.1644/06-MAMM-A-112R1.1.

Ramasindrazana, B., Goodman, S.M., Schoeman, M.C., Appleton, B., 2011. Identification of cryptic species of *Miniopterus* bats (Chiroptera: miniopteridae) from Madagascar and the Comoros using bioacoustics overlaid on molecular genetic and morphological characters. Biol. J. Linnean Soc. 104 (2), 284–302. https://doi.org/10.1111/j.1095-8312.2011.01740.x.

Randrianandrianina, F., Andriafidison, D., Kofoky, A.F., Ramilijaona, O., Ratrimomanarivo, F., Racey, P.A., Jenkins, R.K., 2006. Habitat use and conservation of bats in rainforest and adjacent human-modified habitats in eastern Madagascar. Acta Chiropt. 8, 429–437. https://doi.org/10.3161/1733-5329(2006)8[429:HUACOB]2.0.CO. < http://2.0.CO > ;2.

Rasolofoson, R.A., Ferraro, P.J., Jenkins, C.N., Jones, J.P.G., 2015. Effectiveness of community forest management at reducing deforestation in Madagascar. Biol. Conserv. 184, 271–277. https://doi.org/10.1016/j.biocon.2015.01.027.

Ratnasingham, S., Hebert, P.D., 2007. BOLD: the barcode of life data system. Mol. Ecol. Resour. 7, 355–364. http://www.barcodinglife.org.

Razafindrakoto, N., Harwell, A., Jenkins, R.K., 2010. Bats roosting in public buildings: a preliminary assessment from Moramanga, eastern Madagascar. Madag. Conserv. Dev. 5.

Rocha, R., Virtanen, T., Cabeza, M., 2015. Bird assemblages in a Malagasy forest-agricultural frontier: effects of habitat structure and forest cover. Trop. Conserv. Sci. 8, 681–710. https://doi.org/10.1177/194008291500800307.

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584. https://doi.org/10.7717/peerj.2584.

Russ, J., Bennett, D., 2001. The Bats of Madagascar: A Field Guide with Descriptions of Echolocation Calls. Viper Press.

Russo, D., Bosso, L., Ancillotto, L., 2018. Novel perspectives on bat insectivory highlight the value of this ecosystem service in farmland: research frontiers and management implications. Agric. Ecosyst. Environ. 266, 31–38. https://doi.org/10.1016/j.agee.2018.07.024.

Schnitzler, H.-U., Kalko, E.K.V., 2001. Echolocation by insect-eating bats: we define four distinct functional groups of bats and find differences in signal structure that correlate with the typical echolocation tasks faced by each group. Bioscience 51, 557–569. https://doi.org/10.1641/0006-3568(2001)051[0557:ebieb]2.0.co;2.

Siegenthaler, A., Wangensteen, O.S., Benvenuto, C., Campos, J., Mariani, S., 2018. DNA metabarcoding unveils large-scale trophic variation in a widespread coastal opportunist. Mol. Ecol. https://doi.org/10.1111/mec.14886. in press.

Sikes, R.S., Gannon, W.L., Care, A., Mammalogists, U.Cot.A.So, 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. J. Mammal. 92, 235–253. https://doi.org/10.1644/10-MAMM-F-355.1.

Skaug, H., Fournier, D., Bolker, B., Magnusson, A., Nielsen, A., 2015. glmmADMB: Generalized Linear Mixed Models Using AD Model Builder. R Package v. 0.8. 1.

Stenberg, J.A., 2017. A conceptual framework for integrated pest management. Trends Plant Sci. 22, 759–769. https://doi.org/10.1016/j.tplants.2017.06.010.

Styger, E., Rakotondramasy, H.M., Pfeffer, M.J., Fernandes, E.C., Bates, D.M., 2007. Influence of slash-and-burn farming practices on fallow succession and land degradation in the rainforest region of Madagascar. Agric. Ecosyst. Environ. 119, 257–269. https://doi.org/10.1016/j.agee.2006.07.012.

Swift, J.F., Lance, R.F., Guan, X., Britzke, E.R., Lindsay, D.L., Edwards, C.E., 2018. Multifaceted DNA metabarcoding: validation of a non-invasive, next-generation approach to studying bat populations. Evol. Appl. 11, 1120–1138. https://doi.org/10.1111/eva.12644.

Taylor, P., Bohmann, K., Steyn, J., Schoeman, M., Matamba, E., Zepeda-Mendoza, E., Nangammbi, T., Gilbert, M., 2013a. Bats eat pest green vegetable stinkbugs (*Nezara viridula*): diet analyses of seven insectivorous species of bats roosting and foraging in macadamia orchards at Levubu, Limpopo Province, South Africa. SAMAC Yearbook 21. pp. 37–43.

Taylor, P.J., Monadjem, A., Nicolaas Steyn, J., 2013b. Seasonal patterns of habitat use by insectivorous bats in a subtropical African agro-ecosystem dominated by macadamia orchards. Afr. J. Ecol. 51, 552–561. https://doi.org/10.1111/aje.12066.

Taylor, P.J., Matamba, E., Steyn, J.N., Nangammbi, T., Zepeda-Mendoza, M.L., Bohmann, K., 2017. Diet determined by next generation sequencing reveals pest consumption and opportunistic foraging by bats in macadamia orchards in South Africa. Acta Chiropt. 19, 239–254. https://doi.org/10.3161/15081109ACC2017.19.2.003.

Torrent, L., Lopez-Baucells, A., Rocha, R., Bobrowiec, P., Meyer, C., 2018. The importance of lakes for bat conservation in Amazonian rainforests: an assessment using autonomous recorders. Remote Sens. Ecol. Conserv. https://doi.org/10.1002/rse2.83. Advanced online publication.

Verboom, B., Huitema, H., 1997. The importance of linear landscape elements for the pipistrelle *Pipistrellus pipistrellus* and the serotine bat *Eptesicus serotinus*. Landsc. Ecol. 12, 117–125. https://doi.org/10.1007/BF02698211.

Vieilledent, G., Grinand, C., Rakotomalala, F.A., Ranaivosoa, R., Rakotoarijaona, J.-R., Allnutt, T.F., Achard, F., 2018. Combining global tree cover loss data with historical national forest cover maps to look at six decades of deforestation and forest fragmentation in Madagascar. Biol. Conserv. 222, 189–197. https://doi.org/10.1016/j.biocon.2018.04.008.

Wangensteen, O.S., Turon, X., 2017. Metabarcoding techniques for assessing biodiversity of marine animal forests. In: Rossi, Sergio, L.B, Andrea Gori, Orejas, Covadonga (Eds.), Marine Animal Forests. The Ecology of Benthic Biodiversity Hotspots. Springer International Publishing, Switzerland pp. 445–473.

Wangensteen, O.S., Cebrian, E., Palacín, C., Turon, X., 2018a. Under the canopy: community-wide effects of invasive algae in marine protected areas revealed by metabarcoding. Mar. Pollut. Bull. 127, 54–66. https://doi.org/10.1016/j.marpolbul.2017.11.033.

Wangensteen, O.S., Palacín, C., Guardiola, M., Turon, X., 2018b. DNA metabarcoding of littoral hard-bottom communities: high diversity and database gaps revealed by two molecular markers. PeerJ 6, e4705. https://doi.org/10.7717/peerj.4705.

Wanger, T.C., Darras, K., Bumrungsri, S., Tscharntke, T., Klein, A.-M., 2014. Bat pest control contributes to food security in Thailand. Biol. Conserv. 171, 220–223. https://doi.org/10.1016/j.biocon.2014.01.030.

Wickham, H., 2016. Tidyverse: Easily install and Load 'tidyverse'packages [Software].