ROLE OF BATS AS NATURAL PEST CONTROL AGENTS OF MOTH PESTS (FALSE CODLING MOTH AND CAROB MOTH) ON CITRUS ORCHARD

by

Khavheli Thivhileli

16004875

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Supervisor:

Prof L.H Swanepoel

Co-supervisors:

Prof S.H Foord

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Declaration

I, **<u>Thivhileli Khavheli</u>**, hereby declare that this thesis submitted to the University of Venda for the degree of MSc in Biological Sciences: Zoology, has not been submitted previously for any other degree at this or any other institution, and that it is my own work in design and execution and that all materials contained therein has been duly acknowledged.

Signature: Date:

<u>21 February 2024</u>

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Abstract

Over the years, the change in farming practices has caused a tremendous decline in the diversity of flora and fauna within agricultural landscapes through intensification of land use. An increase in agricultural intensification has resulted in increased farm sizes and reduced landscape heterogeneity. In the past two decades, the interest in services that insectivorous bats provide within agricultural landscapes through insect pest suppression has gained recognition globally. Multiple studies have thoroughly examined the vital role played by insectivorous bats within agricultural landscapes as biological control of insect pests for crops such as rice, cotton, pecan, corn, and macadamia. However, little to none is known about the impact insectivorous bats have within citrus landscapes. This study specifically focuses on how insectivorous bats inhabiting different landscapes respond to insect pest outbreaks, especially that of false codling moth (FCM) and carob moth (CM), important pests of citrus. The study first gives a general introduction of the importance of landscape heterogeneity within agricultural landscapes, beneficial effects that insectivorous bats have in agricultural areas, and the use of advanced molecular methods to analyze the diet of insectivorous bats. Secondly, we investigated the influence of land use heterogeneity, temperature, and insect pest activity on bat species richness and different foraging groups. Our results demonstrate higher bat activity in natural areas (wet riparian zones and orchard edges) compared to orchards, and further illustrate that bat species richness and activity were highly influenced by temperature. Lastly, we investigate the moth pest consumption by different bat species of different foraging groups. We found that all bat foraging groups, open-air and clutter-edge foragers, had a high consumption rate of agricultural pests including FCM and CM. The results of this study emphasize how crucial it is to preserve natural habitats and bat populations in citrus landscapes. Including bats in integrated pest management (IPM) programs can benefit both agriculture and biodiversity.

Keywords: heterogeneity, agricultural landscape, pest outbreaks, biodiversity, flora, fauna

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CHAPTER 1: INTRODUCTION

1.1. General Introduction

Throughout the years, advances in agriculture have resulted in significant improvements in global food security by increasing and stabilizing food production (Gordon *et al*., 2010). However, the need to attain high crop yield and increased crop production has potentially led to an escalation in the use of insecticides and chemical fertilizers in agriculture (Carvalho, 2006). Nonetheless, the extensive application of chemical fertilizers and pesticides is expensive and unsustainable and has led to chemical resistance to pests, and environmental pollution and degradation (Chartres & Noble, 2015). Thus, there is a need to adapt agricultural landscapes to include biodiversity for ecosystem services. In Africa, agriculture is a crucial industry that plays a significant part in economic development (Abate *et al*., 2003). At least 60% of the population earns their livelihood through agriculture, but most farmers engage in small-scale farming, which requires manual labour for crop production (Constantine *et al*., 2020).

Insect pests continue to be problematic to Africa's agricultural production. Most farmers resort to traditional methods to address pest issues, such as planting multiple crops in the same field simultaneously or practicing crop rotation (Imadi *et al*., 2016). These traditional systems promote the growth of natural predators and effectively keep insect pest populations under control (Abate *et al*., 2003). However, government extension programs have been advocating for the use of pesticides, which not only present health risks but also lead to chemical resistance in pests (Imadi *et al*., 2016). Furthermore, modern agricultural systems rely heavily on monocultures of high-yield crop varieties. These monocrops are often treated with synthetic agrochemicals such as herbicides, fungicides, pesticides, and fertilizers, resulting in less diversity and increased resistance to pests. This practice is ecologically unsustainable, leading to pollution of the soil and water, and ultimately causing harm to the ecosystem (Reynolds *et al*., 2015).

Agroecosystems can be defined as the interaction of ecological, socioeconomic, and populations of plants and/or animals within their environments that have been transformed by humans for high yield production in agriculture (Zhu *et al*., 2012). The concept of agroecosystems underlines the importance of maintaining natural habitats

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within the agricultural systems as many conservation schemes are focused on them (Moulton *et al*., 2006; Te Junior, 1999). Modern commercial agriculture is dominated by monoculture systems (Tarigan, 2019), which frequently contribute to low biodiversity and limit the ability of ecosystems to provide vital roles including pollination and insect pest control (Dolezal *et al*., 2019; Rivera-Pedroza *et al*., 2019). Therefore, agroecosystems attempt to balance agricultural productivity with ecosystem conservation through sustainable development (Araujo *et al*., 2021).

Agricultural systems with high-quality habitats are essential for interactions of biodiversity as they provide several benefits to agriculture (Jackson *et al*., 2016). Natural areas adjacent to agricultural systems can serve as habitats for pollinators and predators of pests. Tarigan (2019) reported that increasing crop diversity through polycultures can benefit pollinators and natural pest predators like parasitic wasps, leading to higher populations of these helpful organisms. Landscape heterogeneity within agricultural systems may also increase bat diversity as distinct species can inhabit a given landscape (Monck-Whipp *et al*., 2018). Some studies have indicated that management practices, vegetation structure, and expansion within an agroecosystem can positively and/or negatively impact the species diversity of different organisms (Tilman *et al*., 2001; Tscharntke *et al*., 2005). The extension of farmlands has led to natural habitats being cleared out and fragmented, resulting in the extinction of local species, the introduction of invasive species, and the frequent occurrence of wildfires (Bennett *et al*., 2006). Nevertheless, agricultural landscapes that include natural areas can exhibit high biodiversity and habitat heterogeneity, which benefits many organisms (Benton *et al*., 2003). For example, the citrus orchard in our study area is bordered by different landscapes, including woodland, riparian zones, and rocky outcrops, that not only provide habitat for different animals but are also regarded as the main foraging habitats for a variety of bat species (Lacki *et al*., 2007; Linden *et al*., 2019).

One of the most abundant mammals on earth is Bats (Order: Chiroptera), of which about 70% are insectivorous bats (Monadjem *et al*., 2020). Insectivorous bats are crucial within agricultural landscapes as they provide various ecosystem services including insect pollination, seed dispersal, pest control, and some may serve as indicator species (Boyles *et al*., 2011; Klein *et al*., 2007; Toussaint *et al*., 2021). Conversely, only recently these ecosystem services have been investigated

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thoroughly. Kunz *et al*. (2011) pointed out that the ecological and economic benefits should be highlighted to improve public awareness and perceptions of bats and to help restore or protect bat populations and their habitats.

In agricultural environments, bats have been recommended to provide biological management of crop pests (Kunz *et al*., 2011). Many insectivorous bat species feed on different agricultural insects, including both pests and beneficial insects (pollinators) (Boyles *et al*., 2011; Maas *et al*., 2013). For example, Taylor *et al*. (2018) estimated in a recent study that the avoidable cost in macadamia orchards alone ranged between $57 to 139 per hectare per year due to bat suppression of stinkbugs, specifically the *Bathycoelia distincta* and *viridula*. Linden *et al*. (2019) conducted an empirical study that found that the values of the avoided-cost model were probably underestimated. The study further demonstrated that the disservices caused by vervet monkeys (destroying crops) outweigh the ecological services that bats and birds provide by feeding on insect pests. In agricultural landscapes, the existence of insectivorous bats contributes to sustaining insect pest populations when periodic outbreaks occur (Symondson *et al*., 2002). Several researchers have presented that preventing insectivorous bats from foraging inside farms or orchards may either lower yield or enhance the rate of insect pest infestation (Maas *et al*., 2016; Maine & Boyles, 2015; Taylor *et al*., 2018). However, few studies have extensively investigated the diet of bat assemblage communities within agricultural systems.

The consumption of pest species by bats is an important variable in understanding their role in providing ecosystem benefits. Faecal pellet collection and analysis can provide low-cost, non-invasive information about the insectivorous bats consumption and their function as a pest control (Bohmann *et al*., 2014; Brown *et al*., 2015; Swift *et al*., 2018). Prior studies have analyzed prey fragments in bat pellets using morphological characteristics (Lee & McCracken, 2005; Leelapaibul *et al*., 2005; Whitaker Jr, 1988; Whitaker Jr *et al*., 1996). For example, Lee and McCracken. (2005), used a dissecting microscope to examine the faecal pellets of Brazilian free-tailed bats. However, this method prioritizes hard-bodied insects and the only way to identify the prey consumed is by order and not often by family level (Kunz *et al*., 2011). Recently, a number of molecular techniques, including DNA metabarcoding and next-generation sequencing (NGS), have been utilized to identify different arthropod pests in the faecal pellets of insectivorous bats (Boyles *et al*., 2013; Kemp *et al*., 2019;

Kolkert *et al*., 2020; Taylor *et al*., 2017; Weier *et al*., 2019). A recent research by Weier *et al*. (2019) used a molecular technique to analyze bat pellets from bats captured in macadamia orchards and discovered that at least one pest species (Two-spotted stink bug, *Bathycoelia distincta*) DNA was present in 55% of the pellets.

South Africa is ranked second in global exports of citrus following Spain. Northwest Province, Kwa Zulu Natal, Limpopo, Western Cape, Mpumalanga, Northern Cape, and Eastern Cape are the seven provinces that produce citrus (Citrus Growers Association, 2018). Citrus makes up 32% of South Africa's total fruit production (in terms of volume) and accounts for 45% of all fruit export earnings (Citrus Growers Association, 2022). Exporting almost all of the fruit harvested on the farm to overseas markets, where the farmer can obtain greater prices than on the local market, makes citrus farming more profitable. For smallholder growers, selling citrus to local markets is the only option because export markets demand higher compliance levels with sanitary and phytosanitary regulations (Citrus Growers Association, 2018).

With 99 697 hectares of land covered by citrus in South Africa, Limpopo province is the leading growing region of citrus, accounting for 40% (39 524 hectares) of the total area planted (Citrus Growers Association, 2023). Additionally, the temperature in Limpopo Province is warmer, making grapefruit, Valencia, and Navel oranges more suitable for cultivation. Valencia oranges have a cultivated production area of 30 600 hectares, with Limpopo Province, having more production area of 17 380 ha (57%) than other provinces. The production area for Navel oranges in South Africa is 15 567 hectares, with Eastern Cape having more cultivated area (6 139 ha/ 39%), followed by Limpopo which has a production area for Navel oranges of 4 325 ha (57%).

South Africa's citrus industry regards the false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick), and carob moth (CM), *Ectomyelois ceratoniae* (Zeller), as one of the most dangerous moth pests due to their phytosanitary status (Moore, 2021). These moths are nocturnal and they fly throughout the orchard to find suitable citrus trees to lay their eggs (Moore & Kirkman, 2008; Moore, 2002). They have minimal dispersal outside the citrus orchard, however, Moore and Kirkman (2008) found that they often fly to adjacent natural vegetation to find refuge during spraying. These moth pests deposit their eggs higher up in the trees, one egg at a time, on the surface of leaves or fruit. When the eggs hatch, the larvae immediately burrow into the fruit and undergo five developmental stages (Daiber, 1980). The larval stage is the one that brought about severe damage to the fruit (Daiber, 1980). A wide array of biological methods for controlling FCM and CM are available, such as the FCM egg parasitoid (*Trichogramma cryptophlebiae*), ants (Formicidae), orius bugs (Anthocoridae), assassin bugs (Reduviidae), and a few species of wasp and fly larval parasitoids (Moore, 2002). However, the pest suppression of FCM and CM in citrus orchards by insectivorous bats has not yet been investigated.

1.2. Problem statement

The FCM remains a vital pest of the South African citrus industry, resulting in losses exceeding R500 million per year (Citrus Annual Report, 2022). Currently, FCM and CM control rely heavily on chemical control (Malan *et al*., 2018), the FCM egg parasitoid (*Trichogramma cryptophlebiae*), and granulovirus. However, the use of bats to assist in the biological control of FCM and CM within citrus farming systems has yet to be explored. Therefore, understanding how bats respond to insect pest abundances in a relatively untransformed landscape and whether they target these pest species would provide for the conservation and management of bat populations as part of an integrated pest management approach (IPM) (Maas *et al*., 2016).

1.3. Aims

- To investigate bat activity, species richness, and abundance within citrus orchards and the surrounding landscape.

- To investigate the consumption of FCM and CM by bats.

Objectives

- To determine bat activity and species richness about orchard configuration and landscape elements using bat detectors.

- To estimate consumption of FCM and CM by different bat species across citrus agroecosystem using DNA metabarcoding.

1.4. Significance of the study

Citrus fruit farming is an extremely profitable industry in South Africa. However, due to the zero tolerance of FCM infestation in exported fruits, there is high continued usage

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of chemical pesticides. Consequently, all possible biological control options must be exploited and maximized. It has been recommended that bats play a crucial role, yet unexplored ecological role within agricultural landscapes by suppressing insect pests (Boyles *et al*., 2011; Kunz *et al*., 2011; Taylor *et al*., 2018). Given the landscape heterogeneity and the minimal disturbance in natural habitats surrounding the citrus orchard, these areas provide habitat for different bat species, providing a regulatory service by suppressing insect pests during outbreaks. Therefore, sustaining the orchard's native vegetation can support bat populations. However, limited information is known about the roles or beneficial functions that insectivorous bats play/provide within the citrus fruit farming landscape. This study aims to explore how bat assemblage structure within the citrus agroecosystem is influenced by insect pest abundance, orchard configuration, and specific landscape elements (e.g., woodland, intact riparian zones, human settlements) by investigating the activity and abundance of bats within orchard configuration and landscape elements (Chapter 2) and consumption of FCM and CM by bats (Chapter 3).

1.5. Thesis layout

There are four sections to this thesis: the first chapter is a general backround introduction on bats and citrus pests (Chapter 1). The second part examines bat activity and diversity within the orchard and the surrounding landscape (Chapter 2). The third part is assessing bat diet, using DNA metabarcoding to track bats' consumption of FCM and CM (Chapter 3). The last part is the conclusion and recommendations for the thesis (Chapter 4).

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CHAPTER 2

INSECTIVOROUS BAT SPECIES RICHNESS AND ACTIVITY IN RELATION TO LANDSCAPE CONFIGURATION AND INSECT PESTS WITHIN CITRUS LANDSCAPE

Abstract

Insectivorous bats have been considered one of the most crucial organisms within various agricultural ecosystems. Their widespread distribution, adaptability to human-modified landscapes, and high energy demands enable them to provide valuable biological control services through insect consumption. While there is an increase in evidence for various agricultural systems obtaining the benefits of bat predation, their specific role in eradicating citrus pest remains largely unexplored. In this study, bat activity was acoustically monitored in different landscapes surrounding the citrus orchard, to assess their response to insect activity. Our results show that 19 bat species were found to be foraging across different landscapes, with natural areas having more species than orchards. Temperature, land use heterogeneity, and insect abundance were found to highly influence bat species richness, activity, and foraging groups. Bat species richness and activity were significantly affected by temperature despite the landscape. Bat foraging groups' activity differed with land use, with open-air foragers' activity being high in orchards compared to natural areas, and clutter-edge foragers activity being high in natural areas and orchard edges than in the middle of the orchards. Only the activity of clutter-edge foragers was influenced by insect biomass. This research emphasizes the significance of maintaining landscape heterogeneity within citrus orchards, as they provide habitat for different bat species, which might be important during citrus moth pest outbreaks.

Keywords: acoustic, biological control, landscape, citrus, insect pests

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2.1. Introduction

Modern agriculture has undergone a shift towards intensified practices, due to increases in agricultural productivity (Donald *et al*., 2001). Over the years, the change in farming practices has caused a tremendous decline in the diversity of flora and fauna within agricultural landscapes through the intensive application of agrochemicals and intensification of land use (Tscharntke *et al*., 2005). Consequently, this has detrimental effects on ecosystem service providers such as wild pollinators and insect pest predators (Balvanera *et al*., 2006). The increase in agricultural land use has led to increased farm sizes and reduced landscape heterogeneity (Heim *et al*., 2017). However, the practice of monocultures has led to increased pest infestations and consequently, reduced the effectiveness of biological control by natural enemies in crop-dominated landscapes (i.e. simplified landscapes) (Rusch *et al*., 2016). Sirami *et al*. (2019) have demonstrated that landscape heterogeneity within agricultural landscapes enhances a wide range of diversity and abundance of different insect pest's natural enemies. In addition, farmers are adopting sustainable farming practices as they allow for reduction in chemical and pesticides use as are detrimental to ecosystems and human health (Ekroos *et al*., 2014).

After years of being largely overlooked in pest management research, insectivorous bats have experienced a surge in interest within agricultural landscapes during the last two decades, emerging as crucial ecosystem service providers in regulating pest populations (Ancillotto *et al*., 2024; Bouarakia *et al*., 2023; Nsengimana *et al*., 2023; Tortosa *et al*., 2023). Subsequently, numerous studies have thoroughly assessed the importance of insectivorous bats within agricultural landscapes as predators of insects that feed on crops like cotton (Kolkert *et al*., 2020), rice (Puig-Montserrat *et al*., 2015), corn (Maine & Boyles, 2015), pecan (Braun de Torrez *et al*., 2019), and macadamia (Taylor *et al*., 2017; Weier *et al*., 2018). The success of insectivorous bats as ideal biological control of insect pests is driven by their wide range of diet, wide geographic range, resilience to human-dominated landscapes, rapid metabolic rates related to flight, and particularly, their adaptability to forage in various environments (Maslo *et al*., 2022; Tournayre *et al.,* 2021). Furthermore, the ability of insectivorous bats to be able to adapt to different foraging habitats between seasons makes them exceptionally better to feed on a variety of pests during seasonal eruptions (McCracken *et al*., 2012; Puig-Montserrat *et al*., 2015).

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A number of studies have demonstrated the economic benefits that insectivorous bats indirectly contribute to agricultural landscapes through pest suppression (Boyles *et al*., 2011; Kunz *et al*., 2011; Maas *et al*., 2013; Puig-Montserrat *et al*. 2015; Taylor *et al*., 2018). For example, Boyles *et al*. (2011) reported that the economic value that insectivorous bats provide in a cotton-dominated agricultural landscape annually vetted around 22.9 $ billion (USD). Similarly, Taylor *et al*. (2018) reported that the annual estimated avoided cost values provided by insectivorous bats within macadamia orchards ranged from 0 to 757 $/ha. Furthermore, Linden *et al*. (2019) further reported that the services (insect pest predation) provided by bats and birds within macadamia orchards were more economically beneficial (5000 $/ha/year) than the disservices (crop damage) by monkeys (1600 $/ha/year). Additionally, Ancillotto *et al*. (2024) reported that the presence of insectivorous bats within apple orchards significantly saves an estimated 551 €/ha/year by predating on codling moth, *Cydia pomonella*. Therefore, promoting high bat activity within agricultural landscapes could potentially enhance food security and reduce the use of pesticides.

Both local and landscape scales influence bat composition within agricultural landscapes (Fuentes-Montemayor *et al*., 2013; Kelly *et al*.,2016). At a local scale, insect prey availability and abundance, microclimate, and land use intensity influence bat activity (Wickramasinghe *et al*., 2003). For instance, several studies have previously indicated that bat activity and richness increase with insect abundance within farmlands (Kunz *et al*., 2011; Maine & Boyles, 2015; Taylor *et al*., 2017; Weier *et al*., 2018). At a landscape scale, roost sites availability, foraging habitat structure, proximity to water bodies, and proximity of edge to forest/natural vegetation all appear to influence bat activity and diversity (Avila-Flore & Fenton, 2005; Heim *et al*., 2017; Monadjem *et al*., 2010). Additionally, the effects of landscape characteristics on bat activity differ amongst bat species and foraging groups (Lintott *et al*., 2016). For example, Weier *et al*. (2018) indicated that the activity of clutter-edge foragers increases with distance to settlements and higher bush cover whilst the activity of open-air foragers increases with fallow land and decreases with proximity to water in low season.

The suitability of a landscape for insectivorous bats depends on a number of factors. The most important one is the heterogeneity of the landscape surrounding the crop fields (Bhalla *et al*., 2023). Several studies have highlighted that heterogeneous

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landscapes in agricultural landscapes support higher species richness and diversity of bat communities than homogeneous landscapes (Frey-Ehrenbold *et al*., 2013; Monck-Whipp *et al*., 2018; Rodríguez-San Pedro *et al*., 2019). Physical structures such as windbreaks, remnant vegetation, and hedges have been demonstrated to support elevated levels of bat activity by providing structural heterogeneity, foraging, commuting grounds, roost sites, and protection against the wind as well as possible predator cover (Gelling *et al*., 2007; Hinsley & Bellamy, 2000; Kelm *et al*., 2014). For most bats in agricultural landscapes, woody features are crucial for both commuting and hunting, depending on their echolocation strategy and wing morphology (Frey-Ehrenbold *et al*., 2013; Froidevaux *et al*., 2018; Garin *et al*., 2019), and some bats can forage above farms or orchards (e.g., open-space foragers; Heim *et al*., 2015). Complex landscape diversity and close proximity between roosting and foraging sites provide an ideal environment for bats, increasing their species richness and activity, and minimizing travel time, and energy expenditure (Monck-Whipp *et al*., 2018; Rainho & Palmeirim, 2011).

South African citrus industry exports an estimated annual production of 164.8 million cartons of citrus in 2022 (Citrus Growers Association (CGA), 2023). Pest pressure on citrus by lepidopteran species such as the false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick), and the carob moth (CM), *Ectomyelois ceratoniae* (Zeller) is exceptionally high and combined they cause an estimated loss of R500 million annually as of 2022 (CGA, 2023). Given that these moth pests are nocturnal and that bat species of different foraging guilds feed on different lepidopteran insects (Monadjem *et al*., 2010), we expect that a variety of bat species should forage within citrus orchards. Citrus production plays an essential role in the growth of regional and local economies in South Africa. Given the possibility of expansion in the future , it becomes crucial to conduct more research on habitat selection and the activity of bats within agricultural landscapes such as citrus orchards. Therefore, it is essential to involve insectivorous bats as part of a wider application of IPM approaches as it has already been proven to be effective for rice in South Asia (Boonchuay & Bumrungsri, 2022), pecan farms in United States of America (Braun de Torrez *et al*., 2019; Brown *et al*., 2015), and macadamia in South Africa (Taylor *et al*., 2017; Weier *et al*., 2018).

The study aimed to determine how bat species richness and activity of different foraging guilds/groups respond to pest species abundance, specific landscape elements (e.g. intact riparian zones and woodland), and nighttime temperature within citrus orchards. We predicted that bat species richness and activity of different foraging groups will largely be affected by landscape elements, prey availability, and nightly temperature. The main research questions of this study are: i) How do bat species richness and activity levels change in response to variations in nightly temperature and across different landscapes within citrus orchard landscapes? ii) Within the citrus landscapes, how does the foraging activity of different bat groups vary depending on night temperature, landscape elements, and insect abundance?

2.2. Methods and materials

2.2.1. Study area

The study was conducted in Alicedale estates (-22.60801,-30.17191) in the Limpopo province of South Africa. Alicedale produces grapefruit, mandarins, limes, navel, and Valencia oranges. The orchard, which covers over 800 hectares, is 35 km to the west-southwest of Musina in the Vhembe District Municipality. The orchard is located 400–600 m above sea level. The climate of the area consists of hot summers (October to March) and dry, moderately warmer winters (May to August) (South African Weather Service). The study's geographic area is surrounded by a diversified landscape, dominated by weathered rocky outcrops known as kopjes that provide a habitat for a wide different plants and animals. The area is primarily covered in mopane trees (*Colophospermum mopane*) with the Nzhelele River flowing year-round close to the citrus orchard, providing water and habitats for a variety of foraging animals.

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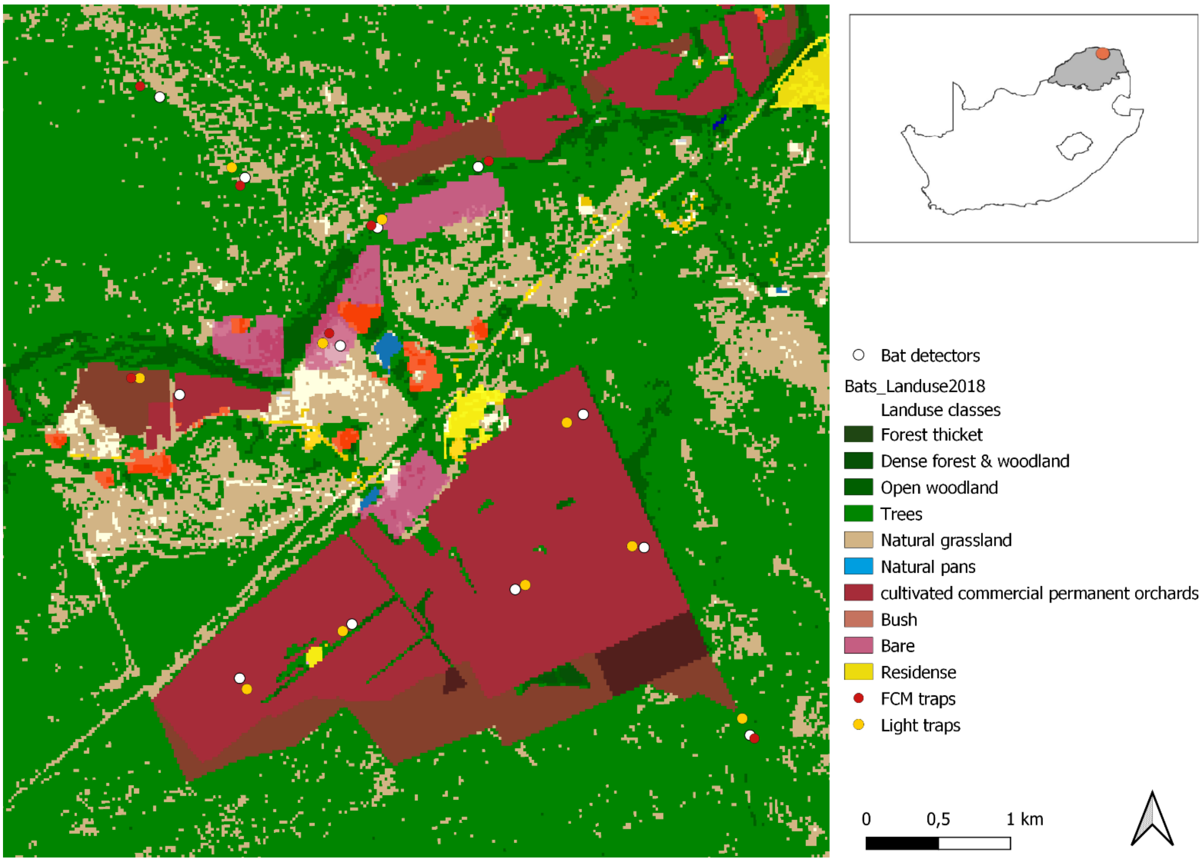

Figure 2.1.The layout of the study area with bat detectors, light, and FCM trap sites within the orchards and the surrounding area.

2.2.2. Orchard management

Effective orchard management practices are the cornerstone of protecting citrus from insect pests. Citrus pest control primarily relies on high volume, full cover spraying regimes applied around 8 and 4 weeks before harvest, coinciding with peak moth activity or egg hatching. This ensures that that most emerging larvae are exposed to the active chemical before damage occurs.

2.2.3. Experimental design

The study area was overlaid with a 6x6 km grid across the orchard and surrounding natural vegetation using the QGIS 3.32.1 software (QGIS Development Team, 2023). A total of 12 sites were stratified across the landscape to account for the landscape variation. Seven sites (N = 7) were selected within the orchard (Orchard 1 and 2; Wet edge 1 and 2; Dry edge 1 and 2) and five sites (N = 5) were selected outside the orchard (Dry riparian; Wet riparian 1 and 2; Woodland 1 and 2). To achieve spatial independence, sites were at least 1 km apart at the time of sampling.

2.2.4. Acoustic sampling

The first bat monitoring took place for three days (15 -18 April 2022) for a preliminary sampling of the study area. Two SM4BAT detectors (Wildlife Acoustics, Inc., Maynard, MA, USA) were used to monitor bat activity for three nights, amounting to 39 hours of sampling effort at two sites (Riparian and woodland). Detectors were automatically configured to record available acoustics upon detection at a full spectrum of sampling rate of 384 kHz, with a record threshold of – 35 Db, an input gain of – 2 Db, and for a maximum recording period of 15 s, and were set to record calls continuously between 5:30 pm and 06:30 am. Detectors were mounted on either a tree trunk or a pole at a height of 1.5-3 m above the ground with external omnidirectional microphones (Wildlife Acoustics, Inc., Maynard, MA, USA), facing in the direction of expected bats' flight paths (Linden *et al*., 2014).

Bat monitoring within the orchard and the surrounding landscape commenced in July-December 2022 (excluding November 2023), with the sampling effort of 7-day surveys for each month. Bat activity was monitored at 12 sites (Fig. 2.1. above) using four Song Meter Mini Bat and eight SM4BAT detectors (Wildlife Acoustics, Inc., Maynard, MA, USA), and the detector's settings were as per the first survey. However, concerning the start and end time of the recording, they were changed to 6:00 pm and 06:00 am. Bat activity was monitored for 24 nights, amounting to 288 hours of sampling efforts at each site.

2.2.5. Acoustic analysis

Recorded calls were manually identified using AnalookW software version 0.3.8.13 (Corben, 2006) after being converted to zero-crossing (ZC) format using Kaleidoscope software version 5.4.9 (www.wildlifeacoustics.com). All calls were identified to species level or assigned as mixed species groups, where the call could not be distinguished from other calls (Torrent *et al*., 2018). The frequency of the calls (minimum, maximum, characteristics, and frequency of the knee), call duration, and slope of each call sequence were used to assign calls to specific species or mixed species groups. All the species identified were assigned according to the reference library of South Africa (Parker & Bernard, 2018; Monadjem *et al*., 2017; Taylor *et al*., 2013). Calls that were unable to be identified were discarded. Identified species were then grouped according to their foraging guilds, clutter – edge foragers (Vespertilionidae), clutter foragers (Rhinolophidae and Hipposideridae), and open-air foragers (Molossidae and

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Emballonuridae). The activity behavior of each species was identified by different call buzzes; searching, foraging, social, and socializing buzzes, call buzzes that could not be distinguished or confidently identified were labelled/noted as “Undefined”. The number of bat call passes were expressed in terms of Miller’s activity index (AI), which accounts for the presence of species occurring during one-minute time intervals (Miller, 2001). The AI is used to estimate bat activity while accounting for bias in identifying individuals several times.

2.2.6. Insect trapping – UV light traps

Insect abundance was estimated using Ultraviolet LED lights (UV Lamp). The UV LED light emits wavelengths between 395 nm and 405 nm (https://www.entosphinx.cz), which are effective wavelengths to trap lepidopteran species and pests (Balamurugan & Kandasamy, 2021; Brehm *et al*., 2021; Knight *et al*., 2023). The light traps were deployed simultaneously with bat detectors the second week of each month and the sampling effort differed with each. In September 2022, the light traps were deployed for six consecutive nights, and in October, they were deployed for seven consecutive nights, and in December for three nights. Custom-made light traps were designed to be easily hung at about 1.5 m above the ground. Plastic bottles (10L) were equipped with UV LED lights connected to a 12-V battery and timers which were programmed to automatically turn on at 18:00 and off at 21:00. The distance between the traps and bat detectors was approximately 200 m (Froidevaux *et al*., 2018). Every morning, each trap bag was labelled and kept in a freezer. We verified if any FCM or CM had been captured, and other insects were classified to the order level. The insects were separated by order such as Lepidoptera, Coleoptera, and Hymenoptera. To estimate the biomass of captured insects at each site, the insects were placed in a convection oven at 60 °C for three days and were then expressed as dry mass (Braun de Torrez *et al*., 2019).

2.2.7. Pest insect (FCM) trapping

The FCM activity and abundance were monitored within the orchard and the surrounding area using yellow delta traps with sticky liners baited with FCM pherolure (species specific, FCM) (https://insectscience.co.za/product/f-c-m-pherolure/) and Chempac pills. The traps were suspended at a height of ~1.5 m above the ground to maximize the spreading of the pheromone in the orchard and the surrounding area (Moore, 2002). The traps were monitored every morning and captured individuals were

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recorded, removed, and stored in a vial. The traps were deployed 200 m away from bat detectors and light traps.

2.3. Data analysis

2.3.1. Estimating species richness

Species richness was estimated using coverage-based rarefaction and extrapolation of species richness (Hill numbers; Chao & Jost, 2012). For accurate comparisons of species richness across samples, the preferred method was using coverage-based rarefaction and extrapolation. This technique adjusts for varying sampling efforts by focusing on sample completeness, leading to more reliable estimates of true richness ratios (Chao *et al*., 2014). To account for incomplete sampling, we use the sample coverage estimator to ensure our estimates of species richness are reliable (Hsieh *et al*., 2016). The iNEXT function in the iNEXT R package (Hsieh *et al*., 2016) was used to create the rarefaction and extrapolation sampling curves using sampling-based abundance data and derive the first 3 Hill numbers (species richness, Shannon diversity, and Simpson diversity). We used observed species richness at equal coverage for subsequent analysis. All sites achieved high sample coverage (>99%; Supplementary Figure S5.1). All statistical analyses were conducted in R software (R core team, 2023) using the package 'iNEXT' (Hsieh *et al*., 2016).

2.3.2. Principal Component Analysis (PCA)

The role of land cover and context in structuring bat assemblages was explored by first extracting 200 m buffers around each site using the South African land cover raster dataset (Fig. S5. 1) (following South African National Land-Cover classes (2013–2014, South African Department of Environmental Affairs, 2016). We then calculated the area for each land-cover class (following South African National Land-Cover classes (2013–2014, South African Department of Environmental Affairs, 2016) encompassing each site. Using a combination of ground truthing and satellite images (Google Earth images; Google Earth), we updated land use values to fit the current land use. We reclassified land-use classes to the following five classes: Open Woodland, Orchard, Dry Riparian, Wet Riparian, and Grassland (Table S5. 1). We then estimated the proportion of each land use at each bat detector station. Since the land use types were highly correlated (variation inflation factor (VIF) > 10) for all land use variables for all modelling scenarios (Table S5. 3), we performed a PCA, generating three principal components. The first two explained 83% of the variation in land cover and were retained (Fig. S5. 2). The advantage of using the PCA is that they are orthogonal to each other and are therefore not correlated. We used R software (R core team, 2023) to establish a circular buffer for/to each site.

2.3.3. Bat species richness and activity

For all the modeling scenarios several Generalized Linear Mixed Effects Models (GLMM) were fitted to account for the repeated counts since the same sites were sampled over time (Bates *et al.*, 2014). Sites were included as a random effect to address potential correlations among observations collected at the same sites throughout the study. In each modelling scenario, overdispersion was evaluated using the full model residuals evaluated against what would be expected under a Poisson or negative binomial distribution (Magnusson *et al*., 2017). If overdispersion was not detected, then a mixed effect model using a Poisson family and "log" link was fitted. If overdispersion was detected, a negative binomial distribution was used to account for the overdispersion (Venables & Ripley, 2002). For the GLMM models, the same structure with sites nested within land use was used.

The models for bat species richness and activity were assessed separately. For each model, the effect of temperature was first evaluated because bat activity is thought to be positively related to average nightly temperatures (Bender & Hartman, 2015). Second, the effect of land use gradient was assessed using PC component 1 (Ringnér, 2008), since different bat species prefer/respond differently to different land cover (Dixon, 2012). A regression model was done first for both temperature and PC1 and then evaluated if adding a quadratic term improved the fit. The addition of a quadric term evaluated if the bat response to temperature and PC1 was non-linear. Several studies have highlighted that bat responses to temperature might be non-linear as different bat species respond differently to nightly temperatures (Arbuthnott & Brigham, 2007; O'Donnell, 2000). These models were contrasted with a null model.

2.3.4. Bat foraging group activity and trophic interactions

We assessed bat foraging groups in two different scenarios. This was done since insect biomass data was only collected from September 2023 to December 2012, while temperature data was available for the complete study period. We therefore used two datasets to evaluate bat foraging groups. In the first scenario, we excluded prey and pest insect data and only focused on the effect of temperature and PC1 (land use variation) on bat foraging group activity. In the second scenario, we added insect biomass and pest species counts to the temperate and PC1 data to evaluate trophic interactions. We only obtained viable sample sizes for open-air and clutter-edge foragers for the trophic interactions.

We additionally used GLMM to evaluate trophic interactions of the different bat foraging groups at the orchard level. We investigated several biologically plausible hypotheses, based on landscape configuration (edge, non-edge; foraging groups respond differently to land use; Puig-Montserrat *et al*., 2015), temperature variation (bat activity is mediated by nightly temperature; Brooks, 2009), pest insect abundance (FCM counts as a proxy; foraging groups have been shown to respond to pest insect abundance; Taylor *et al*., 2013; Heim *et al*., 2018) and insect biomass (from UV light trapping, bat respond positively to insect biomass (Braun de Torrez *et al*., 2019). We fitted these variables alone or in combination, but we did not include interaction terms due to limited data across all combinations. Models were contrasted to a null model. For trophic modelling, final models were selected based on the lowest AICc values using the dredge function (package 'MuMIn' by Barton, 2017). If a model was uncertain, conditional model averaging was used for the models that fall within delta AIC = 2. All statistical analyses were conducted with R (version 4.3.1, R Core Team, 2023). All models were fitted using scaled predictor variables and 'site' as a random factor. Models were also evaluated for multicollinearity (VIF < 5).

2.4. Results

A total of 63,759 bat calls were acoustically recorded during a sampling effort of 25 nights and 319 survey hours, from August 2022 to December 2022 (Table S5. 4). Of these, 51 150 (80.2%) calls were identified and attributed to a species level of 19 species, and 12 609 calls (19.8%) were identified to mixed species groups (Table S5. 4). The most common species detected was *Tadarida aegyptiaca* (50.2%; Table S5. 4), followed by *Chaerephon ansorgei* (12.4%), and *Chaerephon pumilus/Mops condylurus* (11.4%). These species were present during the duration of the study in all sites with higher activity than other species (See Table S5. 4). *Hipposideros caffer* (0.005%), *Sauromys petrophilus* (0.008%), and *Rhinolophus* 70-72kHz (0.01%) were the least recorded species occurring at two or three sites only.

2.4.1. Effect of landscape heterogeneity and temperature on bat species richness and activity

For bat species richness, we did not detect overdispersion with the Poisson distribution models (p-value >= 0.05) and fitted a Poisson mixed effect model. We found support that meanTC (average night temperature)*PC1 interaction had the highest support (*AICwt* = 0.41; Table 2.1; Table S5. 5). While the model with temperature quadratic term and PC1 fell within delta 2 AICc units of the most parsimonious model, the likelihood ratio test suggested that the interaction term provided a better model fit to data (*p* <= 0.000). There was little support for other interaction models or adding quadric terms (Table S5. 7). We therefore retained only the meanTC*PC1 model further inference in bat species richness. There was a significant interaction between meanTC and PC1 (*p* <= 0.000), where the positive effect of temperature on species richness was modulated by land use heterogeneity (variation in PC1). Specifically, the interaction term is negative (*β* = -0.104, *p* = 0.026), suggesting that moving from negative PC1 (Dry riparian, wet riparian, and woodland) to positive PC1 (Dry edge, wet edge, and orchard) weakens the positive temperature bat species richness response (Fig. 2.2 A). This means that the largest effect of meanTC on bat species richness is dry riparian and wet riparian, where increased temperature significantly increased bat species richness, while at orchard level, bat species richness remained constant around temperature variation (Fig. 2.2 A).

For bat activity models, we found no overdispersion in the full model (P >= 0.05), and we therefore fitted (p-value >= 0.05) a Poisson mixed effect model (Kleiber & Zeileis, 2008). There was a significant interaction between meanTC, the temperature quadratic term and PC1 (*AICcWt* = 0.97; Table 2.1; Table S5. 6), where bat activity response to meanTC was mediated by changes in PC1. Specifically, the modulation effect of PC1 on the temperature bat activity response was positive (β = 0.162, p = 0.002), suggesting that moving from negative PC1 (DryRiperian) to positive PC1 (Orchards) strengthens the positive temperature bat activity response (Fig. 2.2 B). This means that the largest effect of meanTC on bat activity is around orchards, where increased temperate significantly increased bat activity, while in the natural areas, this effect was less pronounced.

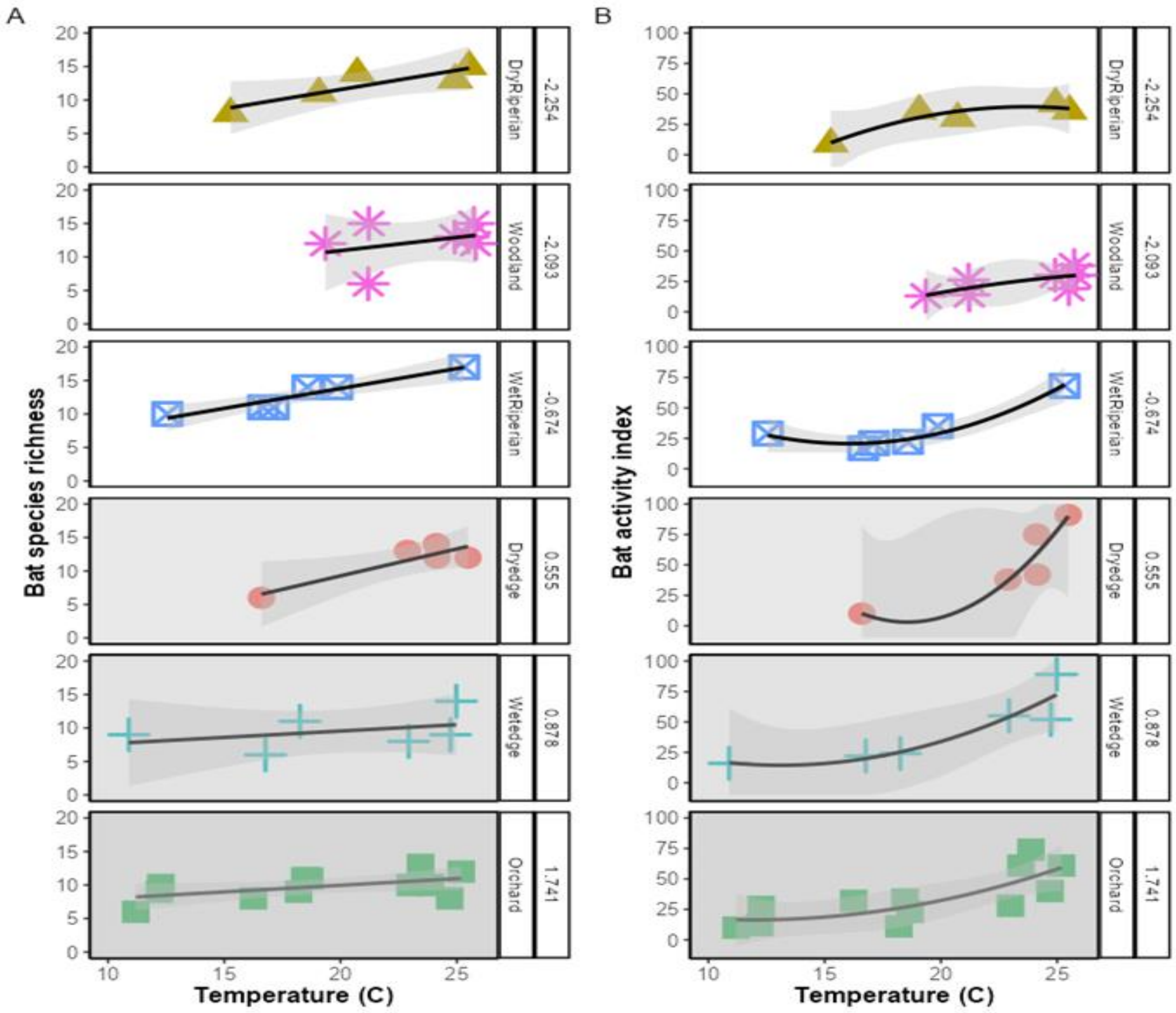

Figure 1.2. Relationship between bat species richness (A) and bat activity (B) across variation in land use expressed as axis 1 (PC1) derived from principal component analysis. Grey blocks indicate orchards, secondary axis represents PC1 scores and their associate with each site.

Table 2.1. Summary of the best generalized linear mixed model assessing the relationship between meanTC and PC1 for bat species richness.

Modnames	*K*	*AICc*	*Delta_AICc*	*ModelLik*	*AICcWt*	*LL*	*Cum.Wt*
Bat species richness models							
meanTCINTPC1	6	211.6	0	1	0.41	-98.7	0.41

meanTCQPC1	6	213.6	1.99	0.37	0.15	-99.7	0.56
Bat activity models							
meanTCQINTPC1	6	395	0	1	0.97	-190.4	0.97
meanTCQPC1	5	402.2	7.19	0.03	0.03	-195.3	1

Abbreviations: meanTC average nightly temperature, PC1 principal component 1, K number of independent variables, AICc Akaike information criterion corrected for small sample sizes, Delta_AICc difference in AICc score, ModelLik model likelihood, AICcWt AICc weight, LL Log-likelihood, CumWt sum of the AICc weights, INT is the Interaction of preceding term, Q is the quadratic term.

2.4.2. Foraging group activity

2.4.2.1. Activity of clutter-edge foragers and trophic interactions

For clutter-edge feeders, there was overdispersion ($p <= 0.05$) and we, therefore, fitted a negative binomial model. Temperature with the added quadratic term (*AICwt* = 0.61; Table 2.2; Table S5. 7) had the highest support. No other variable on their own or in combination affected clutter-edge forager activity (Table S5. 7). Mean temperature had a significant positive effect ($\beta = 0.72$, $p <= 0.0001$) on clutter-edge feeders, with an average activity increase of 2.2 times with one temperature increase (Fig. 2.3 A). However, this effect was non-linear with a positive term ($\beta = 0.269$, $p = 0.009$), suggesting that while activity generally increases with temperature, this rate diminished as temperature continues to rise.

When assessing clutter-edge trophic interactions (temperature, PC, insect biomass, FCM counts) we found great model uncertainty (Table S5. 8), we therefore model averaged the top models using conditional averaging (Barton, 2002). We found that at the orchard level insect biomass (total *AICwt* = 0.475), temperature (*AICwt* = 0.887) and PC1 (*AICwt* = 0.48) (Table S5. 8) contributed to clutter-edge activity. The activity of clutter-edge bats in orchards increased with temperature ($\beta = 0.233$, $p <= 0.000$: Fig. 2.3), insect biomass ($\beta = 0.451$, $p = 0.001$; Fig. 2.4 below), while the effect of PC1 was negative ($\beta = -0.243$, $p = 0.02$; Fig. 2.4), where activity declined from dry edge sites to the wet edge and center of orchards (Fig. 2.4).

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2.4.2.2. Activity of open-air forager and trophic interactions

For open-air forager activity, we found overdispersion in the full model ($p < 0.05$), and we, therefore, fitted a negative binomial model (Kleiber & Zeileis, 2008). The two top-performing models included meanTC and PC1, either as single or multiple fixed effects (Table 2; Table S5. 9). Open-air forager activity increased with temperature ($\beta = 0.447$, $p <= 0.000$; Fig. 2.3 B), and increased with moving from negative PC1 to positive ($\beta = 0.211$, $p = 0.006$), e.g. moving from natural to orchard sites (Fig. 2.3 D). Open-air forager activity had two top-ranking models which included mean temperature and PC1 (Table S5. 10). No other variable had support. We model averaged the top models using conditional averaging and found that at the orchard level, mean temperature (*AICwt* = 0.70) and PC1 (*AICwt* = 0.35) (Table S5.9) contributed to open-air forager activity. The activity of open-air foragers inside orchards increased with temperature ($\beta = 0.457$, $p <= 0.000$: Fig. 2.4), but decreased ($\beta = -0.226$, $p <= 0.029$) when moving from orchard edges (Fig. 2.4) to the middle of orchard (Fig. 2.4).

2.4.2.3. Activity of clutter forager

For clutter forager activity, overdispersion was found in the full model ($p < 0.05$) and we therefore fitted a negative binomial model (Kleiber & Zeileis, 2008). PC1 (*AICcWt* = 0.51; Table 2.2) had the highest support, with a significant negative ($\beta = -1.236$, $p = 0.001$) effect of variation in PC1 scores (land use heterogeneity) (Table S5. 11), which clutter forager activity declining by around 70% when from natural areas to the orchard (Fig. 2.3 C below).

Table 2.2. Best fitting models for different bat foraging groups using mixed effect models fitted to bat acoustic data collected from August to December 2023 in Tshipise (Alicedale), South Africa.

Modnames	*K*	*AICc*	*Delta_AICc*	*ModelLik*	*AICcWt*	*LL*	*Cum.Wt*
Clutter-edge foragers							
meanTCQ	5	422.7	0	1	0.5	-205.6	0.5
meanTCINTPC1	6	424.8	2.03	0.36	0.18	-205.3	0.68
Open-air foragers							

meanTC	4	199.8	0	1	0.44	-94.09	0.44
meanTCPC1	5	199.8	0	1	0.44	-91.91	0.87
Clutter foragers							
PC1	4	239.1	0	1	0.51	-115	0.51
PC1Q	5	241.6	2.53	0.28	0.15	-115	0.66

Abbreviations: meanTC average nightly temperature, PC1 principal component 1, K number of independent variables, AICc Akaike information criterion corrected for small sample sizes, Delta_AICc difference in AICc score, ModelLik model likelihood, AICcWt AICc weight, LL Log-likelihood, CumWt sum of the AICc weights, INT is the Interaction of preceding term, Q is the quadratic term.

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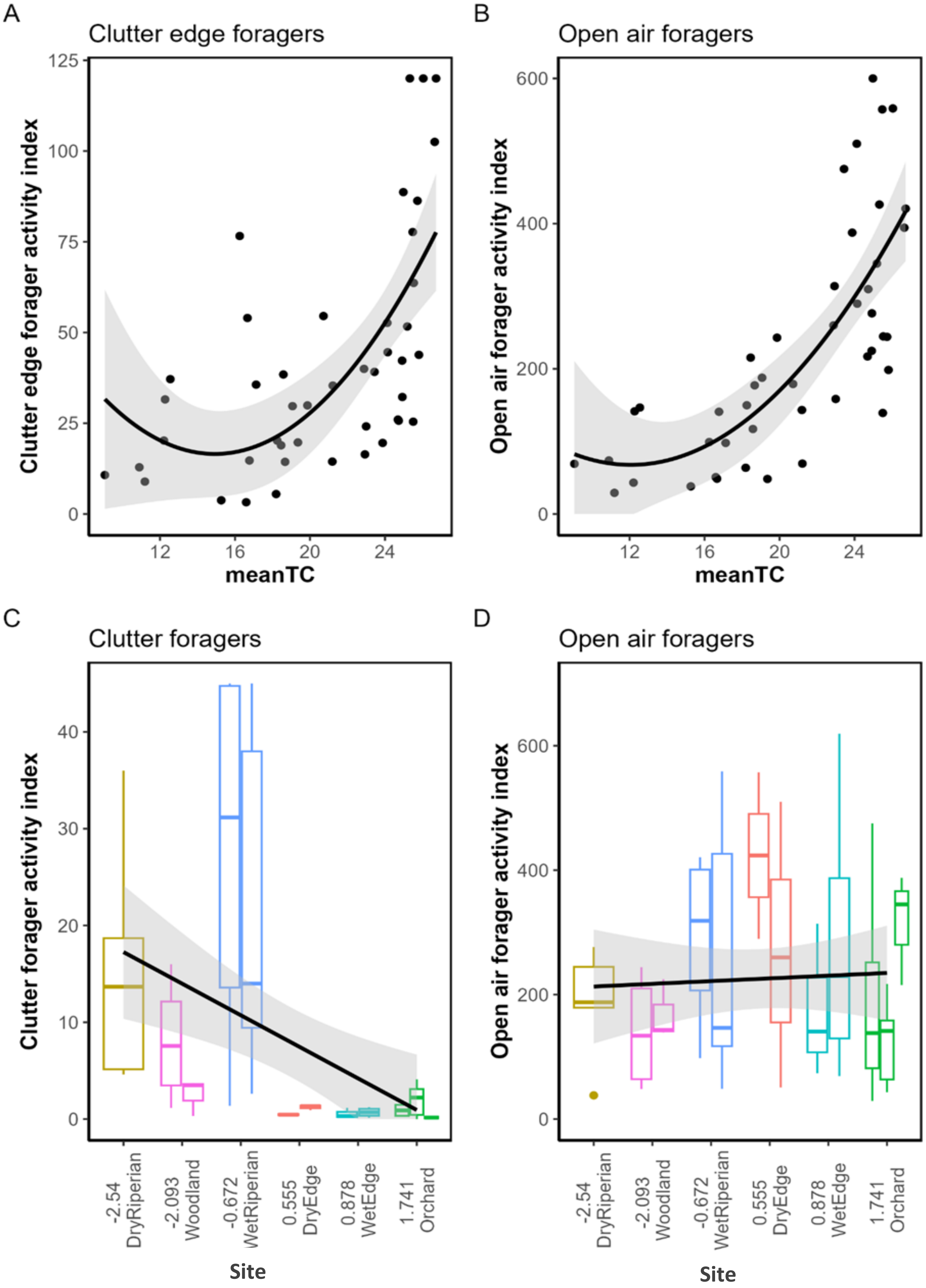

Figure 2.2. Final models showing the relationship between meanTC and different foraging group activity across different sites. The sites were arranged in respect to PC1 scores high vegetation cover to low cover.

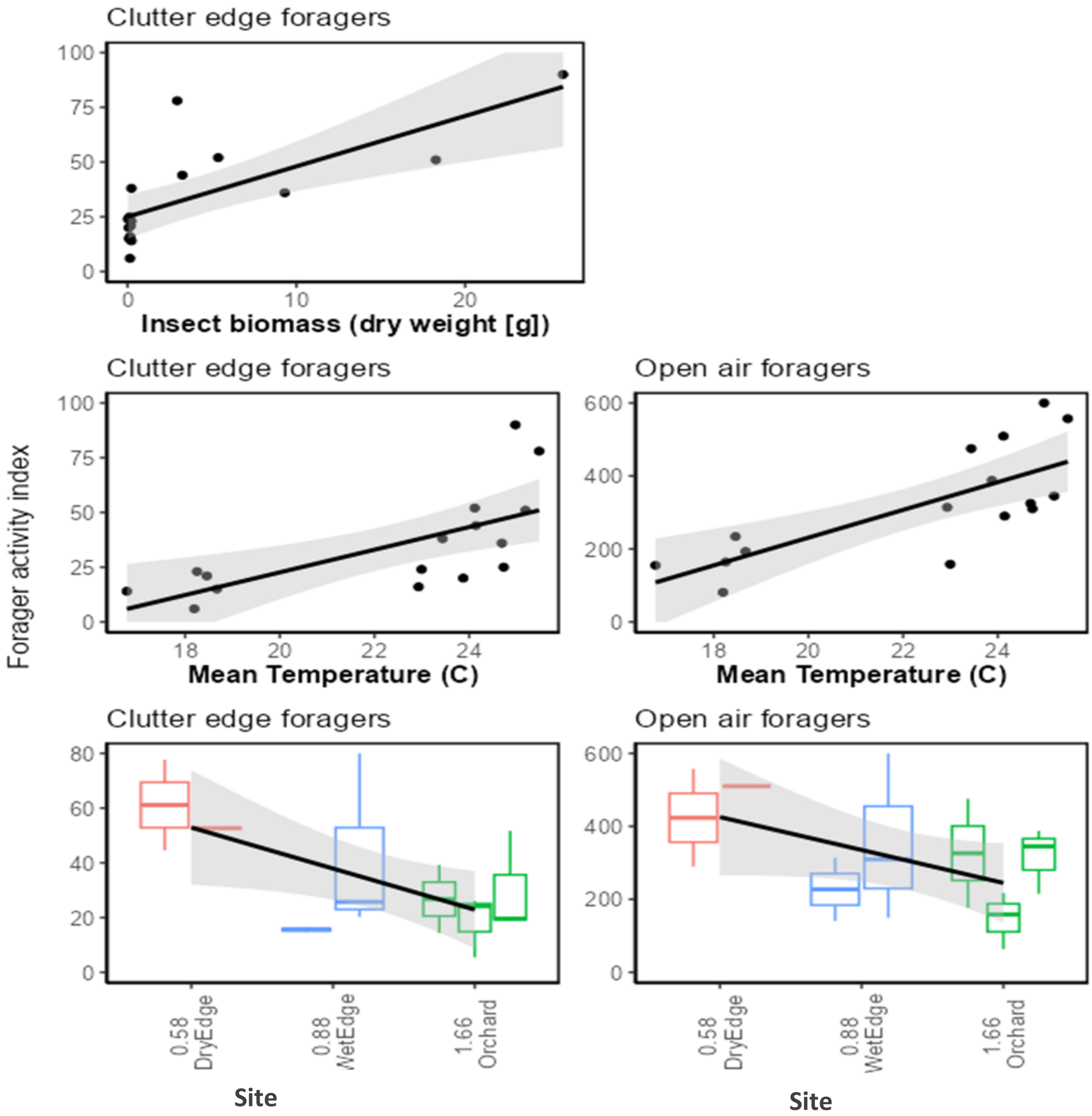

Figure 2.3. Final models showing the relationship between mean temperature, sites and insect biomass (g) about different foraging group activity. The sites were arranged in respect to PC1 scores from low to high level of vegetation cover.

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2.5. Discussion

We investigated the effect of nightly temperature, land use variation, insect biomass, and pest insect count on bat species richness, activity, and bat foraging groups. Our results demonstrated that temperature and land use heterogeneity were the key driving factors influencing bat species richness and activity.

Increased temperature consistently had a positive effect on bat species richness, and activity, for bats as a whole and functional group. This positive trend held for sites located in natural and orchards highlights the importance of temperature for bats. Our results concur with various others. For example, McCain. (2006) reported that bat species richness peaked high with elevated temperature and water availability, and alternatively, declined with low temperature. However, Burles *et al*. (2009) concluded that weather conditions are sometimes highly species-specific, as they found that *Myotis keenii* was not adversely affected by the wet and cool conditions. Several mechanisms could explain the effect of temperature on bat species richness and activity. First, flying insects exhibit temperature-dependent activity patterns, often becoming more active and abundant with increased temperature within their suitable range (Meyer *et al*., 2016). This increased prey availability coincides with optimal temperatures for insectivorous bats, leading to higher foraging activity and prey consumption (Wolbert *et al*., 2014). Second, insectivorous bats require sufficient ambient temperature to maintain body temperature for flight and foraging. Warmer temperatures within their physiological tolerance range (10°C to 25°C; however, differ with species and geographical locations) generally reduce energy expenditure for thermoregulation, allowing them to allocate more energy towards searching for prey (Speakman & Racey, 1989).

Our findings indicate that bat species richness was higher in the natural areas than in the orchard. This is consistent with earlier studies. For example, Wechuli *et al*. (2016) reported that more structurally complex woodland (natural habitat) had the highest bat species richness and diversity compared to farmland. Similarly, Lawer & Darkoh. (2016) found that teak plantations and fallow lands supported a higher number of insectivorous bats and insects than cocoa, cashew, and oil palm farms. High bat species richness in natural areas can be attributed to greater habitat diversity and structural complexity compared to orchards. The structural complexity of natural areas provides a wider range of foraging opportunities, roosting sites, and microclimates

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suitable for various bat species (Avila-Flore & Fenton, 2005). Additionally, this structural heterogeneity supports diverse insect prey communities, and thus, attracts insectivorous bats with varied foraging strategies (Whitaker, 2004). Orchards or farmlands on the other hand, with their monoculture structure and limited vegetation layers, often lack the complexity to support diverse bat communities. While some bat species might adapt to orchards for specific resources, the overall species richness remains lower due to limited ecological niches (Taylor *et al*., 2013).

Our results further demonstrate that bat activity increased more in the orchards than in the natural areas. This observation concurs with the findings of previous studies. For example, Heim *et al*. (2017) reported a high proportion of bat activity above arable fields. Similarly, Taylor *et al*. (2013) found high bat activity in macadamia orchards. However, bat activity in both these studies varied with seasons. In addition, Weier *et al*. (2018) reported that high bat activity in the orchards can be explained by the abundance of insects as orchards usually have high peaks of insects during wet seasons. More importantly, bat activity in orchards can be highly influenced by diverse vegetation structures such as hedgerows, trees, windbreakers, and field margins, which can harbour a variety of insect communities attractive to different bat species (Wickramasinghe *et al*., 2003). Orchard edges were also found to support high bat activity. This corroborates the findings of Wolcott & Vulinec. (2012) that the bat activity was higher in the edges compared to the middle of the farm. This was probably related to edges providing habitat and foraging pathways to different bat species.

Our results suggest that bat forager groups respond differently to temperature, land use heterogeneity, insect biomass and pest counts. This can largely be explained by the fact that insectivorous bats have species-specific guild responses to different landscape elements. This response may be driven by their foraging strategy, wing morphology, and characteristics of echolocation call (Mendes *et al*., 2017). Our findings demonstrated that the activity of clutter-edge foragers was significantly influenced by land use heterogeneity and temperature. Activity of the clutter-edge foragers was significantly high and positively associated with wet edge and riparian zones. This concurs with the recent study by Ancillotto *et al*. (2019) and Katunzi *et al*. (2021) who reported that farm edges that are near riparian zones or water bodies promote high bat activity, especially for clutter-edge foragers, as they provide roosting sites through old and dead trees and ideal temperatures that promote higher insect

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abundance (food sources) (Downs & Racey, 2006). Our results further show that the activity of clutter-edge foragers in orchards was significantly influenced by both temperature and insect biomass. However, their activity decreased as they foraged less from the edges to the centre of the orchard. This concurs with the study by Weier *et al*. (2018) who suggested that clutter-edge foragers increased their activity in the macadamia orchards during the high season while foraging more closely to the edge of the orchards rather than in the middle. This response may have been driven by the lack of tree cover in the middle of the orchard (for protection from predators) (Medina *et al*., 2007).

Our results show a similar pattern of activity in clutter foragers from that of clutter-edge foragers and corroborate that temperature and land use positively influence their activity. The clutter forager's activity was profoundly lower in the orchard, wet edge, and dry edge but high at dry riparian and wet riparian. This suggests that clutter foragers preferentially selected natural vegetation in close proximity to riparian habitats compared to orchards (open areas). The availability of trees might have influenced this response as they not only provide safety against predators but also roosting sites with ideal ambient temperatures, and the availability of water sources that promote high insect diversity (food sources for bats) (Heim *et al*., 2018). This study adds to the majority of studies that have demonstrated increased bat activity along habitat edges. These areas provide a sheltered environment from strong winds, allowing for more efficient hunting, and also attract a wider variety of insect prey (Whitaker *et al*., 2000), a refuge from predators (Lima & O'Keefe, 2013) navigational landmarks and flight conduits (Kalcounis-Rueppell *et al*., 2013).

The activity of open-air foragers showed a marginally significant increase over the orchard, wet edge, dry edge, and wet riparian and a low increase in dry riparian and woodland. This is in agreement with Katunzi *et al*. (2021) who reported that open-air feeders preferentially forage over rice fields compared to forested areas, with Mtsetfwa *et al*. (2018) suggesting that open-air foragers selectively forage more over the sugarcane and less in savanna. In addition, Linden *et al*. (2019) suggested that these open-air foragers search for insects over the orchards rather than the edges or adjacent natural vegetation. The high activity of open-air foragers within the orchard was probably influenced by more open spaces with fewer obstacles, and thus increase their hunting efficiency as they are able to maneuver easily and detect prey at a distance (Monadjem *et al*., 2010). Temperature was the best predictor of bat activity for all foraging groups. Overall bat activity increased throughout the study period (summer), and this is consistent with previous work by Taylor *et al*. (2013) who demonstrated that the increase in bat activity during summer is highly linked to increased activity levels of insects.

Our results demonstrate that insect biomass strongly influenced the activity of clutter-edge foragers than for open-air foragers. This can be probably explained by high insect activity on the orchard edges and riparian zones than over the orchard. Our results correspond with the findings of Rodriguez-San Pedro *et al*. (2019) who reported that natural habitat patches close to water bodies are important in retaining stable bat communities for control of insect pests within agricultural landscapes. Our study concurs with the results from previous studies that highlighted that insect diversity positively influences bat activity (Taylor *et al*., 2013; Heim *et al*., 2018; Weier *et al*., 2018). Although acoustic data for clutter-edge foragers was limited, our results show that they were the more prevalent foraging groups in wet riparian and orchard edges. This can be related to their wing morphology and echolocation strategy in that they can maneuver within complex environments and detect insect prey within intricate vegetation structures (Frey-Ehrenbold *et al*., 2013). Riparian zones and orchard edges are important for clutter-edge foragers as they offer higher insect availability (Downs & Racey, 2006), hence a positive correlation between insect biomass and clutter-edge foragers activity. In riparian and orchard edges, clutter-edge foragers potentially experience less competition from other foraging groups and decreased predation risk (Monck-Whipp *et al*., 2018; Rainho & Palmeirim, 2011).

Multiple studies have shown insectivorous bats to be valuable in controlling pest insects within agricultural landscapes and our results are an exception to that (Braun de Torrez *et al*., 2019; Kolkert *et al*., 2020; Taylor *et al*., 2017; Weier *et al*., 2018). Our study demonstrated high bat diversity and activity within and the surrounding citrus landscape. This indicate is in agreement with IPM practices. The important aspect of IPM is incentivising farmers to maintain landscape heterogeneity by leaving patches of natural vegetation and enabling connectivity of croplands with natural areas as it will favour insects populations and thus increase foraging habitats for bats (Monck-Whipp *et al*., 2018). However, future studies should directly focus on investigating the impact of landscape context on bat populations within cotrus landscapes. Although

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recommendations for management toward the enhancement of bat populations within the macadamia farmlands have previously been highlighted (Linden *et al*., 2019; Taylor *et* al., 2017; Weier *et al*., 2018), however, little is known about bat populations within the citrus landscapes. Therefore, future studies should consider implicating bat houses within the citrus orchards to enhance their activity, as previously reported by Weier *et al*. (2018).

2.6. Conclusion

The results presented in this study demonstrated that land use heterogeneities and their associations with temperature and insect activity within citrus landscapes influenced bat species richness, activity, and foraging group activity. Our study showed a significant differences in bat species richness and activity between natural areas and orchards. Wet riparian and orchard edges supported the high activity of clutter and clutter-edge foragers, while orchards supported the high activity of open-air foragers. The temperature was the key factor affecting bat species richness and all foraging group's activity. The results from this study emphasize that natural and riparian habitats are important for promoting bat species richness and activity and can thus potentially function as stable biological control of major pests within agricultural settings. Our study further emphasizes the need to include insectivorous bats in IPM approaches as it would provide for their conservation and management within intensively managed agricultural landscapes.

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CHAPTER 3

Potential of sympatric bat species to suppress moth pests in the citrus industry

Abstract

Several studies have highlighted the ecological services that insectivorous bats provide within the agricultural landscape. Due to high metabolic demand, insectivorous bats are postulated to consume substantial amounts of insects per night. However, due to their nocturnal and fast moving behaviour, it is quite challenging for direct observation. Advances in molecular techniques have proved to be efficient in assessing bat diets. The citrus industry considers false codling moth (FCM, *Thaumatotibia leucotreta*) and carob moth (CM, *Ectomyelois ceratoniae*) as major important pests of citrus in South Africa. In this study, we captured bats at different landscapes to assess their diet, whether they consume FCM, CM, or other agricultural pests in an industrial citrus orchard, Alicedale, Tshipise. During a sampling effort of four 7-day surveys over six months (July – December 2023), we recorded nine bat species and collected 396 faecal samples. We used DNA metabarcoding to assess the diet of different bat species. The faecal samples were analyzed using polymerase chain reaction (PCR) using universal primer pair LCO 1490 and HCO 2198 region of the COI gene (Folmer primers). We found that all bat foraging groups, open-air and clutter-edge foragers consumed important citrus pests. All foraging groups included agricultural pests, with open-air foragers consuming high volume. All foraging groups consumed had a wide diverse diet, with Lepidopterans being frequently consumed. Our results suggest that insectivorous bats occupying the same landscape as citrus pests can be important for suppressing pests during outbreaks.

Keywords: DNA metabarcoding, nocturnal, faecal samples, polymerase chain reaction, pest suppression

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3.1. Introduction

Numerous studies have indicated the suppression of agricultural insect pests by insectivorous bats (Boyles *et al*., 2011; Cleveland *et al*., 2006; Kunz *et al*., 2011,2019; McCracken *et al*., 2012; Symondson *et al*., 2002; Taylor *et al*., 2018; Weier *et al*., 2019). As such, the immense economic value that insectivorous bats provide in agricultural landscapes is increasingly gaining recognition (Boyles *et al*., 2011; Taylor *et al*., 2018). For example, Wanger *et al*. (2014) showed that wrinkle-lipped bats (*Tadarida plicata*) in Thailand function as natural pest control agents for rice crops, consuming white-backed planthoppers (*Sogathella furcifera*) and preventing an estimated rice loss of more than 1.2 million USD per year. Additionally, studies in North America show that Brazilian free-tailed bats (*Tadarida brasiliensis*) play a significant role in regulating populations of *Helicoverpa zea*, a common pest in cotton and corn fields (Federico *et al*., 2008; Krauel *et al*., 2018). Furthermore, in a recent study, Linden *et al*. (2019) estimated the avoided cost of USD ~5,000 ha/year that bats and birds provide in macadamia orchards by feeding on macadamia pest insect species, like the macadamia nut borer (*Cryptophlebia ombrodelta*) or the green vegetable bug (*Nezara viridula*). Bats can therefore provide viable ecosystem services within agricultural landscapes as potential biological suppressors of various insect pests.

Insectivorous bats within agricultural landscapes can consume a large amount of insects per night due to their energetic demand (Russo *et al*., 2018). Despite bats being voracious insectivores, their dietary preferences differ. While some insectivorous bats specialize in specific insect orders, the majority are opportunistic feeders, readily consuming whichever insects are readily available (Heim *et al*., 2017; Symondson *et al*., 2002). Generalist predators within agricultural landscapes exhibit broader dietary niches compared to specialist predators, contributing to ecosystem stability through enhanced resilience to prey fluctuations and wider pest suppression (Braun de Torrez *et al*., 2019). Generalist predators are therefore important, from a biological control view because they feed on pests during outbreaks and at low abundances (Symondson *et al*., 2002). Multiple studies have documented that insectivorous bats can adjust their predatory behavior based on the availability of insect prey, particularly during outbreaks in agricultural systems (Kennard, 2008; Gonsalves *et al*., 2013; Heim *et al*., 2017). Insectivorous bats have different foraging strategies and wing morphologies that allow them to consume and exploit a broad range of insects, including species that are harmful to the economy or human health (Denzinger & Schnitzler, 2013). Some bat species are adapted to forage in open areas (open-air foragers), and they can have a significant impact on insects that are open-air flyers while others are adapted to forage in cluttered environments and are key predators of insect pests that are adapted to dense vegetation (Maine & Boyles, 2015). Yet, not much is known about the range of factors that affect each foraging group's activity and distribution within an agricultural landscape.

The cryptic nature and volant hunting behavior of insectivorous bats makes it challenging to study their diet, especially through direct observation (Brown *et al*., 2015). Over the years, researchers have used non-invasive methods to get a glimpse on bat diet using faecal pellets. Previous research has depended on traditional methods for dietary analysis, which involve identifying insect fragments found within the bat feces to order or family level (Whitaker, 1998). Additionally, traditional methods are biased towards hard-bodied insects. However, the advances in genetic analysis of insectivorous bats' faecal materials have facilitated the identification of prey species at the taxonomic level (Pompanon *et al*., 2012; Taylor *et al.*, 2017; *Zeale et al*., 2011). This approach has improved our ability to obtain species-level identification of various agricultural pest prey consumed by bats, especially for soft-bodied arthropods like moths. In a recent study, Taylor *et al*. (2017) used Next Generation Sequencing (NGS) to elucidate the diet of six bat species of different foraging groups (clutter, clutter-edge, and open-air foragers) in macadamia orchards and found that one third of all the feces collected of each bat species contained DNA from the macadamia important pest species (Green vegetable bug, *Nezara viridula*)*.* Similarly, Aizpurua *et al*. (2018) used NGS to show that two different bat species (*Miniopterus schreibersii* and *Tadarida brasiliensis*) consume a wide range of agricultural insect pests (44 different insect pest species) across Europe. Some studies have thoroughly investigated the role of bats in insect predation in macadamia (Taylor *et al*., 2013; Weier *et al*., 2019), pecan (Braun de Torrez *et al*., 2019; Brown *et al*., 2015), and cotton (Cleveland *et al*., 2006; Kolkert *et al*., 2020), while little is known about bat predation in citrus farming.

Previous studies demonstrated that a variety of local bat species (*Nycteris thebaica*, *Scotophilus dinganii*, *Mops midas*, *Mops condylurus*, *Chaerephon pumilus*, *Neoromicia nana*, and *Rhinolophus simulator*) forage in macadamia orchards and that they appeared to include important macadamia pests such as green vegetable bug

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and macadamia nut borer and some agricultural pests in their diet (Taylor *et al*., 2013, 2017; Weier *et al*., 2018). Our bat activity results (Chapter 2) show that these bat species occur in our study area. Bat species like *Mops midas*, *Mops condylurus*, and *Chaerephon pumilus* are open-air foragers and their diet comprises mainly of Lepidoptera, Hemiptera, and Coleoptera (Monadjem *et al*., 2010). A recent study by Bohmann *et al*. (2011) shown that *M. condylurus* and *C. pumilus* consume variety of insect pests in sugarcane fields. Species like *Nycteris thebaica*, *Scotophilus dinganii*, and *Rhinolophus simulator* are clutter foragers and their diet comprises mainly of Lepidoptera and Coleoptera (Monadjem *et al*., 2010). Weier *et al*. (20180 reported that *N. thebaica*, *N. nana*, and *S. dinganii* were found to be consuming moth (*Cryptophlebia peltastica*) as well as stinkbug (*B. distincta*) species. Although several studies have successfully documented diet of different bat species within macadamia (Taylor *et al*., 2013, 2017; Weier *et al*., 2018) and pecan orchards (Braun de Torrez *et al*., 2019), but we know nothing about bat predation among different species in citrus orchards.

Two of the major citrus pests, the false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick), and the carob moth (CM), *Ectomyelois ceratoniae* (Zeller), have several morphological characteristics that make them ideal candidates for bat predation. For example, these moths are small and non-tympanate, meaning they are unable to avoid bat frequency (Taylor *et al*., 2017). Furthermore, multiple studies have illustrated that female moths, with their limited lifespans, often fly across open areas to locate suitable fruit to lay eggs (Catling & Aschenborn, 1974; Stotter, 2009). This behavior readily makes them available for open-air insectivorous bats. A recent study by Russo *et al*. (2018) suggested that insectivorous bats attack adult aerial insects, preventing them from laying eggs which are profoundly responsible for crop damage. Due to the cryptic nature of feeding behaviour of FCM and CM, the effects of pesticides are often limited. Producers have started to investigate alternative options to incorporate into pest management programs. This includes the use of various natural enemies of FC and CM, which include egg parasitoid *Trichogrammatoidea cryptophlebiae*, Nagaraja (Hymenoptera: Trichogrammatidae), and granulovirus (Grout & Moore, 2015; Stotter, 2009). Furthermore, breeding disruptions using pheromones are also incorporated into management programs. However, the use the vertebrate predators has yet to be investigated or incorporated into pest management programs (Boersma, 2021; Levi-zada *et al*., 2020).

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This study investigated the diet of an assemblage of insectivorous bats inhabiting different landscapes surrounding a commercial citrus orchard. The main objectives of the study were to first investigate if insectivorous bats consume the two important citrus pets, FCM and CM. The second objective was to examine the comsumption of insect pests by bats of different foraging groups, and, the third objective was to show how diet composition and diversity varied between bat foraging groups, different prey taxa and bat species. It was hypothesized that insectivorous bats would include FCM and CM in their diet based on the morphological and behavioral traits of these moth species that lepidopteran taxa would be included in a high proportion in the diet and that bat foraging groups would be different in prey taxa consumed.

3.2. Methods and materials

The study was conducted with the approval of the animal ethical clearance by Research and Innovation, Office of the Director, University of Venda, with ethical clearance No: FSEA/22/BS/10/2507, Section 20 permit: 12/11/1/1 and 12/11/1/2 (2588SS).

3.2.1. Study area

The research took place in Alicedale estates (-22.60801,-30.17191), the largest citrus orchard in Tshipise, in the province of Limpopo, South Africa. There are different fruits cultivated in the orchard namely navel and Valencia oranges, limes, mandarins, and grapefruit. The study area receives 500 mm of rainfall mostly during summer times (South African Weather Service). During hot summers (October to March), daily temperatures often reach 40°C, whereas in dry winters (May to August), temperatures are slightly warm (15°C-25°C) with cold nights (0°C - 10°C). The landscape is a mosaic of conventional citrus orchards, surrounded by undisturbed habitat types (rocky outcrops, woodland, riparian) dominated by mopane trees (*Colophospermum mopane*). The Nzhelele River, which flows throughout the year close to the orchard, provides a foraging habitat for different animals.

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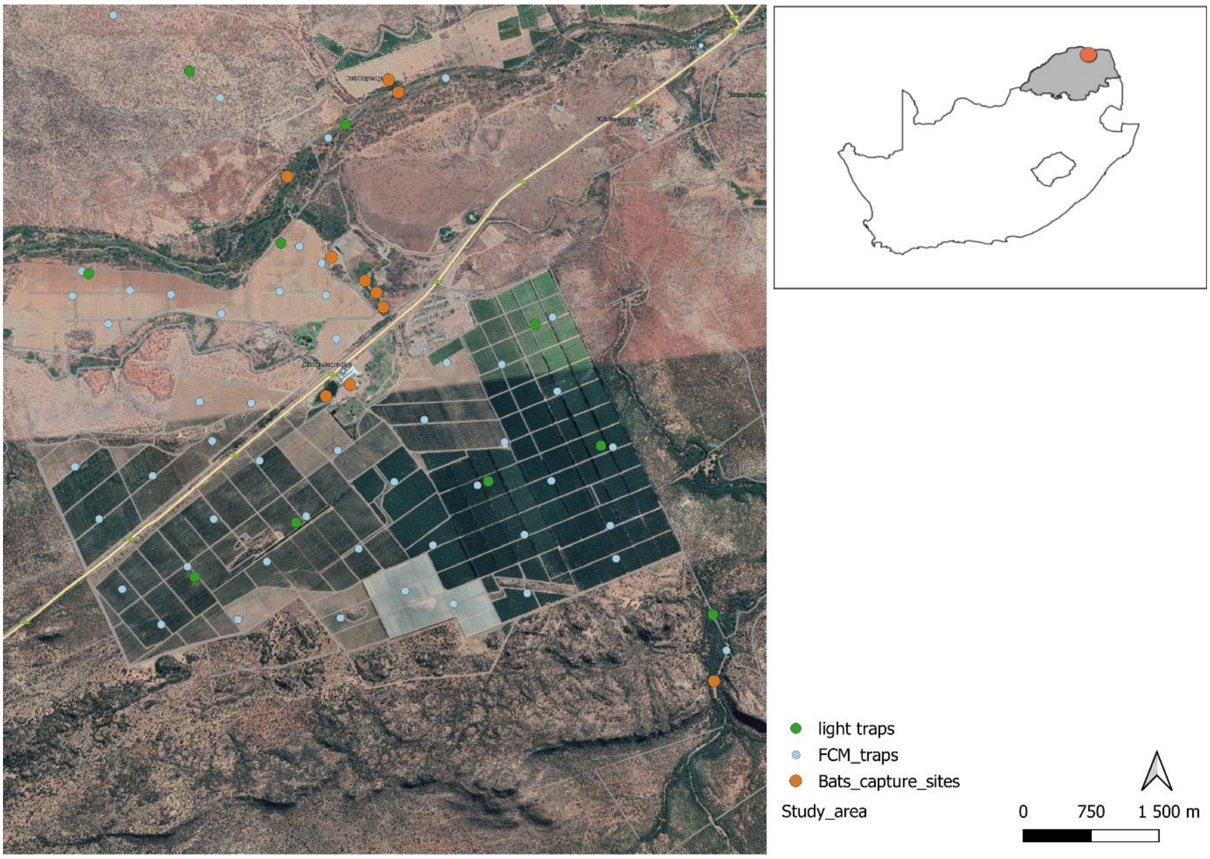

Figure 3.1. The layout of the study area with bat capture, light and FCM trap sites. FCM = false codling moth.

3.2.2. Experimental design

Bats were sampled at different habitat types: citrus orchards, riparian vegetation, dry riparian, and man-made water pan. Within the orchard, bats were sampled at two sites (*n* = 2), the citrus warehouse and an irrigation pond. The warehouse site was selected given that it was an ideal roost site inhabited by several bat species and the irrigation pond because it was assumed that bats would drink water when they return from hunting and thus provide an ideal opportunity to sample for predation around the orchard. It was also assumed that these bats would consume insect pests and other insects that occur in the orchard. The riparian vegetation sites (*n* = 3) were selected close to the river as they provide a clear foraging pathway for different bat species as well as drinking opportunities. The dry riparian site (*n* = 1) was situated close to the rocky outcrops where there were many crevices where bats roosted. A large man-made water pan, close to the orchard edge was chosen since again it will provide foraging and drinking opportunities for bats returning from hunting in natural areas and the orchard edge.

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3.2.3. Faecal collection

Bat capture was conducted the second week of each month for seven days from July-December 2022. The sampling was done during the season in which FCM, and carob moth were at their high peak. Bats were captured at different landscapes (riparian and woodland) and within the orchard at the packhouse and wet_edge (Fig. 3.1). Mist nets (Ecotone) and harp traps (Faunatech, Australia) were used to catch bats. Free-standing mist nets of 9 m, 12 m, and 14 m were strategically extended and positioned along a potential bat foraging pathway. However, on separate sampling nights, a harp trap and a mist net were positioned close to the roost site's entrance/exit at the packhouse. To prevent bias in the capture rate, the mist nets were moved each night to various sample locations. The mist nets were opened 20 minutes after the sunset (usually around 18:20) and compressed/closed at around 22:00. Each captured bat was kept apart in cloth bags until it defecated. Each of the bats that were captured had their species, sex, age, body mass, and forearm length carefully recorded. Faecal pellets (1-10) from each bat were collected using forceps and placed into 2 ml microtubes with DNA/RNA ShieldTM (ZYMO RESEARCH) at room temperature. The faecal samples were labelled per the bat identification code. All bats were released after processing.

3.2.4. Faecal Analysis

3.2.4.1. DNA extraction and sequencing

Bat faecal samples were transported to a Biosafety Level 3+ Laboratory Facility housed at the Centre for Viral Zoonoses, Faculty of Health Sciences, University of Pretoria. DNA from each bat faecal pellet was extracted using DNA Stool Mini Kit (Inqaba Biotechnical Industries), as per the manufacturer’s instructions with few modifications (Weier *et al*., 2018). DNA was extracted from a total of 396 faecal samples from nine insectivorous bat species: *Afronycteris nana, Chaerephon pumilus, Laephotis capensis, Mops condylurus, Myotis tricolor, Molossid, Nycticeinops schlieffeni*, *Scotophilus dingani*, and *Tadarida aegyptiaca.* Faecal samples selected for analysis represented species assemblages at each land cover. Extracted DNA samples were submitted to Inqaba Biotechnical Industries (Pty) Ltd for sequencing for amplification was done in a microfluidic high throughput multiplexed PCR platform (Fluidigm). Genomic DNA samples were PCR amplified through polymerase chain reaction (PCR) using universal primer pair LCO 1490 and HCO 2198 region of the COI gene (Folmer primers). These primers were tagged with PacBio M13 adaptor sequences on both 5’-and 3’-ends to allow barcoding of each amplicon. The resulting amplicons were barcoded with PacBio M13 barcodes for multiplexing through limited cycle PCR.

The KAPA HiFi HotStart Ready Mix program was used to perform PCRs for target amplification. The PCR thermal cycling conditions were set as follows: 3 min at 95 °C, 20 sec at 98 °C, 15 sec for Tm of target-specific primers, and 15–60 sec/kb at 72 °C. The cycle was repeated with 20 sec at 98 °C, 15 sec at 65 °C, and 15–60 sec/kb at 72 °C. Steps 6 to 8 were repeated for 20 cycles for 5 min at 72 °C and it was held at 4 °C. For barcoding M13 primer, the PCR cycling conditions were as follows: 3 min at 95 °C, 20 sec at 98 °C, 15 sec at 60 °C, and 15–60 sec/kb at 72 °C. The second cycle was run for 20 sec at 98 °C, 15 sec at 65 °C, and 15–60 sec/kb at 72 °C. Steps 6 to 8 were repeated for 20 cycles for 5 min at 72 °C and it was held at 4 °C.

The SMRTbell prep kit 3.0 programs were used for Repair and A-tailing, adapter ligation, and nuclease treatment of the target amplification. For repair and A-tailing, the cycle was run as follows: 30 min at 37 °C, 5 min at 65 °C, and was held until the temperature reached 4 °C. For adapter ligation, steps were as follows: 30 min at 20 °C and was held until the temperature reached 4 °C. For nuclease treatment, 15 min at 37 °C and was held until temperature reached 4 °C. Following the manufacturer's instructions, the equimolar and Ampure PB bead-based purification phase was carried out using the quantified and pooled barcoded amplicons. Using the pooled amplicons, the PacBio SMRTbell library was created in accordance with the manufacturing protocol. To get the library ready for sequencing on the PacBio Sequel IIe system, sequencing primer annealing and polymerase binding were carried out in accordance with the SMRT link software protocol (https://www.pacb.com/support/software/).

3.2.5. Data analysis

The taxa were converted into the occurrence and read abundance of the samples matrix of sequence data to quantify food composition, and the data were then summarized using four metrics. Percent frequency of occurrence (FOO) is the percentage of faecal samples that include a specific prey taxon. Percent of occurrence (POO) is the number of occurrences of a particular taxon divided by the total number

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of taxon occurrences. Weighted percent of occurrence (wPOO) can be calculated by dividing the number of weighted occurrences (e.g., if a sample contains two taxa, each will be given one-half the weight) by the total number of samples. The wPOO assigns an equal weight to each sample (Deagle *et al*., 2019). Relative read abundance (RRA) is the total read proportion of a particular sequence (Molecular taxonomic units (MOTU's)) across samples divided by the total number of samples. Dietary metabarcoding studies have made extensive use of the RRA metric (Ando *et al*., 2020), and it is reasonably accurate at the population level (Deagle et al., 2019) but less accurate to species with low-abundance readings (Deagle *et al*., 2019). The insect functional groups were constructed based on the insect specie's feeding behavior. Insects that feed on other insects were classified as "predators," those that damage crops as "pests," those that are problematic to citrus as "citrus pests," and the ones that could not be identified/categorized as pests nor predators were labelled as "others" (see Table. Supplementary (S hereafter) 5). The number of samples that each insect functional group was present in was used to quantify their presence.

The prey diversity in bat diet was estimated by calculating coverage based diversity indexes (Hill numbers; q = 0,1,2) using the iNEXT R package v.2.0.19 (Hsieh *et al*., 2016). The rarefaction and extrapolation curves were constructed for each arthropod taxonomic group (family, order, genus), bat foraging groups (open-air & clutter-edge) and bat species (where sample sizes were adequate) to assess sample completeness. To compare diet diversity, the "raw" indices were converted into "true" diversities, Hill numbers (belonging to the same mathematical family) (Pallmann *et al*., 2012). The diets of the different bat species and foraging groups were compared using two-tailed tests for integral Hill numbers (where sample sizes were adequate). The Shannon entropy index (Hill 1, Hsieh ($q \to 1$) was used to compare diet diversity. About 5000 bootstrap replications were performed to obtain reliable p-values (Cohen *et al*., 2020). The R package "simboot" (Scherer & Pallmann, 2014) was used to compare diet diversities (Pallmann *et al*., 2012). A permutational multivariate analysis of variance (PERMANOVA) implemented in the 'vegan' R package using the 'adonis' function, 'bray' distance matrix and 999 permutations was conducted to assess the difference in diet composition between bat foraging groups. All statistical analyses were performed in R Studio version 4.0.3 (R Core Team, 2023).

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3.3. Results

A total of 60 samples (396 faecal samples) of 49 individual bats representing nine species were captured in orchards, woodland, riparian and orchard/natural edges (wet edge; Fig. 3.1) and were analysed for diet composition and diversity. Only 58 samples (representing 352 faecal pellets; mean = 6 faecal pellets per sample, SD = 4.6) reached the sequencing stage. A total number of 12 samples (95 faecal pellets) were analyzed for *Afronycteris nana*, five samples (50 faecal pellets) for *Chaerephon pumilus*, three samples (40 faecal pellets) for *Laephotis capensis*, ten samples (98 faecal pellets) for family molossid, 21 samples (42 faecal pellets) for *Mops condylurus*, one sample (six pellets) for *Myotis tricolor*, three samples (13 pellets) for *Nycticeinops schlieffeni*, two samples (three pellets) for *Scotophilus dinganii*, and one sample (five pellets) for *Tadarida aegyptiaca*. Sample completeness varied between taxonomic levels, where arthropod order level achieved a sample completeness of 0.98 around 58 samples (Fig. S5. 5 A & B) and 0.96 at 58 samples for family level (Fig. S5. 4 C & D). When considering bat foraging groups (open-air and clutter-edge), observed diversity indexes had a sample completeness of 0.90 (for both foraging groups) for arthropod family level at 37 samples (Fig. S5. 5 A & B) and 1.00 and 0.98, for open-air and clutter-edge foragers respectively, at 17 and 37 samples for arthropod orders (Fig. S5. 5 C & D). In terms of bat species, the diet diversity and comparison analysis were limited to arthropod families and two species having adequate samples and representing an open-air feeder (*Mops condylurus*) and a clutter-edge feeder (*Afronycteris nana*). For bat species, the arthropod family observed diversity indexes reached a sample coverage of 0.84 at 12 samples (Fig. S5. 6 A & B).

3.3.1. Diet composition and diversity.

The results show that bats consume arthropods belonging to seven orders, 18 families, and 23 genera. From the 58 analyzed samples, 22 samples contained four families (15.56%) that were not arthropods and were excluded for further analysis (Table. 3). These include phylum Chordata, Nematoda, “unknown” and thought to be contaminated samples, or sequences amplified to incorrect taxa or DNA related to bat species (Andriollo *et al*., 2021). Among the taxa associated with arthropods, 35% of molecular taxonomic units (MOTUs) were classified as pests, 20% as predators, 23% as citrus pests, and 23% as others (Table 3.1). Pests represent 64% of all replicas (Readouts) found in the faecal samples (Table 3.1). Important citrus pests accounted for 2% of all replicas, with the genus *Drosophila* found within 10 samples (317 readouts), *Paramyiolia* in one sample (13 readouts), *Grapholita* in two samples (7 readouts), and family Pyralidae in two samples (56 readouts) (Table 3.1). Insects' predators were accounted at 4% of all the replicas.

Based on the %FOO, POO, and RRA, the most common insect order found in the faecal samples was Lepidoptera at 33%, followed by Diptera at 29%, Araneae at 18%, Coleoptera at 9%, Psocoptera at 5%, Hymenoptera at 4%, Hemiptera at 1%, and Trichoptera at 1% (Figure. 3.2).

Table 3.1. The DNA samples of different arthropods families/genus detected in the faecal samples of insectivorous bats captured within the citrus landscape, with the different genus of arthropods classified into functional groups (pest status), number of MOTU's, and replicates.

Order/Family/Genus	**Riparian**	**Orchard**	**Woodland**	**Wet edge**	**Functional group**	**MOTUs**	**Replicates**
Araneae							
Theridiidae							
Selkirkiella		1		1	Predator	2	41
Thomisidae							
Xysticus		1			Predator	4	51
Misumena				1	Predator	1	138
Uloboridae							
Octonoba		1			Predator	2	301
Salticidae							
Phintella		1			Predator	1	13
Coleoptera							
Elateridae							
Oedostethus				1	Pest	1	5
Staphylinidae							
Aleochara		1		1	Other	4	265
Diptera							
Calliphoridae							
Hemigymnochaeta		1			Other	1	2
Culicidae							
Culex		1		1	Other	9	7694
Drosophilidae							
Drosophila		1		1	Citrus pest	10	317
Rhiniidae		1			Other	1	1
Syrphidae							
Eumerus		1			Predator	2	308
Tephritidae							
Paramyiolia				1	Citrus pest	1	31
Hemiptera							
Alydidae							
Heegeria		1			Pest	1	74

Hymenoptera							
Chrysididae							
Chrysis				1	Predator	2	24
Formicidae							
Pheidole				1	Predator	1	5
Tapinoma				1	Predator	2	16
Lepidoptera							
Crambidae							
Patania	1		1		Pest	3	254
Mecyna				1	Pest	1	1
Nomophila				1	Pest	1	45
Pygospila			1		Pest	2	85
Erebidae							
Lyclene				1	Pest	4	177
Pyralidae	1	1			Citrus pest	2	56
Tortricidae							
Grapholita		1		1	Citrus pest	2	7
Psocoptera							
Liposcelididae							
Liposcelis		1		1	Pest	9	15608
Trichoptera							
Hydropsychidae		1			Pest	1	1

MOTU's = Represents the number of samples a taxa were detected in. Replicates represent the read outs of a taxa per sample.

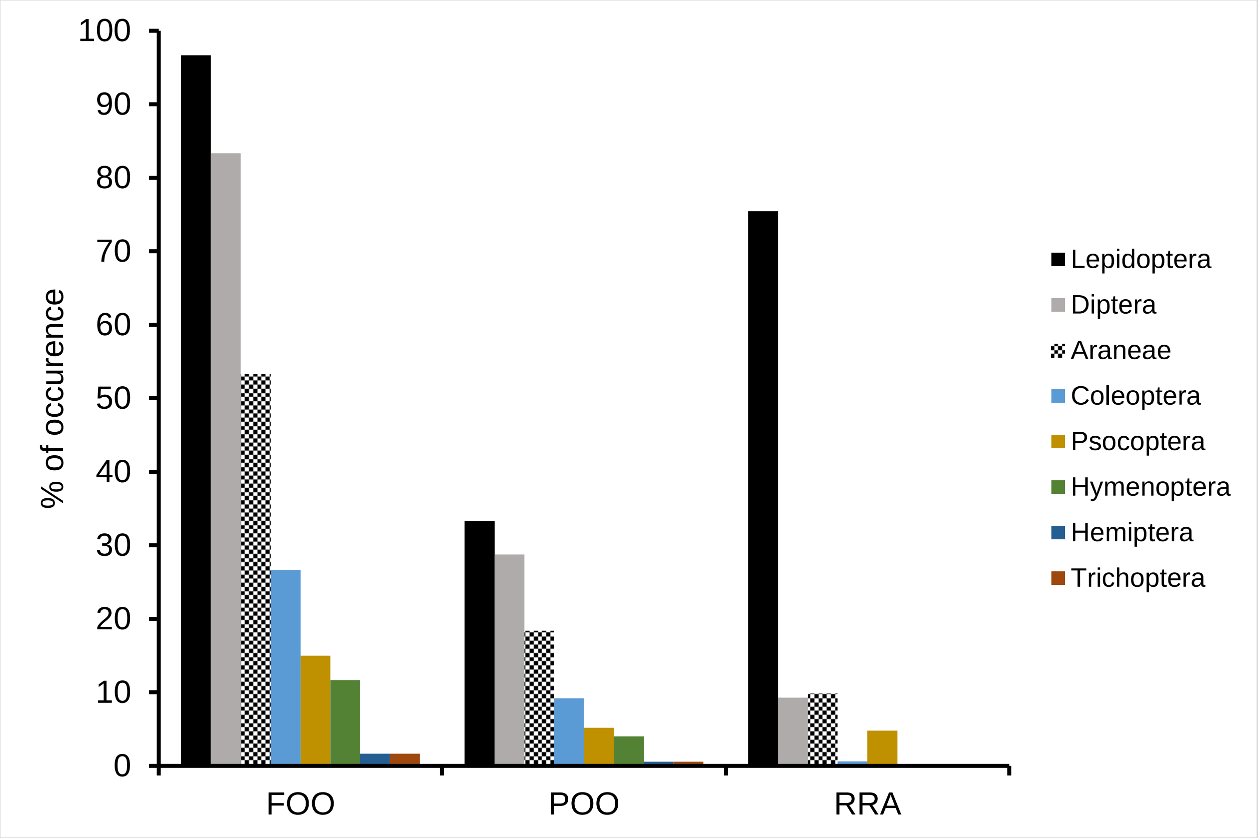

Figure 3.2. Percentage of occurrence of MOTUs of different arthropod orders detected in the faecal samples of insectivorous bats in terms of %FOO, POO, and RRA. %FOO = Percentage frequency of occurrence, POO = Percentage of occurrence, and RRA = relative read abundance.

3.3.2. Diet diversity (Order, Fam, Genus) for bat foraging groups

There was no significant difference for bat foraging groups in the diet composition when looking at arthropod orders (adonis, F1,57 = 0.9913, *p* = 0.399; Fig. 3.3, Fig. S5. 7 A), however, there was a significant difference in diet composition between foraging groups when looking at arthropod families (adonis, F1,57 = 1.92, *p* = 0.018; Fig. 3.3, Fig. S5. 7 B). At order level, the bat foraging groups consumed similar orders (Fig. 3.2), however, at family level, open-air feeders consumed higher diversity of prey items than clutter-edge foragers (Fig. 3.3). Similarly, trophic diversity was similar between foraging groups at order level (Shannon index for open-air, Hsh = 9.67 CI = 7.58-76, and for clutter-edge, Hsh = 7.12 CI = 4.65-9.59, *p* = 0.896; Fig. S5. 8). However, at the family level, there was no significant difference in trophic diversity (Shannon index for open-air, Hsh = 4.69 CI = 4.30-5.09, and for clutter-edge, Hsh = 4.56 CI = 3.93-5.23, *p* = 0.896; Fig. S5. 9).

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The number of MOTUs shows that Lepidopteran insects were the mostly consumed order by all the bats foraging groups, with open-air foragers at 33% and clutter-edge foragers at 34% (Fig. 3.2), followed by Diptera with open-air foragers at 27% and clutter-edge foragers at 29%, Araneae at 22% and 15%. The insects of the order Hymenoptera were only present in the diet of clutter-edge foragers. Coleopterans were consumed by all the foraging groups at 9% and 10%. The order Psocoptera was mostly consumed by open-air foragers at 7% and 2% at clutter-edge foragers, Hemiptera and Trichoptera were only consumed by open-air foragers, but at the lowest rate with only 1% of their fragments present in the faecal samples.

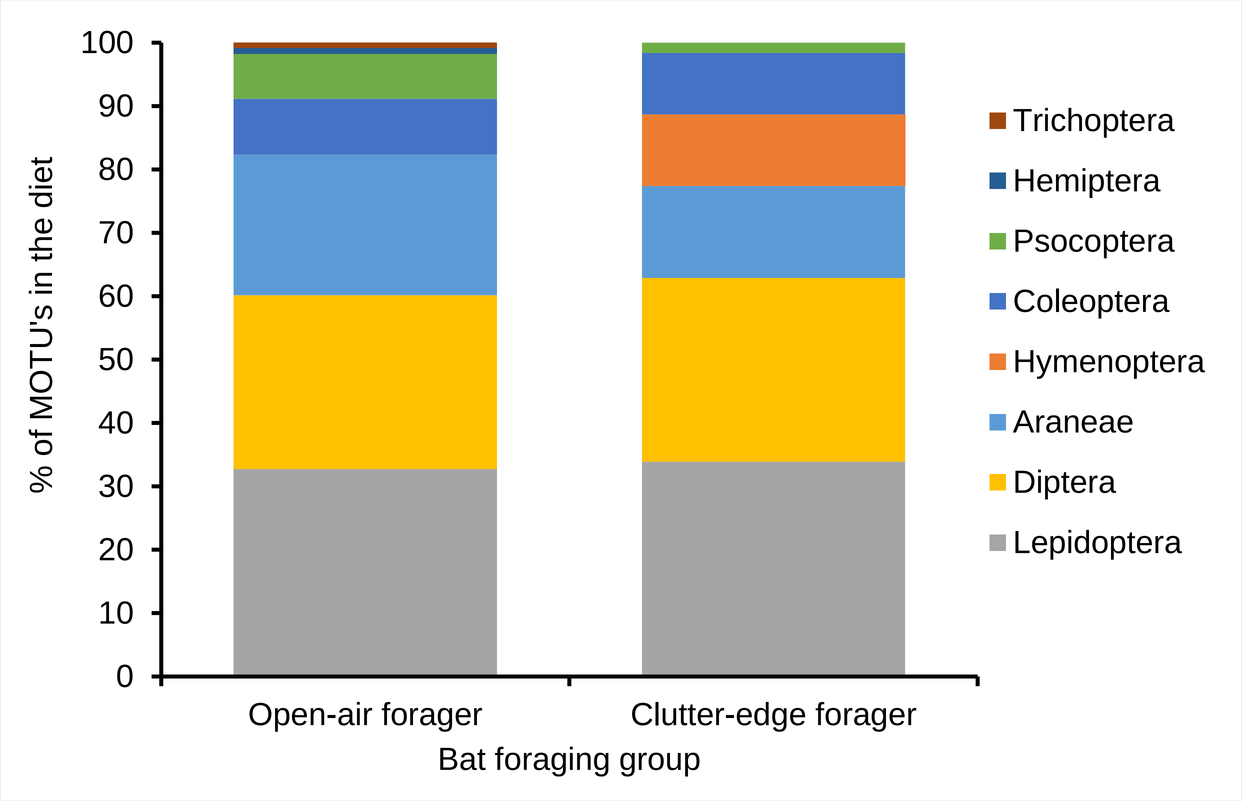

Figure 3.3. Percentage of the MOTU's based on the insect order groups and their presence in faecal samples of different bat foraging groups.

The insect family belonging to Crambidae, Tortricidae (Lepidoptera), Drosophilidae, Elateridae, Calliphoridae, Culicidae (Diptera), Thomisidae (Araneae), Staphylinidae (Coleoptera), and Liposcelididae (Psocoptera) were consumed by all the bat's foraging groups (Fig. 3.4; Table S5. 13). The open-air foragers comsumed more insect families than clutter-edge foragers.

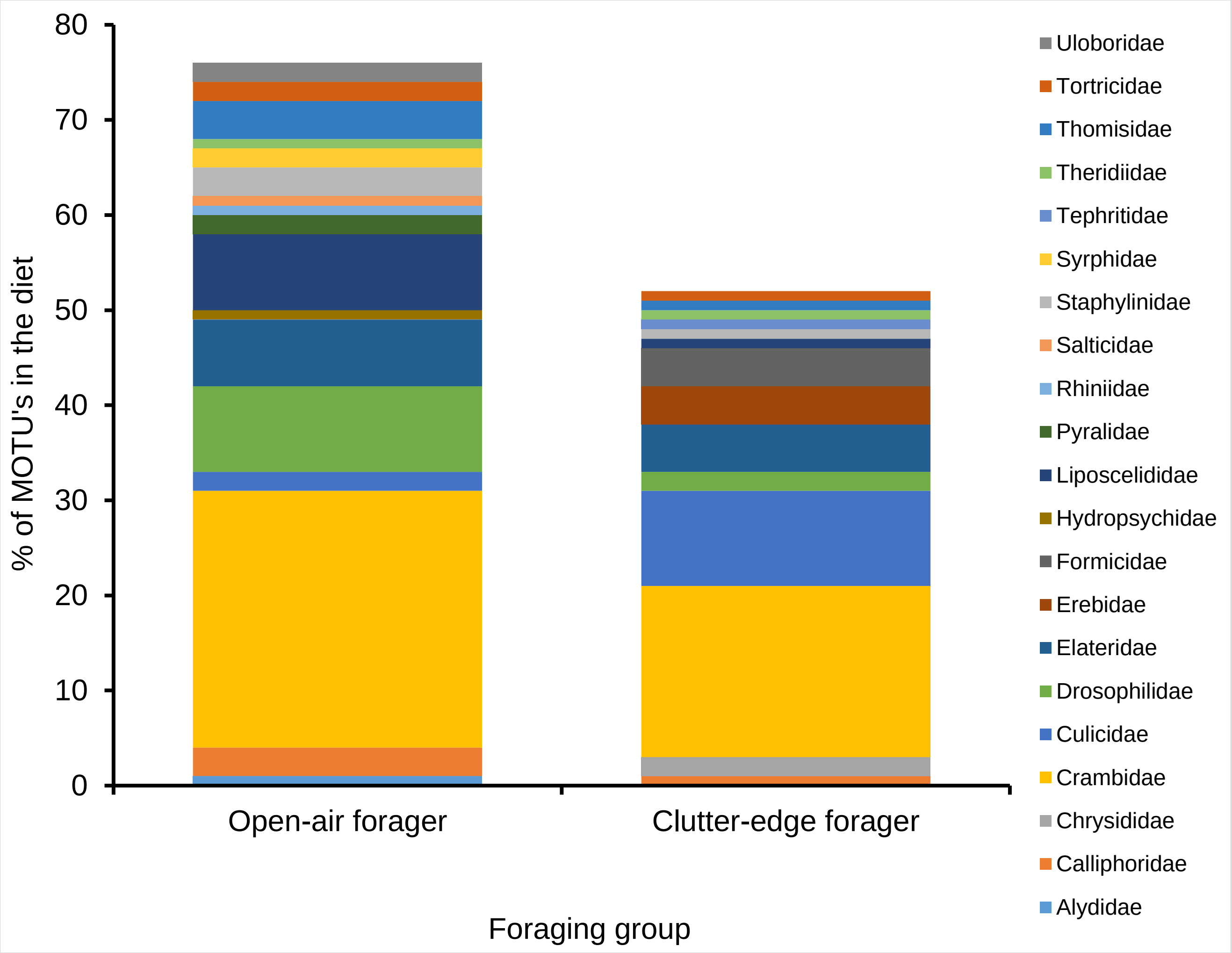

Figure 3.4. Percentage of the MOTU's based on the insect family groups and their presence in faecal samples of different bat foraging groups.

3.3.3. Pest insects in the bat's diet

In total, 23 prey species were identified at the genus level and three at the family level. Among the prey consumed by insectivorous bats of different foraging groups, pest insects were consumed more than beneficial insects (predators). Clutter-edge foragers diet was mainly dominated by others (32%), predators (28%), pests (24%), and a low volume of citrus pests (16%) respectively (Fig. 3.5). Open-air forager's diet included more pests, with citrus pests at 26% and agriculture pests at 35%.

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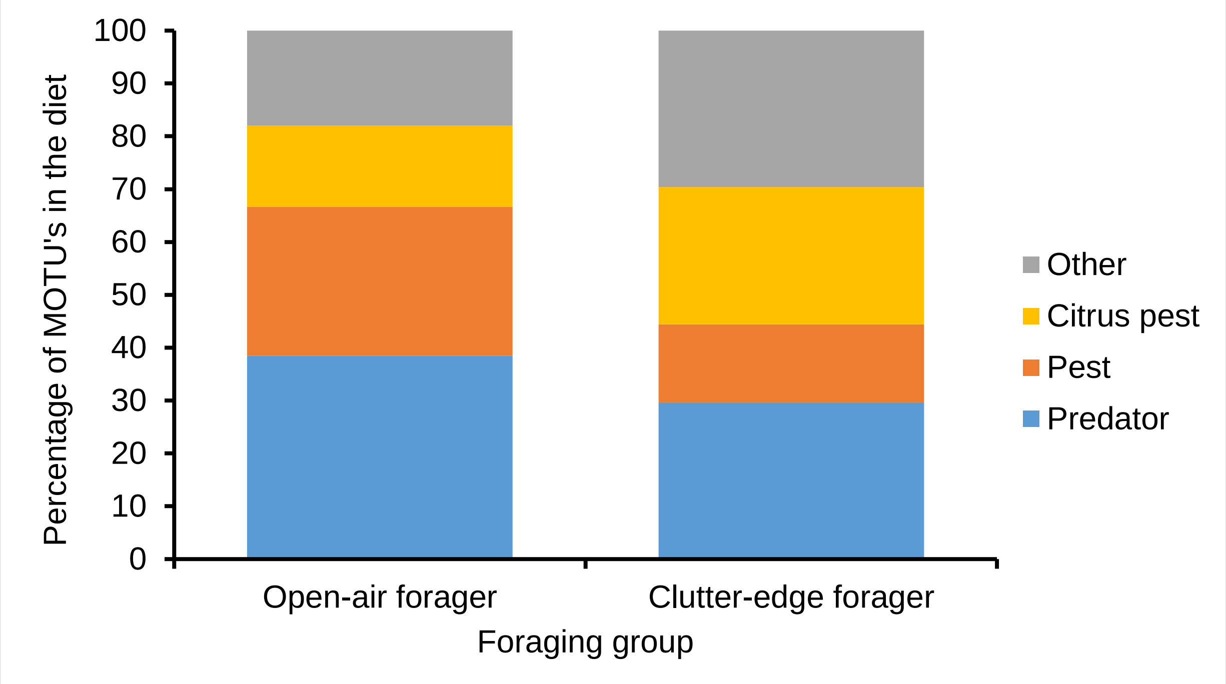

Figure 3.5. Percentage of the MOTU's based on the functional groups (pest status) and their presence in faecal samples of different bat foraging groups.

3.4. Discussion

Our results show that insectivorous bats within the citrus landscapes include a variety of insects in their diets, with different bat foraging groups having a highly diverse diet. Our results illustrate that both bat foraging groups actively feed on Lepidoptera, which

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includes two families (Tortricidae and Pyralidae) that include important citrus pests like FCM and CM. Our results agree with previous studies. For example, Garin *et al*. (2019) reported that bat species of different foraging groups consumed the processionary moth, *Thaumetopoea pityocampa*, the major pest of pine trees. Similarly, Kolkert *et al*. (2020) also detected major cotton pests like Australian bollworm (*Helicoverpa punctigera*) and cotton webspinner (*Achyra affinitalis*) in the diet of seven different bat species. The observed low occurrence of Tortricidae and Pyralidae in the diet of both open-air and clutter-edge foragers may be related to their low abundance within the orchard due to strict management practices such as spraying frequently and orchard sanitation (personal communication). Both open-air and clutter-edge foragers consumed Tortricidae and Pyralidae moths despite their different foraging strategies. This might be explained by the moth's behavior as they tend to fly over the orchard in search of suitable trees to lay eggs and this flight pattern switching behavior makes them readily available to both foraging groups (De Jager, 2013; Ober & Hayes, 2008). In addition, both foraging groups might exhibit opportunistic feeding behavior, switching to readily available prey when preferred options are scarce (Monadjem *et al*., 2020). Our results, therefore, suggest that different bat species of different foraging guilds can opportunistically predate/feed on moth pests during outbreaks.

Our results show no statistical differences between the diet composition of open-air and clutter-edge foragers as they both consumed similar insect orders. However, based on the % of the MOTUs, Lepidopterans were the most prevalent insect order consumed by all bat foraging groups, and this was similar to prior studies (Kemp *et al*., 2019; Kolkert *et al*., 2020; Taylor *et al*., 2017). This suggests that Lepidopterans are an important taxon for different bat species, and this may be due to their erratic and slow flight patterns, high diversity, and high nutritional value for bats with high metabolic demands (Riccucci & Lanza, 2014). Dipterans were the second most frequently consumed order and this concurs with prior studies (Aguiar *et al*., 2021; O'Rourke *et al*., 2021; Wang *et al*., 2023). The occurrence of Dipterans was largely influenced by habitat (vegetation composition), water bodies, and more importantly season (Weterings *et al*., 2015). Our sampling period (July-Dec 2023) might have coincided with a peak in fly abundance or the emergence of Dipterans, temporarily shifting the bat’s dietary focus (Monadjem *et al*., 2020). The higher consumption of Araneae by open-air foragers might be because they are easily detectable in open

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areas due to their larger size and contrasting colors compared to cluttered environments (McAney *et al*. 2000). Additionally, spiders also disperse using ballooning, which can increase their risk for aerial foragers (McAney *et al*. 2000). Both open-air foragers and clutter-edge foragers had high occurrence of Coleoptera. This was not surprising, as both of these foraging groups are known to include beetles as their main source of food (Monadjem *et al*., 2020). Both bat groups could explore unpredictable insects Hemiptera, Hymenoptera, and Tricoptera whenever they are available, suggesting that they are not extremely strict to Lepidoptera and Diptera and can also feed opportunistically on various insect taxonomic groups (Mohamed *et al*., 2023).

Open-air foragers significantly consumed higher insect families compared to clutter-edge foragers. This can be highly influenced by habitat structure that open-air environments typically offer wider, less obstructed flight spaces, allowing bats to cover larger areas and encounter a broader range of insect families compared to the more complex and cluttered landscapes preferred by clutter-edge foragers (Meyer *et al*., 2016). Among the consumed insects, important agricultural pest families such as Crambidae and Drosophilidae (fruit flies) were found to be preyed on by both foraging groups. The Drosophilidae, commonly known as secondary feeders of damaged citrus, were mostly consumed by open-air foragers. This was probably because the open-air foragers were captured within the orchard and were anticipated to have foraged within the orchard. This is consistent with previous studies (Kolkert *et al*., 2020; Wang *et al*., 2023). The order Diptera was found to contain six families, the most common were Culicidae and Drosophilidae. The family Culicidae (mosquitos) is widely known for the risks it poses to human health (malaria) and was highly found in the diet of clutter-edge foragers (Wang *et al*., 2023). The high occurrence of Culicidae was because the clutter-edge foragers were captured at the orchard wet edge close to the water pan, and mosquitos are known to occur in high abundance close to water bodies (Courtney *et al*., 2017). Liposcelididae (booklice) were found highly consumed by open-air foragers and this might be because they were inhabiting the same building (packhouse) with bats which made them readily available. Families like Rhinidae, Salticidae, and Uloboridae were only found in the diet of open-air foragers and at low frequency. Open-air foragers, known for their wider foraging ranges and diverse prey selection, might occasionally encounter and consume these less common insect

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groups alongside their more usual prey (Russo *et al*., 2018). The low volume suggests these encounters might be incidental rather than targeted. Our results suggest that an assemblage of bat species within agricultural landscapes can potentially control a large variety of insect pests.

Generally, the availability of insects has a major impact on the diet of insectivorous bats (Clare *et al*., 2014). As anticipated, our results show high pest consumption by insectivorous bats, with at least one bat individual of both foraging groups feeding on one major citrus pest. This concurs with previous studies (Hughes *et al*., 2021; Kolkert *et al*., 2020; Taylor *et al*., 2017; Weier *et al*., 2018). For example, Taylor *et al*. (2017) demonstrated the potential of insectivorous bats as a biological control for macadamia pest by detecting *Nezara viridula* (Heteroptera) DNA in one-third of faecal samples from five bat species of different foraging groups. Similarly, Kolkert *et al*. (2020) found that the diets of insectivorous bats in cotton-growing regions often include significant amounts of cotton pests, with over 76% of faecal samples containing DNA from the Australian bollworm (*Helicoverpa punctigera*) and the cotton webspinner (*Achyra affinitalis*). These findings highlight the ecological value of diverse bat communities within agricultural landscapes. Despite pest consumption, both bat foraging groups were found to include beneficial insects (predators) (Kolkert *et al* 2020; Wang *et al*., 2023). Most of these insect predators are known to predate on larvae of other insects. For example, the family Chrysididae (Hymenoptera) include cleptoparasitic organisms that feed on the scraps of sphecid nests, which may include dead spiders, true bugs, aphids, or thrips (Kimsey, 2006), and some spiders (Theridiidae, Salticidae, Uloboridae, Thomisidae) are known to be generalist's predators in citrus orchards (Peng *et al*., 2023). However, the relative percentage of beneficial insects consumed by bats was lower than that of pest insects. Therefore, this suggests that insectivorous bats provided more services (pest insect consumption) than disservices within agricultural landscapes.

3.5. Conclusions

Our results show that both open-air and clutter-edge foragers' diets include Tortricidae (FCM) and Pyralidae (CM) moths, therefore, this suggests that different bat species can respond to citrus major pest irruptions. However, similarly to Braun de Torrez *et al*. (2019), the low occurrence of both these moth pests in the diet might not indicate their control, but it does provide important evidence of the potential ecosystem service provided by insectivorous bats. Therefore, further studies are needed to explore the magnitude of FCM and CM control by insectivorous bats within citrus landscapes. Our results showed a diverse consumption of various insects by bats, dominated by Lepidoptera and Diptera. This suggests that insectivorous bats selectively forage on preferred taxa. Therefore, this selective behaviour enables bats to shift their diet and feed on available food sources throughout the growing season. Our results further showed that insectivorous bats mainly consumed pests, some of which are a threat to human health (i.e. Culicidae). This provides evidence for citrus growers to include bats in the IPM approach as they provide essential ecosystem services.

3.6. Reference

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CHAPTER 4: CONCLUSION AND RECOMMENDATIONS

These data on bat activity clearly indicate that temperature and land use variation are the primary drivers of bat species richness and activity. More bat species were found in natural areas compared to orchards, but bat activity was higher in orchards than in natural areas. This is in agreement with previous studies (Fuentes-Montemayor *et al.*, 2011; Weier *et al*., 2018) that natural vegetation promote bat activity and potentially biological control of major crop pests in agricultural landscapes and this would provide for their conservation and include them in IPM approaches. Apart from conserving natural vegetation, it is therefore recommended to create and maintain hedgerows as they favour both bats and insects. Several studies highlight the importance of hedgerows and forested patches in agroecosystems since bats commonly use them

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as corridors and they can act as shelters for arthropods, thus these structures represent valuable foraging habitats for insectivorous bats (Heim *et al*., 2015; RodríguezSan Pedro *et al*., 2018, 2019). Installation of roost sites may attract bats, increasing the ecosystem services they provide. Weier *et al*. (2019) also installed artificial roosts in macadamia orchards in South Africa that were occupied by several species.

Our results of bat diet show that bats consumed a wide range of insects, with all bat foraging groups preferably including lepidopterans in their diet. Major pests of citrus such as FCM and CM were consumed by all foraging groups but in less abundance. Thus, insectivorous bats across different landscapes may respond positively during moth pest outbreaks (Braun de Torrez & McCracken, 2015). However, unfortunately, our study cannot shed more light on the capability of bats to numerically control FCM and CM because these moths pests were fairly infrequently found not in great abundance in the bats' diet. Future studies should investigate the seasonal change in bat diet especieally during peak periods of FCM and CM in citrus orchards to avoid temporal biases which may lead to false conclusion concerning consumption of pest insects by bats. Open-air foragers consumed more diverse insect families than clutter-edge foragers, this was highly influenced by habitat structure. Including tree lines, and windbreakers, and installing bat houses within the orchards can attract different foraging groups within the orchards, which can feed on different insects. Both foraging groups included pest species in their diets, suggesting potential for biological control.

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CHAPTER 5: SUPPLEMENTARY RESULTS

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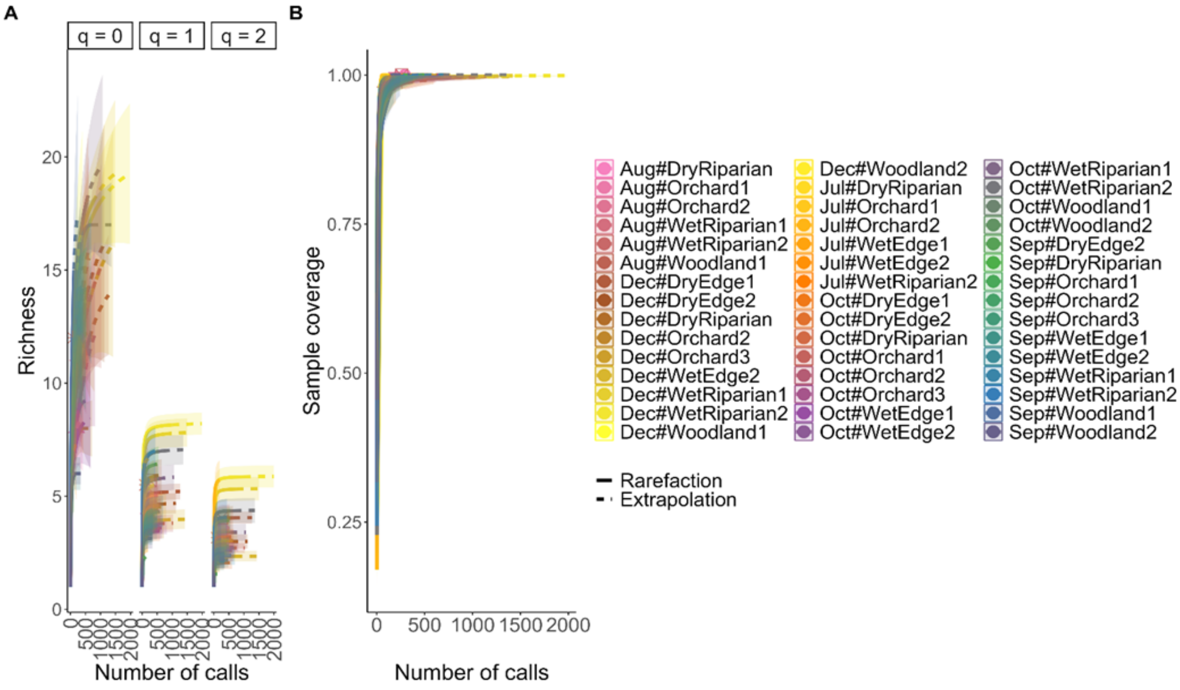

Figure S5. 1. Rarefaction and extrapolation sampling curves with respect to sample coverage (Hill numbers; q = 0 species richness, q = 1 Shannon diversity, q = 2 Simpson diversity).

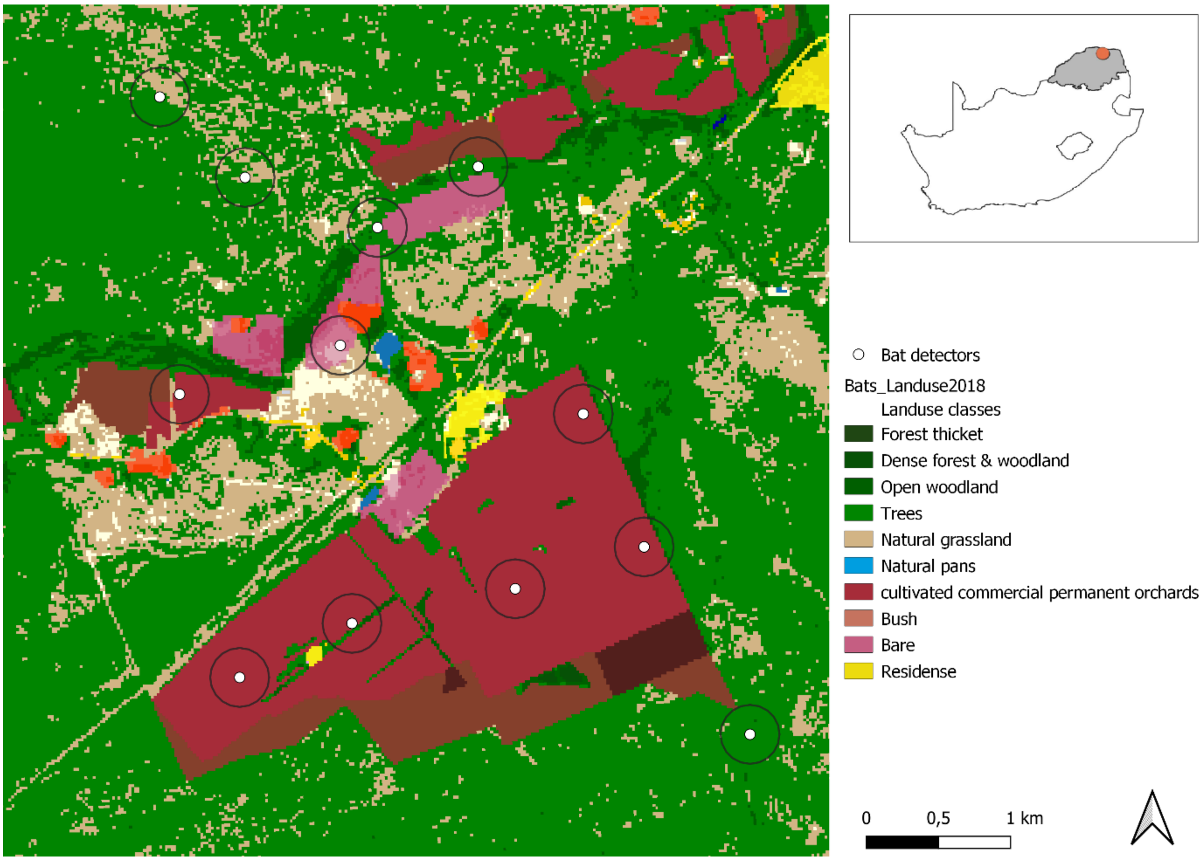

Figure S5. 2. Study area map presented in terms of land-cover classes with bat detector sites buffered within a 200 m circular area.

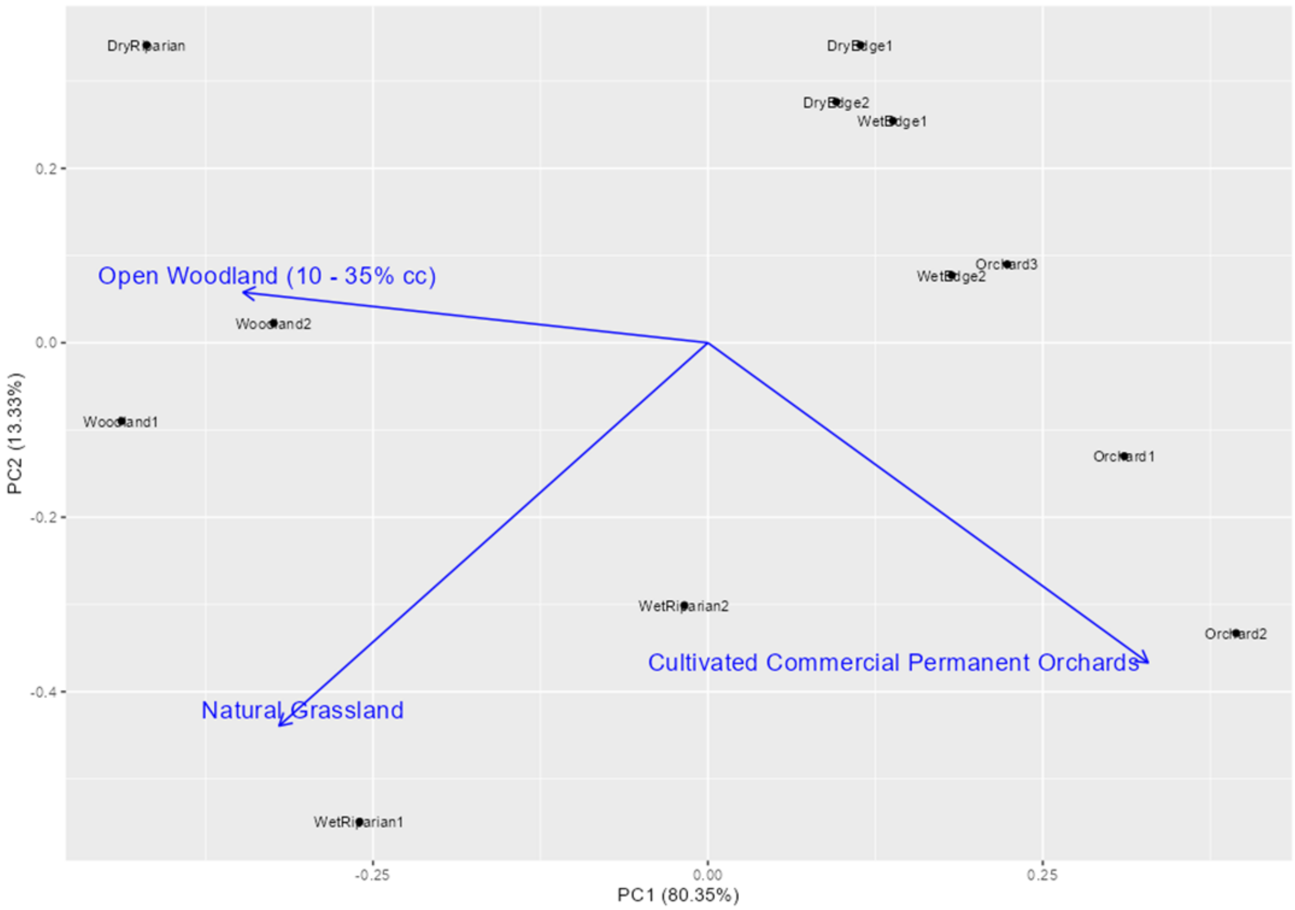

Figure S5. 3. PCA for different land-use classes and study sites.

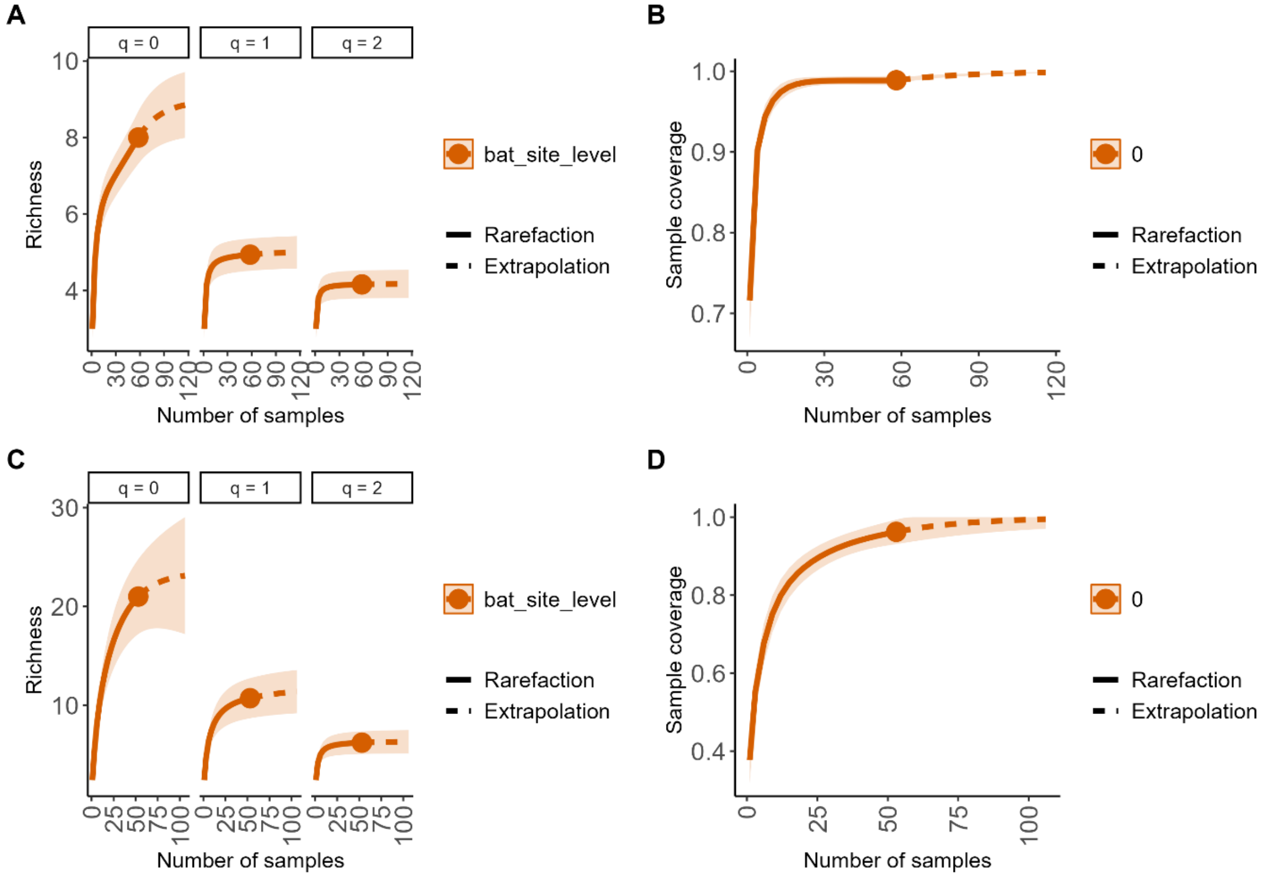

Figure S5. 4. Sample coverage for insect order (A & B) and insect families (C & D) detected in the diet of nine insectivorous bat species (N = 60).

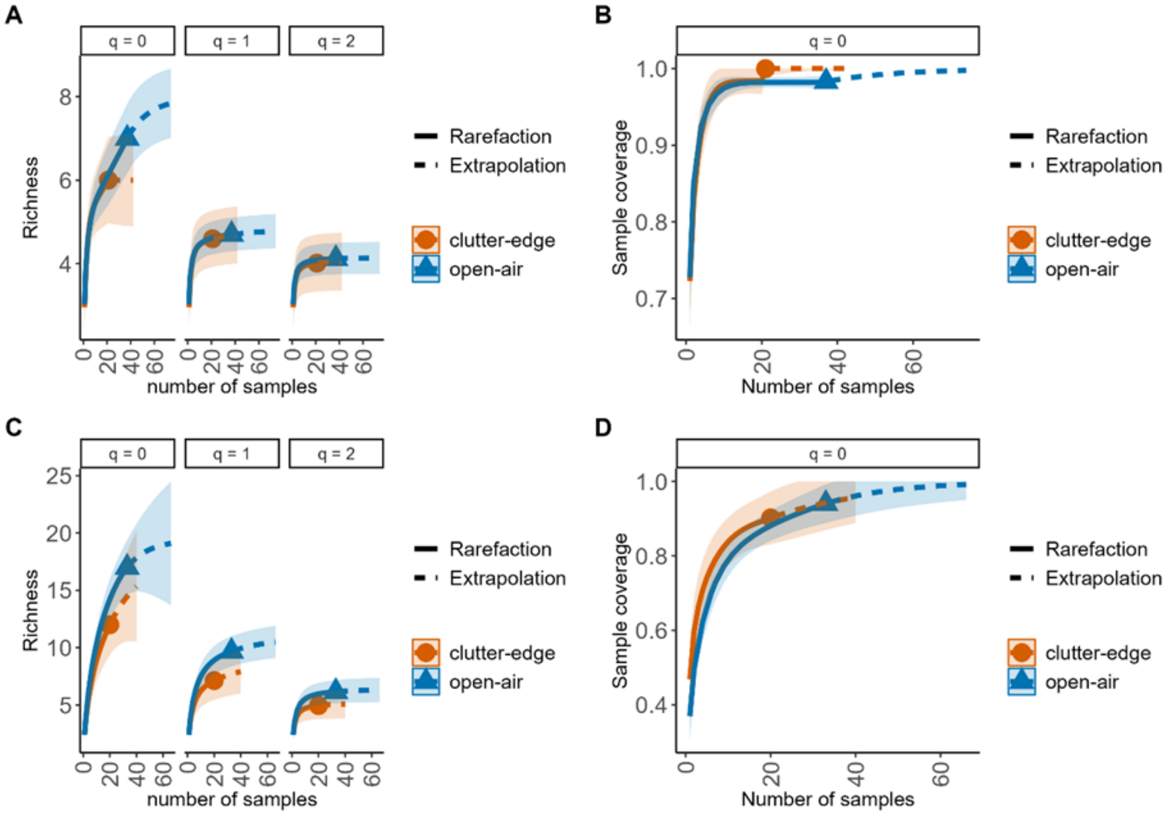

Figure S5. 5. Sample coverage for insect order (A & B) and bat foraging groups and insect families (C & D) and bat foraging groups detected in the diet of nine insectivorous bat species (N = 60). Rarefaction and extrapolation of sample coverage were produced according to Chao’s measure of sample coverage (Hill numbers; q = 0 species richness, q = 1 Shannon’s entropy index, q = 2 Simpson’s concentration index).

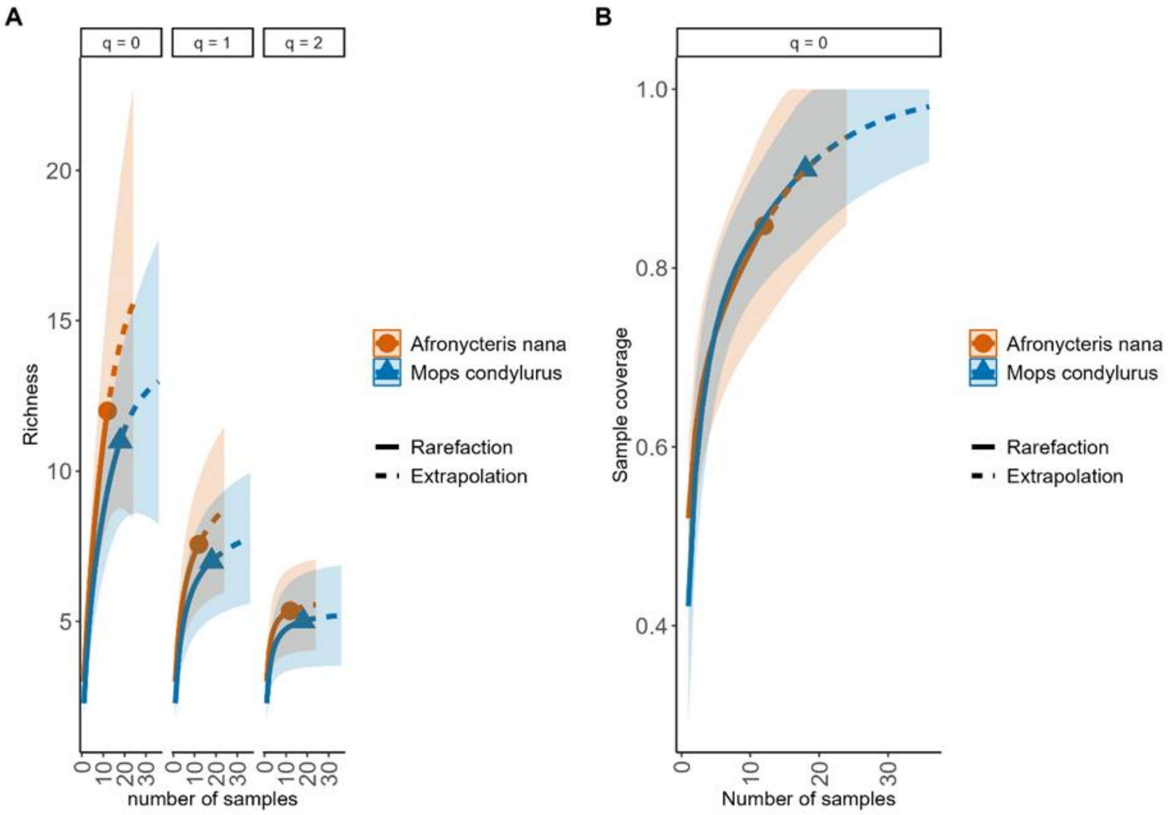

Figure S5. 6. Sample coverage for bat species with the highest capture rate (A & B). Rarefaction and extrapolation of sample coverage were produced according to Chao's measure of sample coverage (Hill numbers; q = 0 species richness, q = 1 Shannon's entropy index, q = 2 Simpson's concentration index).

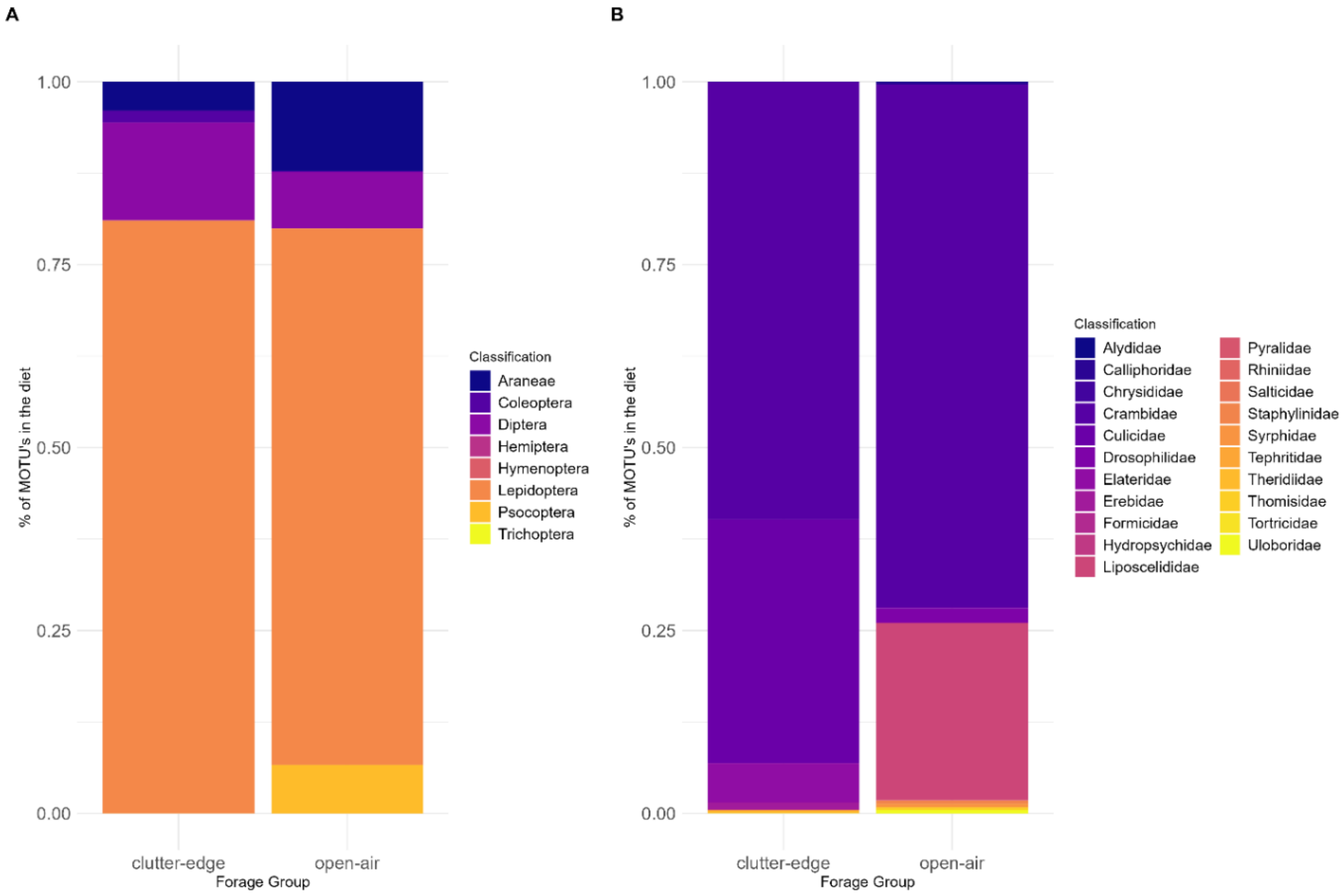

Figure S5. 7. ercentage of the MOTU’s based on the insect order (A) and family (B) groups and their presence in faecal samples of different bat foraging groups.

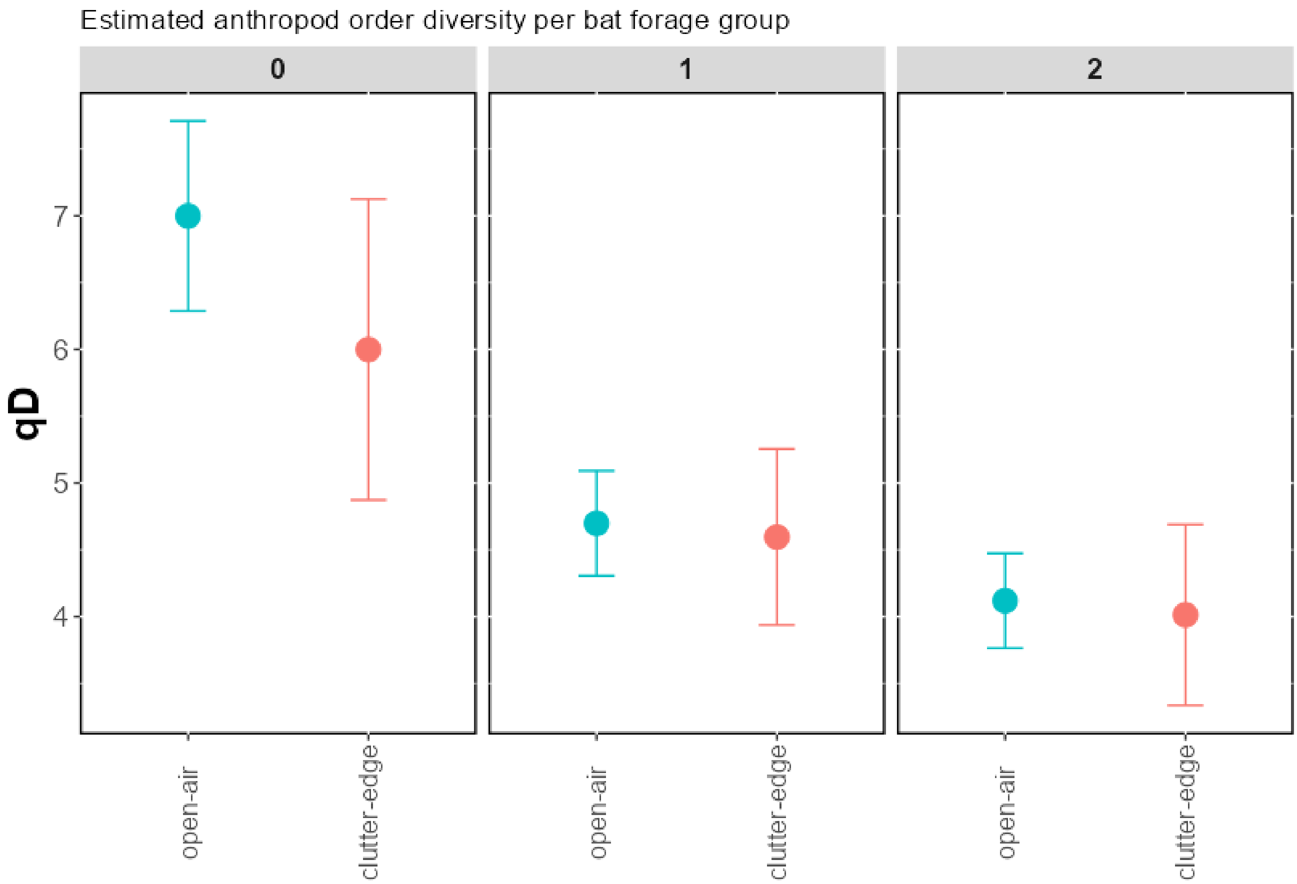

Figure S5. 8. Estimated Shannon index for insect diversity orders per bat foraging group.

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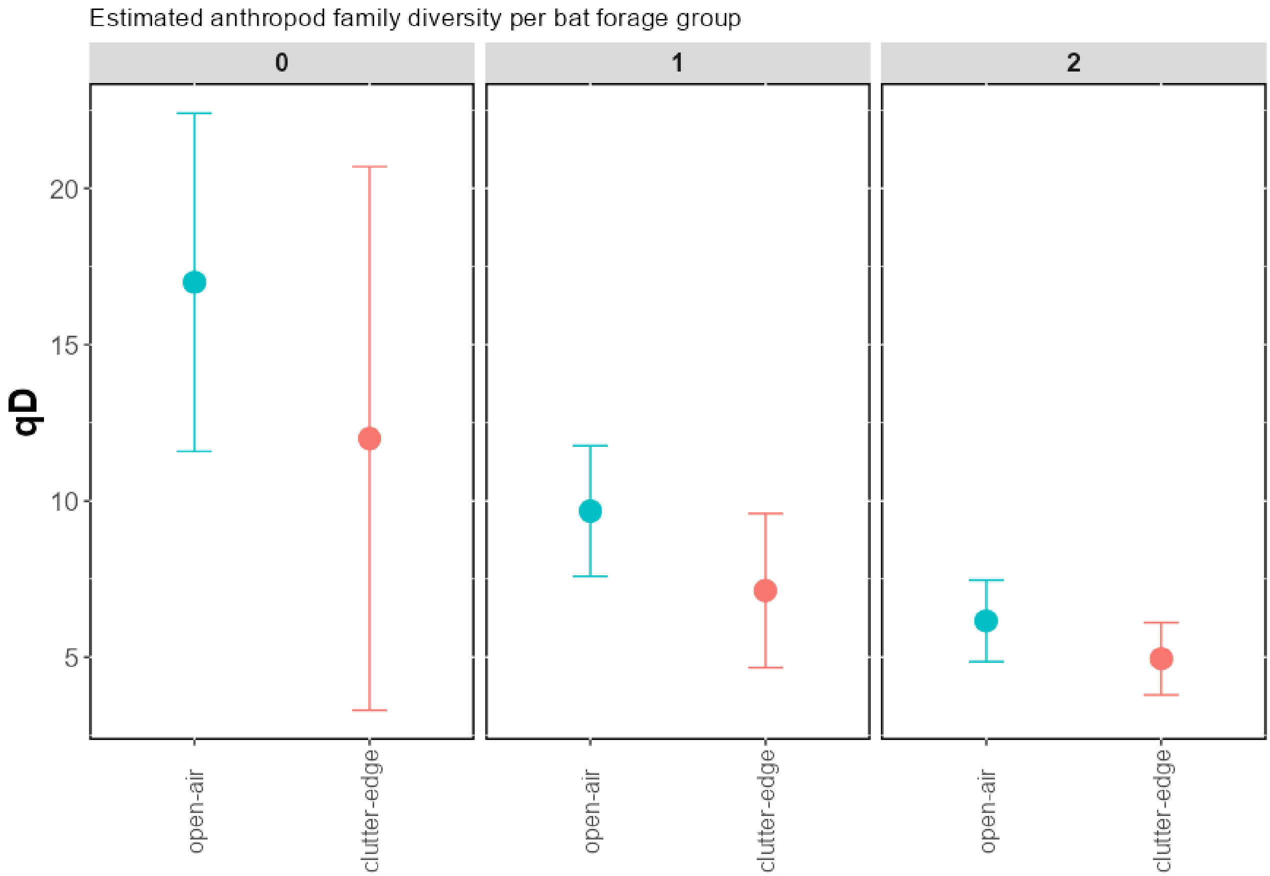

Figure S5. 9. Estimated Shannon index for insect diversity families per bat foraging group.

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Table S5. 1. Combined land use classes from the Department of Environmental Affairs for PCA analysis.

Land-use classes	**National land-cover classes 2013-2014**
Other Bare	Fallow Land & Old Fields
Natural Grassland	Fallow Land & Old Fields
Open woodland	Open & Sparse Planted Forest
Dense Forest & Woodland	Thicket/dense bush, woodland/open bush, low shrubland and grasslands
Cultivated Commercial Permanet Orchards	Commercial Annuals Crops Rain-Fed / Dryland / Non-Irrigated

Table S5. 2. Percentage of each land use (with land cover classes) within the study area.

Land use	**Land cover classes**	**Land-use (%)**
DryRiparian	Dense forest & woodland, Open woodland, Natural grassland	16.36
WetRiparian	Dense forest & woodland, Bare river, Natural grassland, Trees	15.67
Orchard	Cultivated commercial annual crops non-pivot, cultivated commercial permanent orchards and cultivated subsistence crops	17.06
WetEdge	Cultivated commercial permanent orchards, Natural grassland, Forest thicket, Open woodland, Trees, Bush	16.66
DryEdge	Cultivated commercial permanent orchards, Dense forest & woodland, Open woodland, Natural grassland	17.11
Woodland	Open woodland and Natural grassland	17.13

Table S5. 3. Variance Inflation Factors for the different land use types around each detector fitted to a linear model.

```
> vif(all)
```

	GVIF	Df	GVIF^(1/(2*Df))
meanTC	1.1248	1	1.060565
PC1	8.25154	1	2.87255
Orchard	132.888	1	11.527724
DryRiperian	61.0043	1	7.810526
WetRiperian	16.6687	1	4.082729
OpenWoodland	96.4009	1	9.818396
Grassland	97998.1	0	Inf

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Table S5. 4. Total bat activity (Activity index, AI) for each bat species/species group at different landscapes. AI represents activity index and RA represents relative abundance.

Species	**Dry_Edge**	**Dry_Riparian**	**Orchard**	**Wet_Edge**	**Wet_Riparian**	**Woodland**	**AI (Total)**	**RA**
Afronycteris nana	154	96	190	263	1793	209	2705	4,243
Chaerephon ansorgei	1421	546	3441	1066	926	546	7946	12,463
Chaerephon pumilus/Mops condylurus	682	1302	1774	1026	2052	422	7258	11,383
Eptesicus hottentotus/Scotophilus dinganii	184	216	122	76	431	32	1061	1,664
Hipposideros caffer					1	2	3	0,005
Laephotis capensis	26	94	91	92	66	50	419	0,657
Laephotis capensis/Pipistrellus (Vansonia) rueppellii	325	242	811	248	1044	456	3126	4,903
Mops midas	215	62	390	628	344	196	1835	2,878
Myotis tricolor/M. bocagii	73	6	93	16	306	55	549	0,861
Myotis welwitschii	38	29	19	3	96	24	209	0,328

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Neoromicia zuluensis	21	73	112	86	754	123	1169	1,833
Nycticeinops schlieffeni	44	17	45	22	94	70	292	0,458
Otomops martiensseni	213	37	252	134	1283	30	1949	3,057
Pipistrellus hesperidus	5	43	25	7	158	159	397	0,623
Pipistrellus rusticus/Neoromicia hlandzeni	3	30	106	17	382	24	562	0,881
Rhinolophus 70-72kHz			4	1	2		7	0,011
Rhinolophus clivosus				3	298	9	310	0,486
Rhinolophus darlingi/Rhinolophus blasii	6	3		1	42	1	53	0,083
Rhinolophus rhodesiae	1	31	17	1	91	33	174	0,273
Rhinolophus simulator	11	242	21	9	370	48	701	1,099
Rhinolophus smithersi	5	40	20		427	60	552	0,866
Sauromys petrophilus?			5				5	0,008
Tadarida aegyptiaca	3569	2480	8528	5706	7683	4071	32037	50,247
Taphozous mauritianus	80	34	266	15	23	22	440	0,690
TOTAL	**7076**	**5623**	**16332**	**9420**	**18666**	**6642**	**63759**	**100**

Table S5. 5.The final model of the relationship between meanTC and PC1 for bat species richness.

Modnames	*K*	*AICc*	*Delta_AICc*	*ModelLik*	*AICcWt*	*LL*	*Cum.Wt*
meanTCINTPC1	6	211.61	0	1	0.41	-98.7	0.41
meanTCQPC1	6	213.6	1.99	0.37	0.15	-99.7	0.56
meanTCPC1	5	213.65	2.04	0.36	0.15	-101.06	0.71
meanTC	4	214.02	2.41	0.3	0.12	-102.51	0.83
meanTCQ	5	214.06	2.45	0.29	0.12	-101.26	0.95
meanTCQINTPC1	7	215.88	4.27	0.12	0.05	-99.43	1
PC1	4	231.97	20.36	0	0	-111.48	1
PC1Q	5	232.86	21.25	0	0	-110.66	1
null	3	235.88	24.27	0	0	-114.65	1.00

Abbreviations: meanTC average nightly temperature (18:00 to 06:00), PC1 principal component 1, K number of independent variables, AICc Akaike information criterion corrected for small sample sizes, Delta_AICc difference in AICc score, ModelLik model likelihood, AICcWt AICc weight, LL Log-likelihood, CumWt sum of the AICc weights, INT is the Interaction of preceding term, Q is the quadratic term.

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Table S5. 6. The final model of the relationship between meanTC and PC1 for bat species activity.

Modnames	K	AICc	Delta_AICc	ModelLik	AICcWt	LL	Cum.Wt
meanTCQINTPC1	6	394.97	0	1	0.97	-190.4	0.97
meanTCQPC1	5	402.15	7.19	0.03	0.03	-195.3	1
meanTCQ	4	406.84	11.88	0	0	-198.9	1
meanTCPC1	4	411.52	16.56	0	0	-201.3	1
meanTCINTPC1	5	413.87	18.9	0	0	-201.2	1
meanTC	3	415.66	20.69	0	0	-204.5	1
PC1Q	4	734.04	339.07	0	0	-362.5	1
null	2	739.31	344.34	0	0	-367.5	1
PC1	3	740.86	345.89	0	0	-367.1	1

Abbreviations: meanTC average nightly temperature (18:00 to 06:00), PC1 principal component 1, K number of independent variables, AICc Akaike information criterion corrected for small sample sizes, Delta_AICc difference in AICc score, ModelLik model likelihood, AICcWt AICc weight, LL Log-likelihood, CumWt sum of the AICc weights, INT is the Interaction of preceding term, Q is the quadratic term.

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Table S5. 7. Clutter-edge foragers activity and temperature and PC1.

Modnames	*K*	*AICc*	*Delta_AICc*	*ModelLik*	*AICcWt*	*LL*	*Cum.Wt*
meanTCQ	5	422.74	0	1	0.5	-205.6	0.5
meanTCINTPC1	6	424.76	2.03	0.36	0.18	-205.3	0.68
meanTCQPC1	6	425.39	2.65	0.27	0.13	-205.6	0.81
meanTCQINTPC1	7	426.09	3.35	0.19	0.09	-204.5	0.91
meanTC	4	426.61	3.87	0.14	0.07	-208.8	0.98
meanTCPC1	5	429.14	6.41	0.04	0.02	-208.8	1
PC1Q	5	447.59	24.86	0	0	-218	1
null	3	447.69	24.95	0	0	-220.6	1
PC1	4	448.78	26.04	0	0	-219.9	1

Abbreviations: meanTC average nightly temperature (18:00 to 06:00), PC1 principal component 1, K number of independent variables, AICc Akaike information criterion corrected for small sample sizes, Delta_AICc difference in AICc score, ModelLik model likelihood, AICcWt AICc weight, LL Log-likelihood, CumWt sum of the AICc weights, INT is the Interaction of preceding term, Q is the quadratic term.

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Table S5. 8. Clutter-edge and trophic interactions.

	cond(Int)	disp(Int)	cond(biomass_std)	cond(fcm_count)	cond(meanTC)	cond(PC1)	df	logLik	AICc	delta	weight
14	3.34	+	0.21	NA	0.4	-0.24	6	-57.63	136.59	0	0.25
13	3.36	+	NA	NA	0.52	-0.25	5	-60.33	136.67	0.08	0.24
6	3.38	+	0.25	NA	0.36	NA	5	-60.38	136.76	0.17	0.23
5	3.39	+	NA	NA	0.56	NA	4	-62.83	137.29	0.71	0.17
7	3.38	+	NA	-0.07	0.59	NA	5	-62.68	141.36	4.77	0.02
2	3.43	+	0.41	NA	NA	NA	4	-64.92	141.47	4.88	0.02
8	3.38	+	0.26	0.07	0.32	NA	6	-60.27	141.87	5.28	0.02
15	3.36	+	NA	-0.01	0.52	-0.25	6	-60.33	141.99	5.41	0.02
10	3.39	+	0.39	NA	NA	-0.27	5	-63.31	142.61	6.02	0.01
16	3.34	+	0.22	0.03	0.38	-0.24	7	-57.58	143.16	6.57	0.01
4	3.4	+	0.36	0.21	NA	NA	5	-64.01	144.01	7.42	0.01
12	3.37	+	0.36	0.19	NA	-0.29	6	-62.2	145.74	9.15	0
9	3.47	+	NA	NA	NA	-0.33	4	-67.88	147.39	10.8	0
1	3.51	+	NA	NA	NA	NA	3	-69.88	147.76	11.17	0
3	3.45	+	NA	0.3	NA	NA	4	-69.05	149.74	13.15	0
11	3.46	+	NA	0.2	NA	-0.37	5	-67.03	150.06	13.47	0.000295

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Table S5. 9. Open air activity, PC1 and temperature.

Modnames	*K*	*AICc*	*Delta_AICc*	*ModelLik*	*AICcWt*	*LL*	*Cum.Wt*
meanTC	4	199.82	0	1	0.44	-94.09	0.44
meanTCPC1	5	199.82	0	1	0.44	-91.91	0.87
meanTCQ	5	203.96	4.14	0.13	0.06	-93.98	0.93
meanTCINTPC1	6	204.76	4.94	0.08	0.04	-91.71	0.97
meanTCQPC1	6	205.12	5.3	0.07	0.03	-91.89	1
meanTCQINTPC1	7	210.65	10.83	0	0	-91.32	1
PC1	4	212.88	13.06	0	0	-100.6	1
null	3	213.85	14.03	0	0	-102.9	1
PC1Q	5	216.57	16.75	0	0	-100.3	1

Abbreviations: meanTC average nightly temperature (18:00 to 06:00), PC1 principal component 1, K number of independent variables, AICc Akaike information criterion corrected for small sample sizes, Delta_AICc difference in AICc score, ModelLik model likelihood, AICcWt AICc weight, LL Log-likelihood, CumWt sum of the AICc weights, INT is the Interaction of preceding term, Q is the quadratic term.

Table S5. 10. Open air foraging activity and trophic interactions.

	cond(Int)	disp(Int)	cond(biomass_std)	cond(fcm_count)	cond(meanTC)	cond(PC1)	df	logLik	AICc	delta	weight
5	5.64	+	NA	NA	0.47	NA	4	-94.09	199.82	0	0.36
13	5.62	+	NA	NA	0.45	-0.23	5	-91.91	199.82	0	0.36
15	5.61	+	NA	-0.15	0.5	-0.2	6	-90.52	202.36	2.54	0.1
7	5.64	+	NA	-0.14	0.52	NA	5	-93.48	202.96	3.14	0.07
6	5.65	+	0.07	NA	0.43	NA	5	-93.66	203.31	3.49	0.06
14	5.62	+	0.06	NA	0.42	-0.22	6	-91.51	204.35	4.53	0.04
8	5.65	+	0.08	-0.13	0.47	NA	6	-93.01	207.34	7.52	0.01
16	5.61	+	0.05	-0.15	0.47	-0.2	7	-90.29	208.57	8.75	0
9	5.69	+	NA	NA	NA	-0.3	4	-100.62	212.88	13.06	0
1	5.73	+	NA	NA	NA	NA	3	-102.93	213.85	14.03	0
10	5.67	+	0.2	NA	NA	-0.27	5	-98.97	213.95	14.13	0
2	5.7	+	0.23	NA	NA	NA	4	-101.32	214.27	14.45	0
11	5.69	+	NA	0.02	NA	-0.3	5	-100.6	217.21	17.39	0
3	5.73	+	NA	0.02	NA	NA	4	-102.91	217.46	17.64	0
4	5.7	+	0.23	-0.01	NA	NA	5	-101.32	218.63	18.81	0
12	5.67	+	0.2	-0.01	NA	-0.27	6	-98.96	219.26	19.44	2.14E-05

Table S5. 11. Clutter forager, PC1 and temperature.

Modnames	*K*	*AICc*	*Delta_AICc*	*ModelLik*	*AICcWt*	*LL*	*Cum.Wt*
PC1	4	239.05	0	1	0.51	-115	0.51
PC1Q	5	241.57	2.53	0.28	0.15	-115	0.66
meanTCPC1	5	241.58	2.53	0.28	0.14	-115	0.8
meanTCINTPC1	6	243.45	4.4	0.11	0.06	-114.6	0.86
meanTCQINTPC1	7	243.74	4.7	0.1	0.05	-113.4	0.91
meanTCQPC1	6	244.04	4.99	0.08	0.04	-114.9	0.95
null	3	244.45	5.41	0.07	0.03	-118.9	0.99
meanTC	4	246.74	7.69	0.02	0.01	-118.9	1
meanTCQ	5	248.75	9.7	0.01	0	-118.6	1

Abbreviations: meanTC average nightly temperature (18:00 to 06:00), PC1 principal component 1, K number of independent variables, AICc Akaike information criterion corrected for small sample sizes, Delta_AICc difference in AICc score, ModelLik model likelihood, AICcWt AICc weight, LL Log-likelihood, CumWt sum of the AICc weights, INT is the Interaction of preceding term, Q is the quadratic term.

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Table S5. 12. The total number of families found in the faecal samples of insectivorous bats captured at the Alicedale citrus orchard landscape.

Family	**Common name**	**No. samples**	**Relative abundance**
Alydidae	Broad-headed bugs	1	0.44
Bovidae	Bovids	16	7.11
Calliphoridae	Blow-flies	4	1.78
Cervidae	Deer	4	1.78
Chrysididae	Cuckoo Wasps	2	0.89
Crambidae	Crambid snout moths	46	20.44
Culicidae	Mosquito	12	5.33
Drosophilidae	Fruit Flies	11	4.89
Elateridae	Click beetles	12	5.33
Erebidae	Tussock moths	4	1.78
Formicidae	Ants	4	1.78
Hydropsychidae	Net-spinning caddisflies	1	0.44
Liposcelididae	Booklouse	9	4.00
Molossidae	Free-tailed bat	46	20.44
Rhabdiasidae	Lungworms	2	0.89
Pyralidae	Snout moths	18	8.00
Rhiniidae	Nose flies	1	0.44
Salticidae	Jumping Spiders	1	0.44
Staphylinidae	Rove beetles	4	1.78
Subuluridae	Roundworms	12	5.33
Syrphidae	Hoverfly	2	0.89
Tephritidae	Fruit Flies	1	0.44
Tortricidae	Leafroller moths	3	1.33
Uloboridae	Cribellate Orb-weavers	2	0.89
Thomisidae	Crab spiders	5	2.22
Theridiidae	Cobweb weavers	2	0.89

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Table S5. 13. The DNA samples of different arthropod orders/families/genus were detected in the faecal samples of different bat foraging groups, with the different genus of arthropods classified into functional groups (pest status) and a number of MOTU's.

Order/Family/Genus	Functional group	Open-air forager	Clutter-edge forager	MOTUs
Araneae		25	9	34
Theridiidae		1	1	2
Selkirkiella	Predator	1	1	2
Thomisidae		4	1	5
Xysticus	Predator	4		4
Misumena	Predator		1	1
Uloboridae		2		2
Octonoba	Predator	2		2
Salticidae		1		1
Phintella	Predator	1		1
Coleoptera		10	6	16
Elateridae		7	5	12
Oedostethus	Pest		1	1
Staphylinidae		3	1	4
Aleochara	Other	3	1	4
Diptera		31	18	49
Calliphoridae		3	1	4
Hemigymnochaeta	Other	1		1
Culicidae		2	10	12
Culex	Other	2	7	9
Drosophilidae		9	2	11
Drosophila	Citrus pest	8	2	10
Rhiniidae	Other	1		1
Syrphidae		2		2
Eumerus	Predator	2		2

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Tephritidae			1	1
Paramyiolia	Citrus pest		1	1
Hemiptera		1		1
Alydidae		1		1
Heegeria	Pest	1		1
Hymenoptera			7	7
Chrysididae			2	2
Chrysis	Predator		2	2
Formicidae			4	4
Pheidole	Predator		1	1
Tapinoma	Predator		2	2
Lepidoptera		37	21	58
Crambidae		27	18	45
Patania	Pest	3		3
Mecyna	Pest		1	1
Nomophila	Pest		1	1
Pygospila	Pest	2		2
Erebidae			4	4
Lyclene	Pest		4	4
Pyralidae	Citrus pest	2		2
Tortricidae		2	1	3
Grapholita	Citrus pest	1	1	2
Psocoptera		8	1	9
Liposcelididae		8	1	9
Liposcelis	Pest	8	1	9
Trichoptera		1		1
Hydropsychidae	Pest	1		1

MOTU's = Represents the number of samples taxa were detected in.

Table S5. 14. The DNA samples of different arthropod orders were detected in the faecal samples of different bat foraging groups, with a number of MOTU's (Read outs), RRA, %FOO, POO, and wPOO.

Classification	**Forage_group**	**n**	**n.taxa**	**sum_Perc**	**wP**	**distinct_samples**	**MOTU**	**RRA**	**FOO**	**POO**	**wPOO**
Araneae	clutter-edge	9	9	0.16	2.8	58	3583	2.76E-03	0.16	0.05	0.05
Araneae	open-air	23	23	0.75	7.1	58	28459	1.29E-02	0.40	0.13	0.12
Coleoptera	clutter-edge	5	5	0.06	1.5	58	1507	9.64E-04	0.09	0.03	0.03
Coleoptera	open-air	11	11	0.01	3.2	58	476	2.00E-04	0.19	0.06	0.05
Diptera	clutter-edge	19	19	0.51	6.8	58	12212	8.85E-03	0.33	0.11	0.12
Diptera	open-air	31	31	1.15	10.4	58	17993	1.98E-02	0.53	0.18	0.18
Hemiptera	open-air	1	1	0.00	0.2	58	74	2.93E-05	0.02	0.01	0.00
Hymenoptera	clutter-edge	7	7	0.00	2.1	58	77	4.83E-05	0.12	0.04	0.04
Lepidoptera	clutter-edge	21	21	2.42	7.6	58	73959	4.18E-02	0.36	0.12	0.13
Lepidoptera	open-air	37	37	5.16	13.6	58	171658	8.90E-02	0.64	0.21	0.23
Psocoptera	clutter-edge	1	1	0.00	0.3	58	8	6.90E-06	0.02	0.01	0.01
Psocoptera	open-air	8	8	0.80	2.2	58	15600	1.38E-02	0.14	0.05	0.04
Trichoptera	open-air	1	1	0.00	0.3	58	1	1.72E-06	0.02	0.01	0.00

MOTU's = Represents the number of samples taxa were detected in. n = number of samples that a taxa were detected. RRA = Relative read abundance. FOO = Percentage of frequency of occurrence. POO = Percentage of occurrence. wPOO = weighted percentage of occurrence.

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Table S5. 15. The DNA samples of different arthropod families were detected in the faecal samples of different bat foraging groups, with a number of MOTU's (Read outs), RRA, %FOO, POO, and wPOO.

Classification	Forage_group	n	n.taxa	sum_Perc	wP	distinct_samples	MOTU	RRA	FOO	POO	wPOO
Alydidae	open-air	1	1	0.002	0.2	53	74	3.21E-05	0.019	0.008	0.004
Calliphoridae	open-air	4	4	0.004	0.9	53	147	7.74E-05	0.075	0.031	0.017
Chrysididae	clutter-edge	2	2	0.001	1.0	53	24	1.89E-05	0.038	0.015	0.019
Crambidae	clutter-edge	18	18	0.337	7.9	53	14274	6.36E-03	0.340	0.138	0.149
Crambidae	open-air	27	27	0.874	14.8	53	46070	1.65E-02	0.509	0.208	0.280
Culicidae	clutter-edge	10	10	0.351	3.4	53	7959	6.62E-03	0.189	0.077	0.064
Culicidae	open-air	2	2	0.003	0.6	53	124	6.42E-05	0.038	0.015	0.011
Drosophilidae	clutter-edge	2	2	0.000	0.5	53	11	7.55E-06	0.038	0.015	0.009
Drosophilidae	open-air	9	9	0.042	3.7	53	1217	8.00E-04	0.170	0.069	0.070
Elateridae	clutter-edge	4	4	0.051	2.3	53	1279	9.55E-04	0.075	0.031	0.044
Elateridae	open-air	8	8	0.002	3.5	53	66	4.15E-05	0.151	0.062	0.066
Erebidae	clutter-edge	4	4	0.006	1.3	53	206	1.19E-04	0.075	0.031	0.025
Formicidae	clutter-edge	4	4	0.001	2.0	53	32	2.08E-05	0.075	0.031	0.038
Hydropsychidae	open-air	1	1	0.000	0.3	53	1	1.89E-06	0.019	0.008	0.006
Liposcelididae	clutter-edge	1	1	0.000	0.3	53	8	7.55E-06	0.019	0.008	0.005
Liposcelididae	open-air	8	8	0.799	3.0	53	15600	1.51E-02	0.151	0.062	0.056

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Pyralidae	open-air	2	2	0.003	0.7	53	56	6.04E-05	0.038	0.015	0.013
Rhiniidae	open-air	1	1	0.000	0.2	53	1	1.89E-06	0.019	0.008	0.004
Salticidae	open-air	1	1	0.000	0.2	53	13	5.66E-06	0.019	0.008	0.003
Staphylinidae	clutter-edge	1	1	0.000	0.3	53	4	3.77E-06	0.019	0.008	0.005
Staphylinidae	open-air	3	3	0.006	0.7	53	261	1.15E-04	0.057	0.023	0.013
Syrphidae	open-air	2	2	0.016	0.5	53	308	2.94E-04	0.038	0.015	0.009
Tephritidae	clutter-edge	1	1	0.001	0.3	53	31	2.64E-05	0.019	0.008	0.005
Theridiidae	clutter-edge	1	2	0.008	0.5	53	70	1.51E-04	0.019	0.008	0.009
Theridiidae	open-air	1	2	0.001	1.0	53	12	1.13E-05	0.019	0.008	0.019
Thomisidae	open-air	5	5	0.005	1.5	53	211	9.62E-05	0.094	0.038	0.027
Tortricidae	clutter-edge	1	1	0.000	0.5	53	5	3.77E-06	0.019	0.008	0.009
Tortricidae	open-air	2	2	0.000	1.2	53	9	7.55E-06	0.038	0.015	0.022
Uloboridae	open-air	2	2	0.037	0.7	53	301	7.00E-04	0.038	0.015	0.013

MOTU’s = Represents the number of samples taxa were detected in. n = number of samples that a taxa were detected. RRA = Relative read abundance. FOO = Percentage of frequency of occurrence. POO = Percentage of occurrence. wPOO = weighted percentage of occurence.