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BAT DIVERSITY, HABITAT USE AND POTENTIAL EXPOSURE TO PPPs IN AN AGRICULTURAL LANDSCAPE OF CENTRAL PORTUGAL

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Bat diversity, habitat use and potential exposure to PPPs in an agricultural landscape of central Portugal

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Abstract

With the worldwide increase in the use of plant protection products (PPPs) and the conversion of forest habitats into agricultural land, bats are at greater risk of exposure than ever before. This study serves as a pilot project aiming at understanding bat habitat use and their potential exposure to plant protection products (PPPs) in agricultural landscapes dominated by permanent crops, specifically vineyards and olive groves. The study was developed in central Portugal, at Bairrada, one of the main wine-producing regions. The primary objectives were to assess the differences in bat species richness and habitat use between two crop types in contrasting landscape contexts and to evaluate the potential exposure of bats to PPPs through their insect prey. The research was conducted in two types of agricultural landscapes with different levels of complexity: low-intensive farmland characterised by smaller patches interspersed with natural and semi-natural structures and forest, and highly intensive farmland dominated by contiguous crop patches, and less interspersed with natural and semi-natural habitats. In both vineyards and olive groves, bat activity was recorded using Audiomoth devices, ground-dwelling arthropods were sampled with pitfall traps, foliage-dwelling arthropods were sampled via suction, and night-flying arthropods were sampled with the help of a white sheet and a suction device. Bat faeces were also collected in a nearby roosting site of the species *Miniopterus schreibersii*. Both faeces and arthropods were chemically analysed for traces of PPPs. In total, 13 bat species were detected across the study areas. Important species included *Pipistrellus pipistrellus*, which was the most abundant, *Pipistrellus kuhlii* which was particularly abundant in olive groves, and *Eptesicus isabellinus*, which showed high abundance in vineyards. Other species such as *Hypsugo savii* and *Miniopterus schreibersii* were also recorded, though *Miniopterus schreibersii* was less prevalent in agricultural areas. Bat activity and species richness were positively correlated with landscape complexity, with higher activity and species richness found in more complex landscapes. The same pattern was observed on arthropod richness. Both landscapes showed significantly different communities, being the major driver in explaining bat species assemblages; crop type played a less significant role. Most of the PPPs applied by the farmers were detected on the arthropods, showing potential exposure via prey consumption. However, no direct evidence of PPP exposure was found in bat faeces. This study highlights the need for further research, including the installation of bat boxes in agricultural areas, to better understand bats' exposure to PPPs and ensure their conservation in agricultural landscapes.

Resumo

Com o aumento da utilização de produtos fitofarmacêuticos (PPP) a nível mundial e a conversão de habitats florestais em terrenos agrícolas, os morcegos possuem um risco de exposição a estes compostos maior do nunca. Este estudo constitui um projeto-piloto que visa compreender a utilização do habitat pelos morcegos e a sua potencial exposição a produtos fitofarmacêuticos em paisagens agrícolas dominadas por culturas permanentes, nomeadamente vinhas e olivais. Este estudo foi desenvolvido no centro de Portugal, na região da Bairrada, uma das principais regiões produtoras de vinho. Os principais objetivos foram avaliar as diferenças na riqueza de espécies de morcegos e na utilização de habitats entre os dois tipos de culturas em contextos paisagísticos contrastantes e avaliar a exposição potencial destes mamíferos aos produtos fitofarmacêuticos via as suas presas. A investigação foi realizada em dois tipos de paisagens agrícolas com diferentes níveis de complexidade: áreas agrícolas caracterizadas por pequenas manchas intercaladas com estruturas naturais e seminaturais e floresta, e áreas agrícolas dominadas por manchas de culturas contíguas, com menor incidência de habitats naturais e seminaturais. Em ambos os tipos culturas, vinha e olival, a atividade de morcegos foi registada usando dispositivos Audiomoth, os artrópodes que vivem à superfície do solo foram amostrados com armadilhas de queda, os artrópodes que vivem na vegetação foram amostrados utilizando um método de sucção e artrópodes voadores noturnos foram amostrados com a ajuda de um lençol branco e também via um dispositivo de sucção. Fezes de uma colónia da espécie *Miniopterus schreibersii* foram também coletadas num local de nidificação perto das xonas de amostragem. As amostras de fezes e de artrópodes foram analisadas para detetar resíduos de produtos fitofarmacêuticos. Um total de 13 espécies de morcegos foi detetado em todas as áreas amostradas. Espécies importantes incluíam *Pipistrellus pipistrellus*, que foi a espécie mais abundante, *Pipistrellus kuhlii* espécie particularmente abundante nos olivais, e *Eptesicus isabellinus*, que mostrou alta abundância em vinhas. Outras espécies como *Hypsugo savii* e *Miniopterus schreibersii* também foram registadas, embora *M. schreibersii* tenha sido menos prevalente em áreas agrícolas. A atividade dos morcegos e a riqueza de espécies encontraram-se positivamente correlacionadas com a complexidade da paisagem, com maior atividade e riqueza de espécies encontradas em paisagens mais complexas. O mesmo padrão foi observado ao nível da riqueza de artrópodes amostrados. Ambas as paisagens mostraram comunidades de morcegos significativamente diferentes, sendo o principal fator explicativo da composição das espécies; já o tipo de cultura desempenhou um papel menos significativo. A maioria dos produtos fitofarmacêuticos aplicados pelos agricultores foi detetada nos artrópodes, demonstrando uma potencial exposição através do consumo de presas. No entanto, não foi encontrada evidência direta de exposição a produtos fitofarmacêuticos nas fezes recolhidas. Este estudo salienta a necessidade de mais investigação, que passa pela instalação de caixas de morcegos em zonas agrícolas, para melhor compreender a exposição dos morcegos aos produtos fitofarmacêuticos e garantir a sua conservação em paisagens agrícolas.

Contents

1. Introduction

Bats are mammals belonging to the order 'Chiroptera', literally hand – wing in Greek, highlighting the order's main feature, i.e., the presence of wings arising from the forelimb's adaptation to flight, some other mammals, such as flying squirrels, may glide but not fly. Bat's wings make them the only mammalian group capable of powered flight. Bats may fly over long distances and for extended periods of time, and due to their outstanding dispersal ability, they are often the only native mammals on remote islands (Conenna et al., 2017). With more than 1300 recognised species, bats contribute to a significant portion of the mammal's diversity (Fenton and Simmons, 2014; Dietz and Kiefer, 2016). Due to their ability to disperse over water, when compared to other mammals, about 60% of bat species diversity occurs on islands, and about 25% of all species are insular endemics, a percentage that is higher in bats than in other mammals (Fleming and Racey, 2010).

1.1 The Remarkable Adaptations and Longevity of Bats

Despite having a similar body plan, all bat species have spread throughout all continents except Antarctica through various physiological, ecological, and behavioural adaptations. These habitats range from temperate forests to rainforests and farmland to urban areas. Bats are nocturnal, and this behaviour is believed to have arisen as an evolutionary response to predation by diurnal avian predators that rely on vision (Mikula et al., 2016). While some bat species may occasionally fly during the day, these are usually confined to oceanic islands that are inaccessible to these predators; even more rarely, daytime flight occurs at mainland locations where the benefit of feeding before dusk outweighs the risk of being caught (Russo et al., 2011). The biosonar, or echolocation, is the most striking adaption to the nocturnal lifestyle of the bat; most extant bats emit ultrasonic pulses and listen for their echoes to explore their surroundings for orientation, navigation, feeding or performing social behaviour (Fenton and Simmons, 2014).

The body size of bats shows a remarkable range, spanning from the 2-g bumblebee bat (*Craseonycteris thonglongyai*) to the largest flying foxes (Pteropus genus) that can weigh over one kg and showing a wingspan larger than 1.5 m (Russo and Fenton, 2023). However, most species are small: for example, most European bat species weigh less than 20 g (Dietz and Kiefer, 2016). In Portugal, the most common species weigh between 4g and 8g. Bats are K-strategists and often give birth to one, more rarely two or more young per year. The offspring are born already quite large and then milked for several weeks, after which their body size equals that of their mother. When compared to small mammals such as rodents or shrews, female bats mature sexually later, give birth to bigger and fewer offspring, show longer interbirth intervals, longer gestation, and lactation. Furthermore, compared to ground-dwelling small mammals, bats live much longer; on average, bats could live more than 15 years, and up to 38 years have been recorded in some species (Brunet-Rossinni and Austad, 2004). Such longevity is very unusual compared to small mammals. For mammals, the rate of living theory states that there is a constant mass-specific lifetime energy expenditure, meaning that animals with higher metabolic rates tend to have

shorter lifespans. However, bats defy this theory, as they live much longer than other small mammals despite having high metabolic rates during flight (Sacher, 1959). However, bats spend, on average, more than twice the amount of energy in a lifetime compared to non-flying mammals (Austad and Fischer, 1991). This implies that longevity should be inversely correlated with metabolic rate and, thus, body size. The longevity quotient (ratio of observed longevity to longevity predicted by body size) for Chiropteran is 3. This means that, on average, bats live three times longer than they should based on body size (Austad and Fischer 1991), contradicting the rate of living theory. There is no scientific consensus on the explanation of the increased longevity of bats; it is believed to be a mix of physiological, genetic, and environmental factors, plus some ecological variables (Wilkinson and South, 2002; Foley et al., 2018).

As stated previously, bats' bodies are powered by high metabolic rates. During flight, bats can exhibit more than 250 bpm, and these rates can vary among species and seasonally. Species living in geographical regions with large seasonal temperature fluctuations, such as in Europe, are typically heterothermic, hibernating to survive long winters without food, and daily torpor to reduce energy expenditure while roosting (Dietz and Kiefer, 2016). During hibernation, the metabolic rate of bats may drop to one-hundredth of the awake rate; in some bat species, it can drop as low as a few bpm. During the day, depending on the ambient temperature, the metabolic rate might rise to one-tenth of the waking rate (Dietz and Kiefer, 2016). Active bats frequently adopt torpor during the day when they are roosting; this is known as "daily torpor" since it lasts for less than 24 hours before being followed by nocturnal foraging. During the winter months of the year, when bats hibernate, they go into a state of torpor that lasts for many days to weeks (Ruf and Geiser, 2014; Geiser et al., 2020). During this time, they metabolise fat accumulated during the active months to provide them with energy (Ruf and Geiser, 2014). During hibernation, torpor can be maintained for several days, weeks, or even months, allowing bats to save vast amounts of energy (Stawski and Geiser, 2010; Shankar et al., 2023). According to Ruf and Geiser (2014), bats can lower body temperature, heart rate, respiration rate, oxygen consumption rate, and metabolic rate and impede blood supply to the primary organs.

1.2 Seasonal Breeding and Maternity Roosts of European Bats

The primary food source for European bats is insects, which are sporadically found throughout the year (Vaughan, 1997). Every species has a yearly cycle that begins with springtime births and ends with migration or hibernation to survive the winter (Figure 1). Around May, females begin congregating in what are known as maternity roosts or nurseries, where they form breeding colonies. Here, they give birth to their offspring, which is frequently a synchronous process. This synchronisation is most likely the consequence of all females' bodies warming up in the spring, which causes ovulation and the subsequent fertilisation of eggs with sperm stored over the winter. Species in the Mediterranean region often give birth a bit earlier, around mid-May, than those in central Europe, which typically do so in June. Some species found in northern regions, including Northern and Brandt's bats, require three to four weeks to raise their young, while others require 4 to 8 weeks. Adult males rarely inhabit nursery

roosts. Although solitary males can be found in the roosts of some species, for example, in the roosts of the Greater Mouse-eared Bat, males are primarily geographically separated in the attics. More males can be found in nursery roosts of long-eared horseshoe and Daubenton's bat species, particularly in chilly regions found in the north of their range or the mountains. The presence of the males could help raise the temperature of the swarm in case of especially hard conditions (Kerth, 2008). Most male bats either establish male groups or spend the summers alone. By the time of the first mating, the lone animals among the males frequently already have a highly territorial nature in the summer.
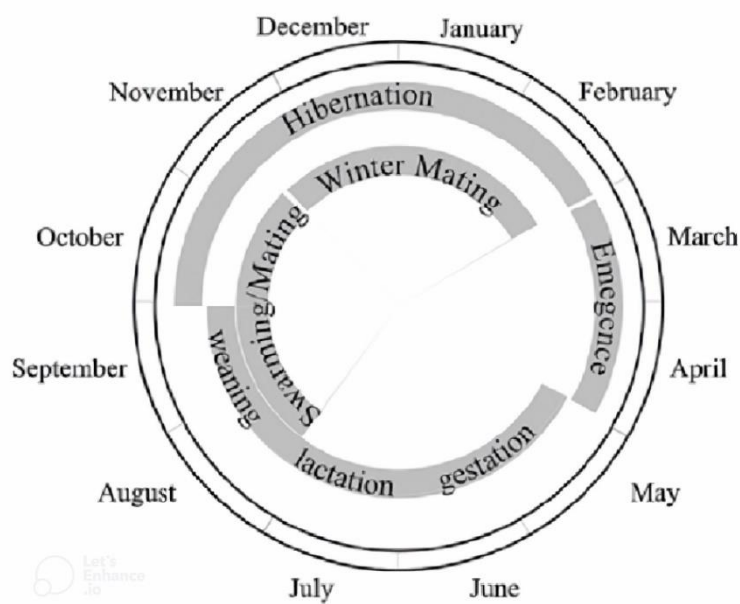
Figure 1. The typical yearly cycle of European insectivorous bats. The cycle depends on species, weather, geographical location, and the physiological status of bats it is only an approximation. Taken from (Altringham et al., 2011).

1.3 Ecology and behaviour

Bats are widespread in every habitat in Europe, and they exhibit a variety of species-specific foraging tactics that enable them to reach habitats with varying degrees of structural complexity. A species' capacity to utilise a particular habitat structure for food gathering depends on a combination of wing morphology, echolocation call design and food availability. Bats consume a large quantity of food, which peaks at particularly taxing stages of the life cycle like breastfeeding, when females may eat up to 130% of their body mass in insects in a single foraging night (Kurta et al., 1989; Russo and Fenton, 2023). Usually, bats are most active at night. Some species, like pipistrelles, begin feeding just after sunset (or sometimes even earlier) and are particularly active in the early evening, taking advantage of the peak in crepuscular insect abundance (Jones and Rydell, 1994).

The design of echolocation calls varies throughout species because they are adapted to make use of distinct habitat structures and hunting strategies (Bogdanowicz et al., 1999; Jones and Holderied, 2007;

Russo et al., 2018). Compared to species that hunt in congested environments, those that hunt in open spaces broadcast their echolocation calls at lower frequencies and with narrower frequency bandwidths. While calls with high frequencies and a broader bandwidth experience strong atmospheric attenuation, which limits their operational range, they provide many details about the surroundings, which is crucial in structurally complex environments (Jakobsen et al., 2013; Jacobs and Bastian, 2016). Low frequency and narrow bandwidth calls probe long distances but provide low resolution, so they perform well in open habitats but cannot handle the complexity of dense vegetation (Lawrence and Simmons, 1982). By design, some bat species are tied to specific types of habitats and feeding behaviour.

For example, according to Jones and Rayner, 1989, *Rhinolophus spp.* frequently use a sit-and-wait tactic known as "perch-hunting" in forest habitats or at the boundary between forest and open habitat. In this strategy, the bat hangs onto a small branch, uses its specialised, high-frequency biosonar to scan the area (mainly using Doppler-shift compensated calls that detect movement), and then takes off to capture any prey it spots. Usually, the prey is dismembered and eaten while perching (Jones and Rayner, 1989).

The easiest way to measure predation to determine daily food intake is to observe captive individuals, however this method is likely to yield only a significant underestimation of the real energy needs of free-living bats. A single bat would consume up to 25% of its body mass in insects each night, according to research done on North American bats in captivity (Brisbin, 1966; O'Farrell et al., 1971; Coutts et al., 1973). Those studies also suggest that bats drink large quantities of water compared to their body size; O'Farrell et al., (1971) suggest that some species, such as *Myotis thysanodes*, have a water turnover of almost half the total body water per day. In contrast, it would be equivalent to humans drinking more than 20 litres of water daily. However, more recent research suggests that the amount of food consumed by free-living bats is estimated to be significantly larger; however, this varies according to the physiological state of the bat and is typically highest for females during the peak of their lactation and for males during spermatogenesis. Encarnação and Dietz (2006) suggested that insect intake of *Myotis daubentonii* female bats was 8.0 g during pregnancy and 4.9 g per day during post-lactation, providing 5.0 and 3.0 kJ of ingested energy per gram body mass per day. For male bats, the calculated intake was 3.6 g of insects per day during late spring and 8.0 g during the period of intensive spermatogenesis, providing 2.6 and 5.7 kJ of ingested energy per gram body mass. *Myotis daubentonii* weighs between 6 and 15 grammes, making a daily intake of about 80% of body mass during lactation or spermatogenesis and approximately 50% after lactation.

Kurta et al., (1989)a and Kurta et al., (1989)b estimated that breastfeeding *Myotis lucifugus* consumes up to 130% of its body mass in insects by analysing the turnover of doubly labelled water, in other species such as a lactating *Tadarida brasiliensis*, this figure would be as high as about 70%. The number of prey items consumed is significantly higher than that anticipated from the biomass consumed alone because large preys are often dissected and their wings, heads, and legs are frequently discarded. It could explain the discrepancy found in different studies. For example, in their study, Anthony and Kunz (1977) examined the body weights of *M. lucifugus* before and after feeding (approximately 10 g body mass) as the animals returned to their roosts following two major nocturnal foraging sessions. They found that

pregnant females consumed 2.5 g of prey per night, lactating females consumed 3.7 g, and juveniles consumed 1.8 g. Overall, experimental data suggests that wet food intake can reach 50% of body mass daily.

1.4 Habitat Preferences and Foraging Behaviours

European bat species exhibit diverse habitat preferences and foraging behaviours. While bats are present in every European habitat, each species favours certain environments. These preferences are believed to be influenced by echolocation call structure and wing morphology, as some predictions were confirmed in specific bat species (Vaughan et al.1997). However, more than this theory is needed to explain all the variation in habitat use. For example, de Jong (1995), showed that among the 11 species studied, most completely avoided open habitats, while others, like *Pipistrellus pipistrellus*, did not avoid them. No morphological or echolocation trait would explain the avoidance of open habitats. The study also demonstrated that most species actively use open space corridors in fragmented landscapes to move through forest patches and hunt. Insect abundance is recognised as the primary factor influencing the distribution of hunting bats (Jong and Ahlen, 1991). It could explain the abundance of bats in tree corridors as they probably act as a barrier for insects that are washed by the wind (Jong and Ahlen, 1991). Bat species richness appears to be positively correlated with the area of deciduous woodland due to the high availability of food and roosting sites (de Jong, 1995). Kusch and Schmitz (2013) examined the difference in habitat use of three sympatric bat species, *P. pipistrellus*, *Pipistrellus. nathusii* and *P. pygmaeus*, and found that even in species showing very similar morphological traits, exploitation of habit could differ. It was shown that *P. pipistrellus* had a more extensive distribution than the limited distribution of two other species. Vaughan et al. (1997) demonstrated that bat activity positively correlates with air temperature, highlighting the importance of accounting for this variable when assessing bat habitat use. Polyakov et al. (2019) found that bat activity was 1.5 times greater at isolated trees compared to open sites in vineyards. Remnant oak trees offer edge-space bat species access to the interior of vineyards. These trees likely provide bats with habitat, enhanced foraging opportunities, cover, and connectivity.

To accurately assess bat habitat and use, it is necessary to analyse at the species level to understand their distribution. Although bats typically select habitats suited to their echolocation calls and morphological traits, this is only sometimes the case. Studies have shown that bats' foraging behaviour and habitat selection are heavily influenced by the abundance and type of prey available, in each habitat (Heim et al., 2017; Tortosa et al., 2023).

1.5 Dietary Habits and Foraging Strategies of Some Relevant European Bat Species

Pipistrellus pipistrellus is considered the most widespread and abundant bat species in continental Europe. Its diet primarily consists of Diptera and Lepidoptera, though it exhibits significant dietary variety, adapting to various environments (Arlettaz et al., 2000; Galan et al., 2018). Rydell (1992)

speculated that the success of *P. pipistrellus* could be linked to its ability to prey around light posts, where insect density is high, allowing it to outcompete other European species (Arlettaz et al., 2000). The detailed diet composition of *P. pipistrellus* is illustrated in Figure 2.

Until recently, the pipistrelle bat *Pipistrellus pipistrellus* (Schreber, 1774) was considered a single biological species. However, studies by Barratt et al. (1995), Barlow (1997), and Barlow and Jones (1999) revealed two reproductively isolated, cryptic species within Europe: *P. pipistrellus* and *P. pygmaeus*. The diet of *P. pygmaeus* is similar to that of *P. pipistrellus*, with Diptera (suborder Nematocera) as the main food item, followed by Trichoptera and Lepidoptera (Barlow, 1997; Bartonička, et al., 2008). The primary difference in their diets lies within the Diptera order, with *P. pygmaeus* consuming smaller flies than *P. pipistrellus* (Barlow, 1997). The diet composition of *P. pygmaeus* is also shown in Figure 2.

The feeding behaviour of *Myotis myotis* is characterised by opportunistic predation, with carabid beetles comprising 46% of their diet in the Swiss Alps (Arlettaz, 1996). Pereira et al. (2002) found similar results, with carabid beetles in 52% of pellets, crickets in 43%, and spiders in 34%. While crickets were the preferred food, beetles were more abundant throughout the year, making them the most consumed item. *Myotis myotis* primarily gleans prey from the soil surface while flying, demonstrating flexibility in foraging behaviour (Arlettaz, 1996). Audet (1990) found that *M. myotis* prefers foraging in woodland habitats, suggesting that grass presence may hinder their ground-gleaning capacity. The diet composition of *M. myotis* is depicted in Figure 2.

Miniopterus schreibersii was analysed by Presetnik and Aulagnier (2013) through a morphological study on prey remnants found in droppings from a roost in northern Slovenia (Central Europe) during spring and fall of 2000. Lepidoptera made up the majority of the diet with an average percent volume (APV) of 79% and a year-round dominance. Neuroptera (mainly Chrysopidae) were the second most consumed (APV 9.2%), followed by Diptera (APV 7.4%), Trichoptera (APV 2.2%), and Coleoptera (APV 1.4%). The variety of the diet peaked in late October. *M. schreibersii* appears to be an airborne hawker that specialises in consuming moths, yet it is opportunistic enough to transition to other seasonally abundant species. Similar results were found by Galan et al. (2018), with Lepidoptera as the main prey and Neuroptera as the second most consumed prey. Overall, it pursues small to medium-sized winged prey (wing length: 2–18 mm), the majority of which are tympanate insects. Furthermore, *M. schreibersii* are known to travel considerable distances from their roosts (4 to 29 km) to feed in specific small areas. Urban areas lit by white streetlamps were used extensively. Some bats also foraged selectively in deciduous or mixed woodlands, with habitats containing hedgerows being preferred (Vincent et al., 2011). Given the unique feeding behaviour and habitat preferences of *M. schreibersii*, conservation efforts must be planned on a large scale. Protecting a network of roosts is essential to ensure the survival of this species (Vincent et al., 2011).

Nyctalus leisleri, commonly known as Leisler's bat, is a European species found across Europe and parts of Asia. It prefers habitats such as woodlands and parklands. This bat species primarily feeds on Diptera and Lepidoptera, as well as Arachnida and Hemiptera. The diet composition of *N. leisleri* is also shown

in Figure 2. Leisler's bat is known for its fast and agile flight, often foraging in open spaces and around streetlights, similar to *P. pipistrellus*.
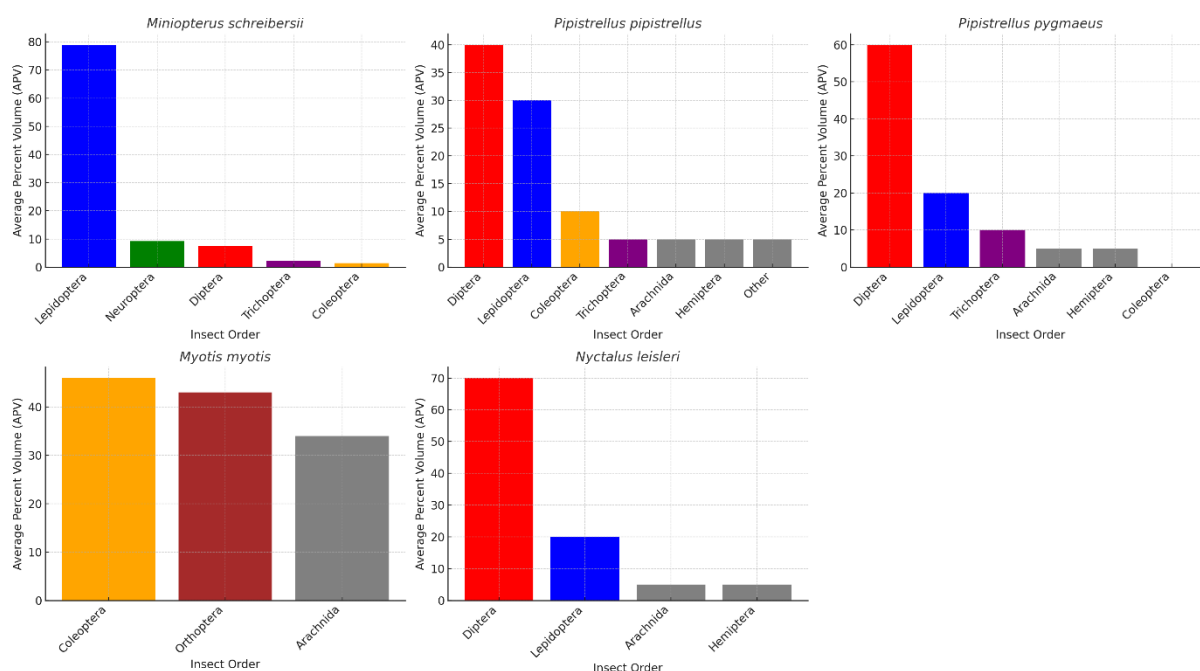

Figure 2. Diet composition of various European bat species, Miniopterus schreibersii, Pipistrellus pipistrellus, Pipistrellus pygmaeus, Myotis myotis, and Nyctalus leisleri. The data are presented as the average percent volume (APV) of different insect orders found in the diet of each species. The diet composition data were normalised to the taxonomic order level for consistency across different studies. Miniopterus schreibersii: data from Presetnik and Aulagnier (2013); Pipistrellus pipistrellus: based on Ware et al., 2020; Pipistrellus pygmaeus: according to Ware et al., 2020 and studies by Barlow (1997) and Bartonička et al. (2008); Myotis myotis: data from Arlettaz (1996) and Pereira et al. (2002); and Nyctalus leisleri: From Ware et al., 2020.

1.6 The Role of Bats in Natural Pest Control

Worldwide it is estimated that around 30% to 50% of the crop yield is directly lost to pathogen and pests (Oerke, 2005; Savary et al., 2019). Arthropod pests represent 18–26 % of annual crop production loss, at a value of more than $ 470 billion, with 13 – 16% of the loss occurring before harvest (Culliney, 2014).

Using synthetic pesticides is essential for controlling insect populations in the vast majority of crops and geographical areas worldwide. But in addition to having a negative impact on the environment, the use of pesticides has led to the development of pest resistance and the secondary emergence of diseases and pests (Pimentel et al., 2012; Pimentel and Burgess, 2014). Hence, enhancing natural pest management services in agricultural landscapes seems to be a viable strategy in the current environment to reduce pesticide use as the current EU strategy demands (Wesseler, 2022). Agricultural productivity can be positively impacted by the natural pest management offered by a wide range of vertebrate and invertebrate taxa, including parasitic wasps, predatory mites, beetles, spiders, bats, and birds. According to recent research (Dainese et al., 2019), boosting the diversity or number of natural enemies typically results in increased pest control services and decreased production losses.

However, the benefits of more species-rich or abundant predator groups are not always linked to a higher pest control service, indicating that predator-prey interactions—such as intra-guild predation—may have a negative effect on this ecosystem service (Letourneau et al., 2009). Therefore, knowledge about predator diets and behaviour is needed to better understand relationships between natural enemy communities and pests.

Bats are frequently mentioned as one of the most effective predators among all possible natural enemies, helping to prevent the growth of agricultural pests. They reduce crop damage and yield losses from insect pests (McCracken et al., 2012; Wanger et al., 2014, Baroja et al., 2019; Charbonnier et al., 2021; Herrera et al., 2021).

In vineyards, the high abundance of certain bat species is directly associated with the emergence of specific grapevine moths (Charbonnier et al., 2021). Moth species like *Lobesia botrana, Eupoecilia ambiguella, and Sparghanotis pilleriana* (Lepidoptera: Torticidae) are responsible for the most damage caused by arthropods in vineyards and are also potential prey for bats that feed on lepidoptera (Thiéry, 2011)

The biocontrol role of bats is closely connected to the surrounding environment of the farmland. In olive groves many studies have found that the biocontrol capacity of bats is directly related to the presence of tall hedgerows or forest patches in their surrounding (Costa et al., 2020; Herrera et al., 2021; Puig-Montserrat et al., 2021; Jiménez-Navarro et al., 2023). Jiménez-Navarro et al. (2023) discovered that landscape complexity was the most influential factor affecting bat abundance. While chemical applications had a stronger negative impact on birds, landscape intensification was the most significant factor for bats.

A small number of studies have evaluated insectivorous bats' daily food intake (Brisbin, 1966; O'Farrell, Studier and Ewing, 1971; Coutts et al., 1973; Anthony and Kunz 1977; Kurta et al., 1989a and Kurta et al., 1989b). While estimates in the literature are subject to discussion, it is undeniable that bats ingest many arthropods each night, which has significant implications for oral pesticide exposure (Stahlschmidt and Brühl, 2012, Brooks et al., 2022)

Evidence of pesticide exposure in bats was first discovered in the 1960s and 1970s, a period of widespread use of organochlorine pesticides (Jayaraj et al., 2016). Some of these pesticides were responsible for the significant mortality of several bat species, as demonstrated by field and laboratory studies in northern America and Europe (Jefferies, 1972; Clark et al., 1978). A study made in 1991 in Spain found residues of fenitrothion (organophosphate) in common pipistrelles (*Pipistrellus pipistrellus*) following agricultural applications (Stahlschmidt and Brühl, 2012). Since then, the majority of highly deadly and persistent pesticides have been replaced, and it is likely that the effects of current pesticides on bats are underestimated, more challenging to prove, and have not been thoroughly researched (O'Shea and Johnston, 2009). Many more studies found that bats were exposed to pesticides, however, most of them used destructive methods involving capturing and euthanising the bat (Schmidt

et al. 2000, Senthilkumar et al., 2001, Allinson et al., 2006; Kannan et al., 2010, Stechert et al., 2014, Valdespino and Sosa, 2017), a procedure not currently allowed due to bats conservation status.

The use of PPPs has nearly doubled since 1990. This increase is not uniform across all world regions; generally, developed countries have reduced their usage, while developing countries have seen a significant rise (Figure 3). For example, since 1990, Africa has nearly tripled its consumption, with similar trends observed in Asia and South America. Although PPP use in Portugal appears to have declined over the past 10 years, this is not reflective of the global trend, and pressure on bats is increasing.

For all the above, it is unclear at this time whether bats are protected by the existing risk assessment scheme, which solely considers birds and ground-dwelling mammals (Hernández-Jerez et al., 2019). Bats may provide a greater indication of risk than birds and ground-dwelling mammals due to their ecology and traits. Furthermore, under "The Convention on Migratory Species (UNEP/CMS)," 53 species of European bats are protected under the "Conservation of Populations of European Bats" agreement (Annex I to the Agreement on the Conservation of Populations of European Bats, EUROBATS, 19911). These factors make it imperative that a particular risk assessment encompassing bat species should be developed, or that bats should be included as vulnerable species in current risk assessment for birds and mammals.

Pesticide breakdown by type, 1990 to 2021

Pesticide use, broken down by product type, measured in tonnes of active ingredient.

Legend: Rodenticides, Plant growth regulators, Other pesticides, Insecticides, Fungicides and bactericides, Herbicides

World / Europe (FAO) / Portugal / Africa (FAO)
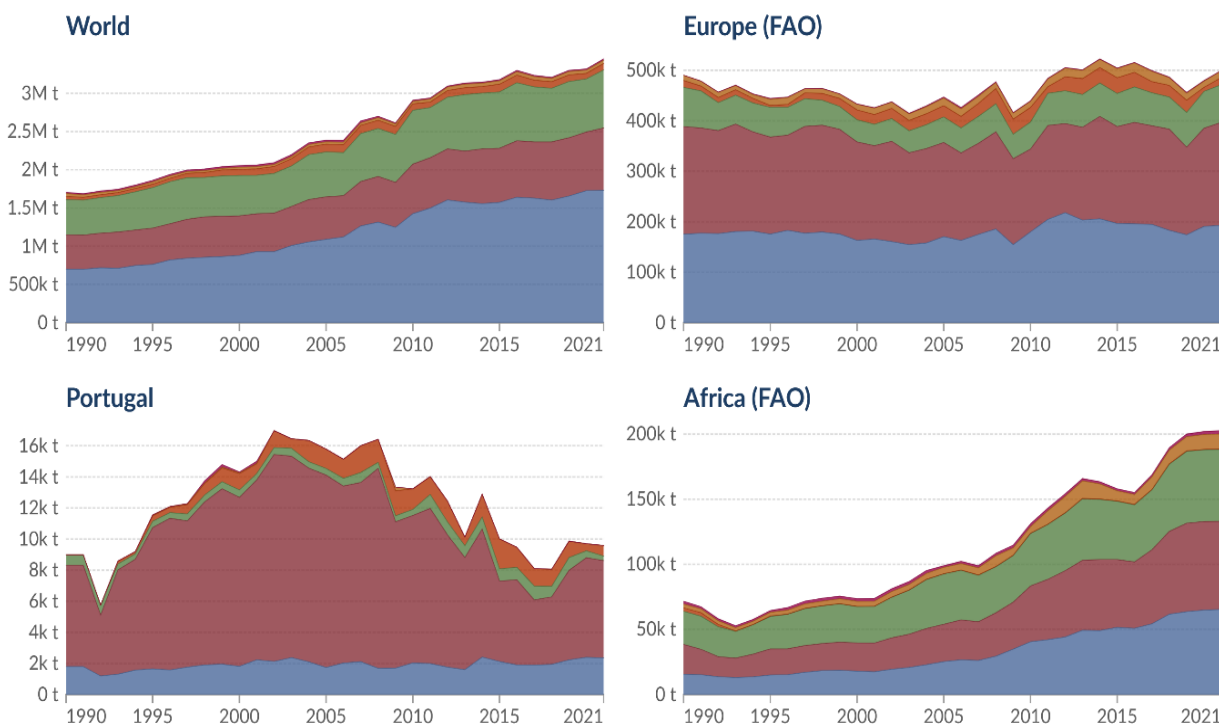
Data source: Food and Agriculture Organization of the United Nations (2024)

OurWorldInData.org/pesticides | CC BY

Figure 3. Plot of the PPPs used by type, in the world, Europe Portugal and Africa (Food and Agriculture Organization of the United Nation (2024).

1.7 Placing this thesis into context: aims and working hypotheses

This thesis serves as a pilot study aimed at understanding bat habitat use and their potential exposure to plant protection products (PPPs) in agricultural landscapes dominated by permanent crops, specifically vineyards and olive groves.

The first goal was to assess differences in species richness and habitat use between these two crop types in two different landscape contexts, i.e., an area with continuous vineyard area vs. an area with smaller vineyard patches interspersed with forest patches. I have hypothesised that a higher bat species richness and abundance (using the number of echolocations calls as a proxy) will occur in the more complex landscape due to increased habitat structure and prey availability.

The second goal was to assess bats' potential exposure to PPPs by analysing pesticide residues in potential bat prey. I have hypothesised that bats feeding in both crop types are exposed to PPPs due to the presence of pesticide residues on insect prey.

2. Methodology

2.1 Sampling Sites and Experimental Design

The area selected for this study is located inside the Bairrada wine producing region in central Portugal (Figure 4).
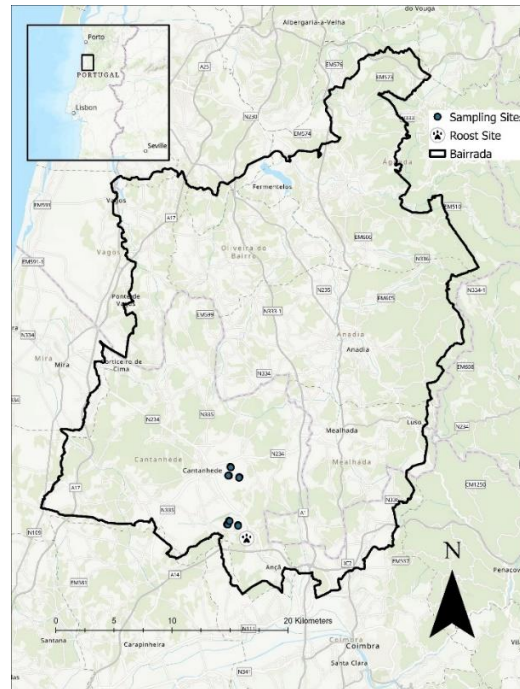

Figure 4. Map of the study area in central Portugal Region.

The research was carried out across two types of farm intensification landscapes: low-intensive farmland located in the extreme south of the location (from now on designated as South) and a highly intensive farmland located a few kilometres north (hereafter designated as North) (Figure 4). It is important to note that the term "intensification", in this context, does not refer to the agricultural practices adopted in each area of each farm but is instead a qualitative measure of the proportion of farmland within the landscape. Within each farm intensification system, two permanent crop types were examined: vineyards and olive groves. Both locations have vineyards (dominant crop) with nearby (sometimes contiguous) small olive groves. The big difference between both locations is that the vineyard patches in the south were generally smaller and, besides the olive patches, they were interspersed with different natural/semi-natural structures and forest patches. In contrast, the northern vineyard patches were larger and/or continuous, and the existence of natural/semi-natural structures was lower.

For each farm intensification gradient, three vineyard fields and three olive grove fields were selected. Each field contained three sampling points, resulting in a total of 36 sampling points. In the low-intensive farmland landscape in the South and the highly intensive farmland landscape in the North (Figures 5 and 6), there were 3 vineyard fields with 9 sampling points (3 per vineyard) and 3 olive grove fields with 9 sampling points (3 per olive grove).
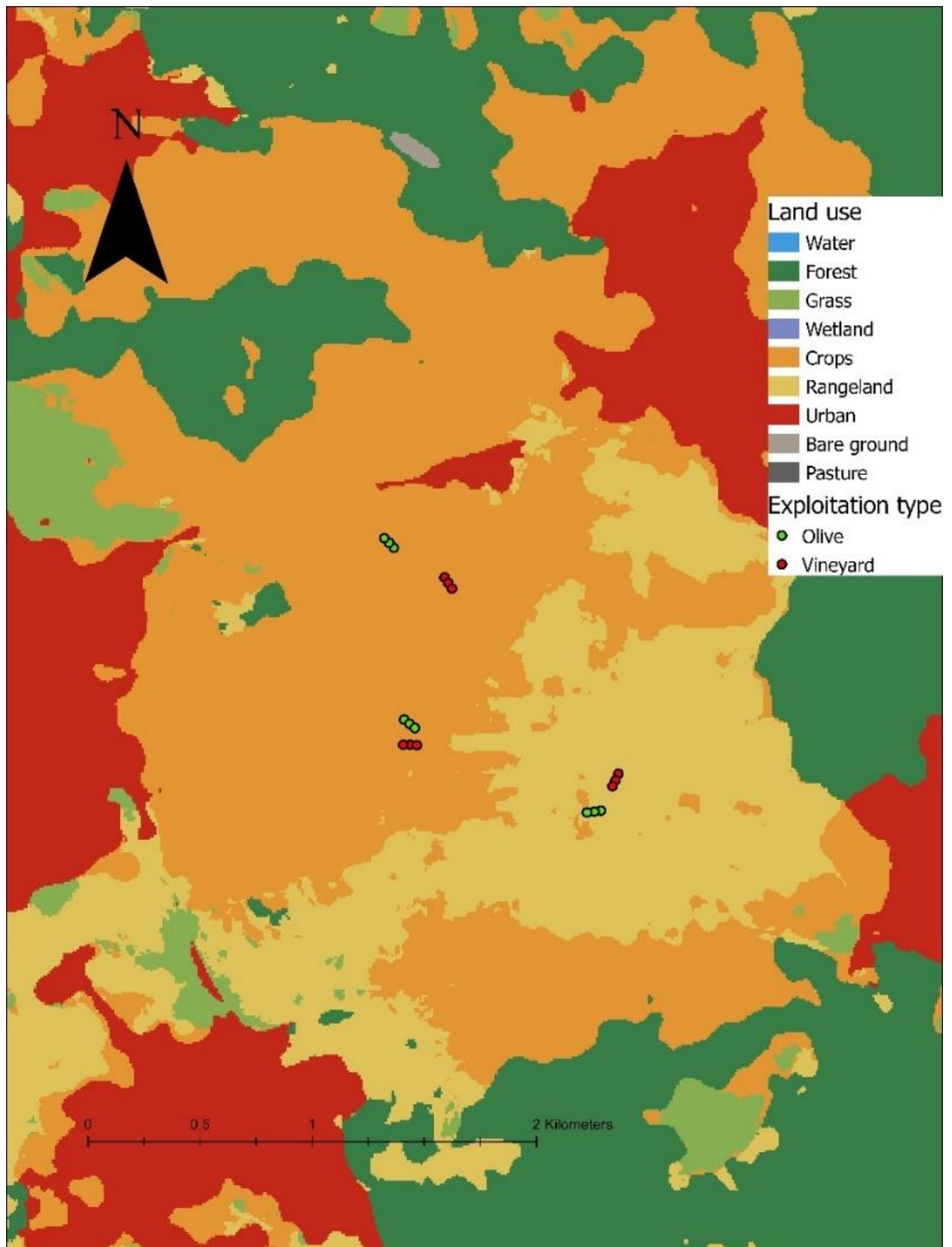
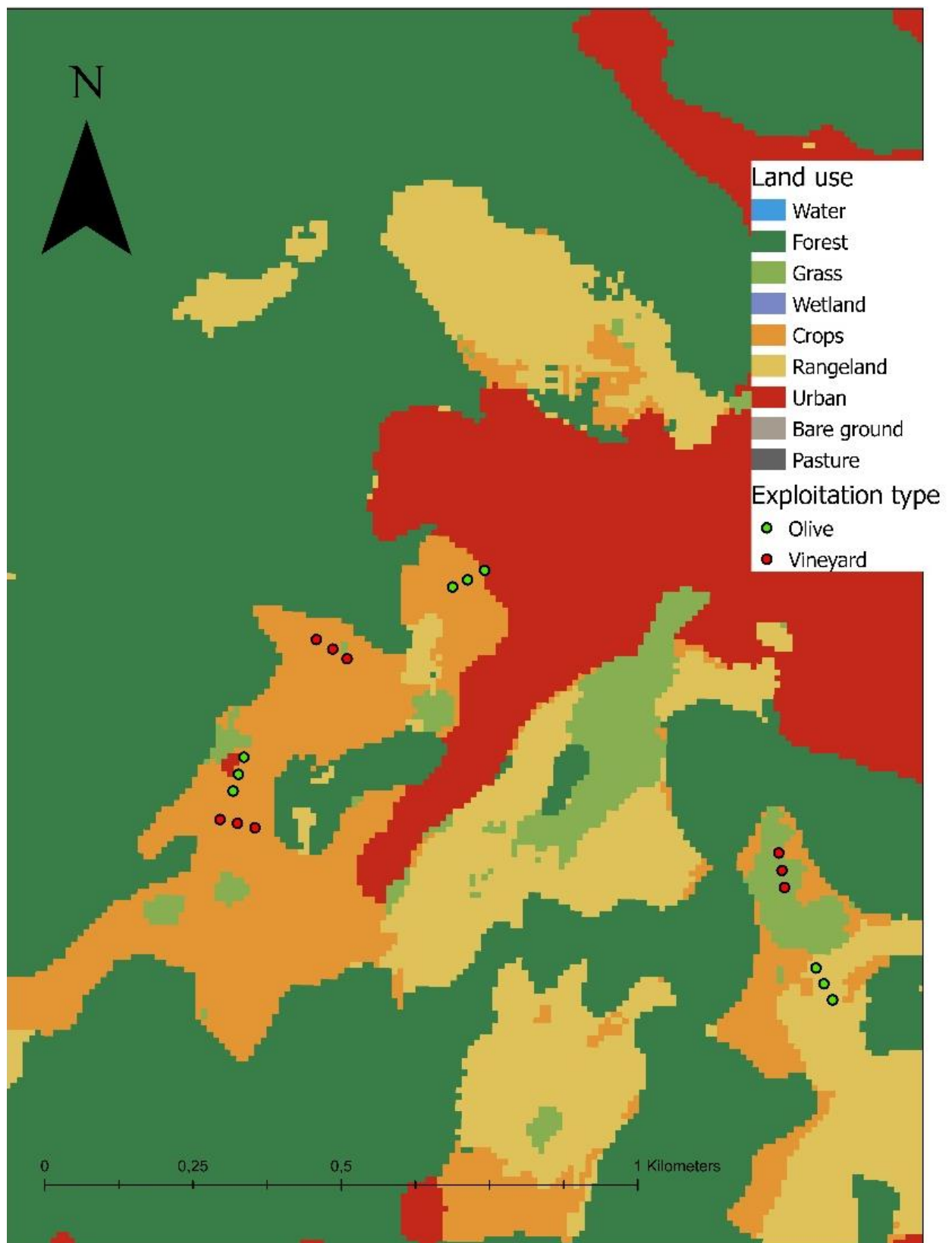*Figure 5. Map of the northern sites, with the CORINE land cover layer.*

Figure 5. Map of the South sites, with the CORINE land cover layer.

Characterising landscape complexity is a key process of the study design. Figures 4 and 5 highlight the difference in landscape complexity between the North and South sites. In the North, farmland tends to be a very large patch surrounded by rangeland, which is an open area with some medium-sized trees. In contrast, the Southern farmland is smaller, fragmented, and surrounded mostly by forest.

2.2 Field Sampling Methods and Equipment Deployment

Audiomoth Devices: AudioMoth devices were used to detect bat calls and they deployed on 22nd April and retrieved on 14th May, spanning a total of 22 days. A total of 36 devices were used, with one device per sampling point. These devices were arranged linearly, following the rows of vineyards or olive plantations, spaced 30 meters apart and centred in the middle of the field to minimise confounding effects from landscape elements known to affect bat activity, such as hedgerows, ponds, dwellings, or lamp posts. AudioMoths version 1.2.0 from Open Acoustic Devices were set to record from 8:15 PM until midnight, operating with a cycle of sampling for 60 seconds followed by a sleep period of 180 seconds. The sampling rate was configured to 386kHz, with the gain set to medium (30.6 dB).

Dry Pitfall Traps: Dry pitfall traps were used to collect surface-dwelling arthropods (potential prays of some bat species) for pesticide residue analysis. They were set up on the 22nd of April and checked on 26th and 29th of April, with final retrieval on 2nd and 3rd of May. A total of 36 pitfall traps were used one per sampling point. Dry pitfall traps were utilised to avoid the use of a killing/preserving solution (pitfall traps usually contain a solution like pet-safe anti-freeze, soapy water, or ethyl alcohol) which could dilute the PPPs. Samples were recovered from the ground and placed in falcon tubes, transported in a cool box, and preserved at -20ºC on the same day until further processing.

Vacuum Suction for Foliage-dwelling Insects: Vacuum suction sampling was conducted on the 2nd and 3rd of May, resulting in a total of 36 samples, one per sampling point. The suction device was used along the vine foliage for three intervals of one minute each, and for olive groves, it was used on three different trees with one minute of sampling time per tree. Samples were transported in a cooler and preserved at -20ºC on the same day. Additionally, when possible, suction samples were taken from the ground if a grass layer was present, with five samples in total.

Nocturnal Flying Insects (Blank Sheet Method): Sampling for nocturnal flying insects was conducted on the 2nd and 3rd of May, with a total of 12 samples, one per field (6 vineyards and 6 olive groves). A white sheet measuring 2 by 2 meters was positioned in the centre of the field for 30 minutes. Powerful lights were directed at the white sheet, and after 30 minutes, the insects were collected using a suction device and placed into a Lycra sock. The sampling was performed at night; on each day, the first sampling started at 10PM and ended at 1AM.

Bat faeces: Bat faeces were collected on May 9 and June 3 from a nearby roost of *Miniopterus schreibersii*. The samples were gathered from six different points along the cave floor to maximise recovery from multiple locations within the cave. Subsequently, the samples were pooled to create a single composite sample for each collection date.

2.3 Chemical Analysis of Arthropod Bulk Samples and Faecal Pellets

The individuals collected from the pitfall traps and the suction apparatus were identified at the order level. The samples were combined to match the weight restriction of 4 g needed for the chemical analysis. Five pooled samples were created: Olive North, Olive South, Vineyard North, Vineyard South, and Suction. It must be noted that the suction sample includes all arthropods collected from the white sheet and suction methods, encompassing both north and south locations and olive and vineyard sites. The pooled samples were then sent for pesticide analysis at a private laboratory, where MR GC-MS/MS and MR UPLC-MS/MS techniques were used to screen for more than 90 pesticides with a detection limit of > 0.01 mg/kg. The bat faecal samples were also assessed, and the same screening was performed.

2.4 Analysis of Echolocation Calls

The identification of echolocation calls was carried out using Kaleidoscope software v5.5.0, and all echolocation calls were manually identified using the continental Portuguese key (Rainho et al., 2011). Some species with similar echolocation calls could not be distinguished and are listed in Table 6 – Annex I. Social and hunting calls were excluded due to insufficient information given by them to clearly identify the species. The species that were group together are *Miniopterus schreibersii*/*Pipistrellus pygmaeus*, *Eptesicus isabellinus*/*Eptesicus serotinus* and *Plecotus spp.*

2.5 Data Analysis

Non-metric Multi-Dimensional Scaling (NMDS), PERMANOVA, and SIMPER analyses were conducted using Primer 6.1.13 with Bray-Curtis dissimilarity statistic. PERMANOVA was employed to assess significant differences in bat abundance across different locations and exploitation types. NMDS was utilized to evaluate the resemblance between sites. SIMPER analysis identified the most relevant species contributing to the dissimilarity between the North and South locations.

Redundancy Analysis (RDA) and *p*RDA were performed using Canoco 4.5. Data manipulation and the construction of a Rarefaction curve were conducted in R, utilising the "INEXT" package (Hsieh et al., 2016). RDA provided insights into the most significant environmental parameters explaining the variability between sites.

3. Results

3.1 Bat Species Diversity and Abundance based on Echolocation Calls

A total of 13 bat species (or groups of species) were acoustically identified in this study, whereas only 10 species were previously identified in Bairrada according to the Portuguese bat atlas (Instituto da Conservação da Natureza e das Florestas, 2012). The newly identified species include *Plecotus spp.*, *Nyctalus lasiopterus*, *Eptesicus isabellinus/Eptesicus serotinus*, *Hypsugo savii*, and *Myotis myotis*. However, due to the low number of recordings for some of these species, this result should be interpreted with caution.

Pipistrellus pipistrellus was the most dominant bat species recorded across all sites, followed by *P. kuhlii*, which was highly dominant in olive south but not very present at the other sites. Except for *Tadarida teniotis* and *Nyctalus leisleri*, which were found equally across all sites, more bats were detected in the southern sites than in the northern ones.

Species richness varied notably across the different habitats. Both vineyards and olive groves in the south exhibited high species richness, respectively with 12 and 13 species recorded. Conversely, vineyards and olive groves in the north recorded the lowest species richness with 7 and 8 species, respectively (Table 1). The Shannon index, which accounts for both abundance and evenness of species, showed that olive groves in the south had the highest diversity with a value of 1.46. Vineyards in the north followed closely with a Shannon index of 1.42. Vineyards in the south and olive groves in the north had Shannon index values of 1.35 and 1.16, respectively, indicating moderate diversity. The Margalef index, which measures species richness relative to the logarithm of the number of individuals, indicated that olive groves in the south had the highest value at 1.77. Vineyards in the south had a Margalef index of 1.71, followed by olive groves in the north with 1.44. Vineyards in the north had the lowest Margalef index at 1.20. Pielou's evenness index, reflecting how evenly individuals are distributed across different species, showed that vineyards in the north had the highest evenness with a value of 0.73, indicating a more balanced species distribution. Olive groves in the north had the lowest evenness index at 0.53. Vineyards in the south and olive groves in the south had evenness indices of 0.54 and 0.57, respectively. Regarding species abundance, *Pipistrellus pipistrellus* was the most abundant in vineyards in the south with 398 calls. This species was also the most common species in the northern vineyards, with 43 calls. Olive groves in the south had the highest abundance of *Pipistrellus kuhlii*, with 448 calls, while in the northern olive groves, *Pipistrellus pipistrellus* was the most abundant, with 160 calls.

Table 1. A summary table for each group of sites, with species richness, biodiversity, and evenness indexes as well as species count per site. Species codes can be checked in Annex 1 – Table 6.

	SPECIES	SHANNON	MARGALEF	PIELOU	EPTISASER	MINSCHPIPPYG	NYCLAS	NYCLEI	PIPKUH	PIPPIP	TADTEN	HYPSAV	PLE	RHIFER	MYODAU	MYOMYO	MYONAT
VINE SOUTH	12	1,35	1,71	0,54	13	42	29	25	60	398	52	1	5	1	4	1	0
VINE NORTH	7	1,42	1,20	0,73	2	5	4	29	1	43	40	0	0	0	0	0	0
OLIVE SOUTH	13	1,46	1,77	0,57	17	79	12	23	448	227	50	13	5	1	7	2	1
OLIVE NORTH	8	1,16	1,44	0,53	2	3	8	24	5	160	54	1	0	1	0	0	0

The rarefaction and extrapolation curves further support these observations (Figure 7). Regarding species richness, olive groves in the south reached higher values than other habitats, followed by vineyards in the south. Olive groves in the north and vineyards in the north had lower species richness. The Shannon index curves similarly showed that olive groves in the south and vineyards in the south had higher diversity indices, with vineyards in the north demonstrating moderate diversity and olive groves in the north having the lowest diversity. The rarefaction curves indicate that, as the number of sampling units increases, the species richness and diversity indices stabilise, reflecting the adequacy of the sampling effort in capturing the richness in these habitats. In this study, the extrapolation curve shows more completeness for the northern sampling than for the southern one. Conversely, the Shannon index shows more nuanced results, almost reaching completeness for all sites.
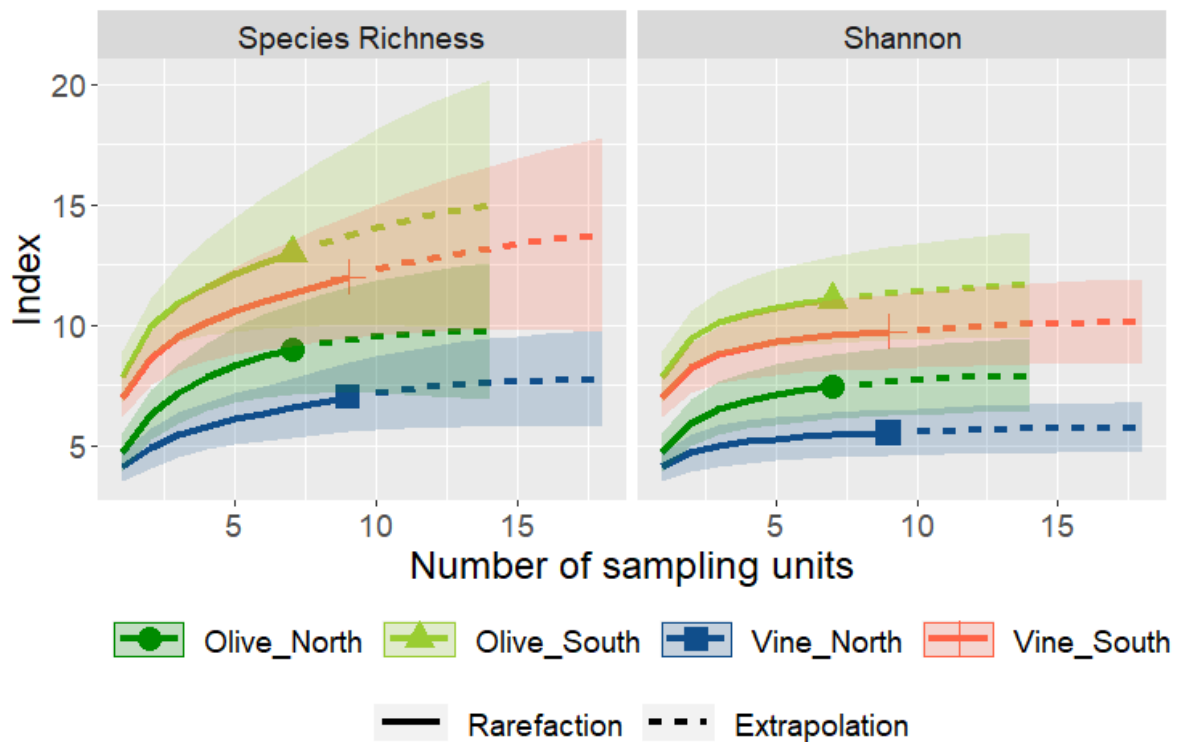
Figure 6. Rarefaction curve of the Audiomoth Bat's data. Each Audiomoth is considered a sampling unit, leading to nine sampling units per group.

The Non-metric Multi-Dimensional Scaling analysis performed to assess the resemblance between sampling sites using bat calls (Figure 8) revealed a clear separation between the North (on the left side of the graph) and South sites located on the right side.

This indicates that the primary differences between the sites are linked to location, with land use (olive groves or vineyards) having a minimal effect on species composition. The general trend shows a higher abundance (using a number of calls as a proxy) and richness at the southern sites, as indicated by the species clustering towards the right side of the graph. Species such as *Miniopterus schreibersii/Pipistrellus pygmaeus* and *Eptesicus isabellinus/serotinus*, located furthest to the right, show a greater preference for the southern sites. In contrast, species like *Tadarida teniotis*, which has a more central gradient, demonstrate that location does not significantly explain the differences in their abundance.

Figure 7. This is a plot of the Non-metric Multi-Dimensional Scaling. VS represents the 3 vineyard sites located in the south, VN represents viney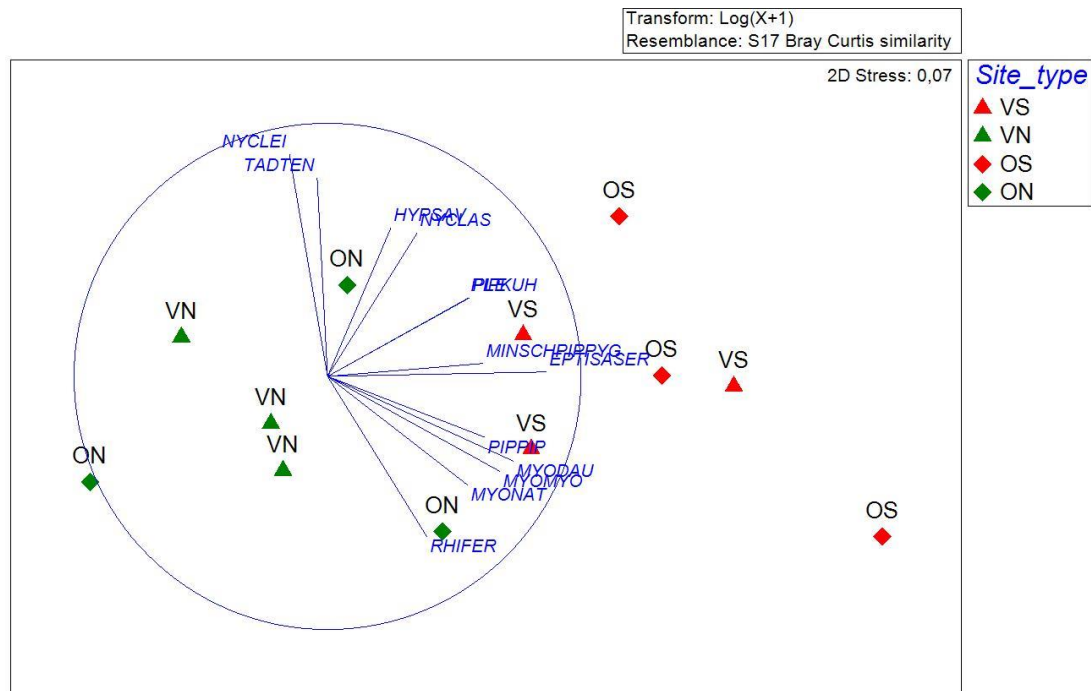ards located in the north, OS represents olive groves located in the south, and ON represents olive groves located in the north. The species gradients are represented in blue.

The two-factor PERMANOVA performed to detect potential differences between zones and crop types (and their interaction) just indicated significant differences between zones (North vs South) with crop types and the interaction between factors providing non-significant differences (Table 2)

Table 2. Summary of the results of the PERMANOVA analysis

FACTORS	PSEUDO-F	P-VALUE
ZONE	8.81	0.001
CROP TYPE	0.38	0.785
INTERACTION	0.24	0.924

The SIMPER analysis performed afterwards (to understand the most relevant species contributing to the dissimilarity between North and South locations) revealed a dissimilarity between zones of 42,03 % with the species *Pipistrelus kuhlii, Miniopterus schreibersii/Pipistrellus pygmaeus and Pipistrelus pipistrelus* being responsible for most of this dissimilarity (percentage of importance of 24,71%, 14,16% and 10,79%, respectively) and presenting a higher abundance on the southern zone.

When looking at the dissimilarity between Vineyards and Olives, a lower value was found (33,54%), but the species responsible for it were the same as those mentioned above: *Pipistrelus kuhlii* (20,68%), *Miniopterus schreibersii/Pipistrellus pygmaeus* (13,58%) and *Pipistrelus pipistrelus* (12,38%).

The results obtained when performing a partition of variation (using successive RDAs and *p*RDAs) indicated that the two factors analysed (Zone and Crop type) significantly explained 51.5% (F= 4.784; p=0.002). As expected, most of this variation (47.2%) was significantly explained by Zone (F= 8.771; p=0.002), whereas Crop type only explained a low percentage (4.3%), being non-significant (F= 0.798; p=0.628).

3.2 Arthropod Diversity and Abundance Using Pitfall and Suction Sampling Methods

Regarding the pitfall data, Coleoptera was the most common group except in the olive groves in the northern location, where Hymenoptera was dominant. In olive groves, Isopoda was much more prevalent compared to vineyards. Ground-dwelling arthropods were more abundant in the olive groves, with 97 individuals recorded at the northern sites and 96 at the southern sites, compared to 68 and 71 individuals in the northern and southern vineyard sites, respectively (Table 3). The suction data revealed a higher order richness than the pitfall data, with Diptera being the most dominant group, followed by Hemiptera and Hymenoptera. Overall, the data indicate a few dominant species and numerous rare orders.

Table 3. Results from the identification to order level of the pitfall and suction data.

|	ACARII	ARANEAE	BLATTOEDA	CHILOPODA	COLEMBOLA	COLEOPTERA	DIPLOPODA	DIPTERA	GASTROPODA	HEMIPTERA	HYMENOPTERA	ISOPODA	LARVAE	LEPIDOPTERA	LUBRICIDAE	NEUROPTERA	OPILIONES	ORTHOPTERA	TRICHOPTERA	TOTAL
OLIVE NORTH	0	11	0	8	0	26	0	0	0	0	39	11	2	0	0	0	0	0	0	97
VINEYARD NORTH	0	8	0	6	0	31	0	0	0	0	17	5	1	0	0	0	0	0	0	68
OLIVE SOUTH	0	8	1	3	0	66	2	0	1	0	2	12	0	0	1	0	0	0	0	96
VINEYARD SOUTH	0	9	0	4	0	45	1	0	1	0	2	4	1	0	0	0	0	4	0	71
SUCTION	2	24	1	0	10	30	0	724	0	129	98	0	5	21	0	2	2	3	3	1054
TOTAL	2	60	2	21	10	198	3	724	2	129	158	32	9	21	1	2	2	7	3	
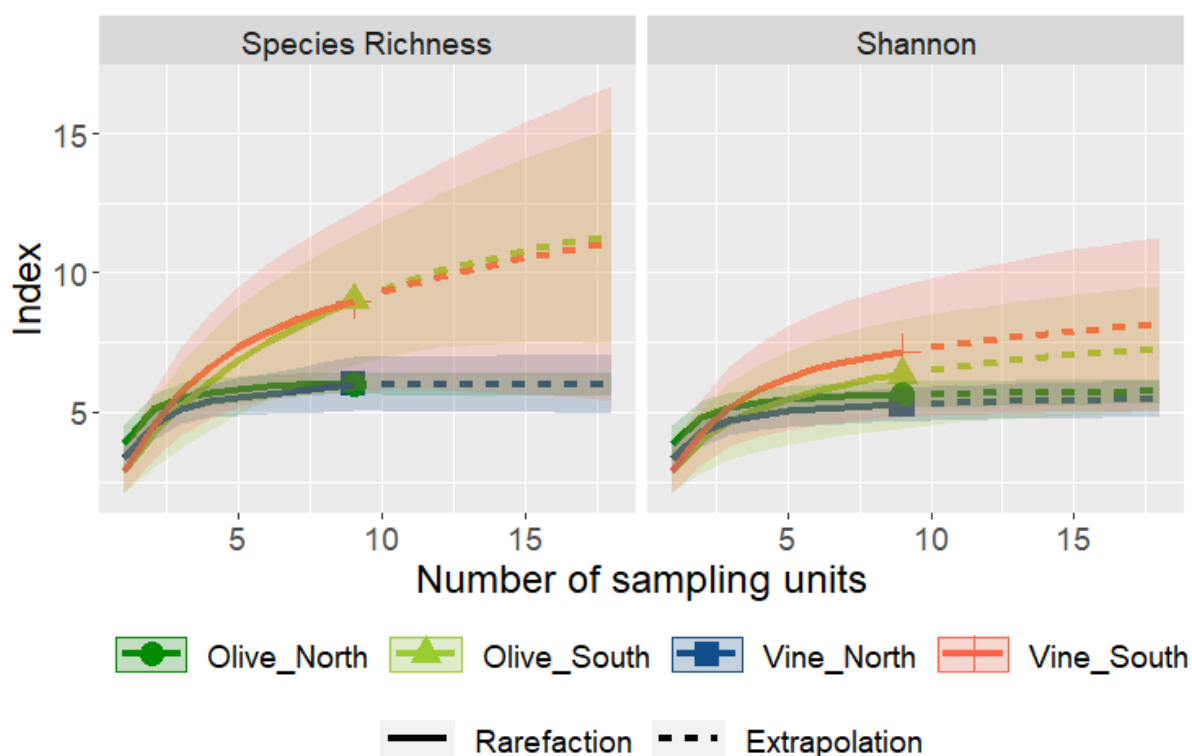
Figure 8, Rarefaction curve of the pitfall data, each pitfall is a sampling unit, leading to 9 sampling unit per group.

Species richness is equally high in both crop types in the southern zone, and similarly low on both northern crops. The extrapolation curve indicates completeness for the northern samples but suggests incompleteness for the southern samples. Conversely, the Shannon index shows more nuanced results, with the difference between north and south being less pronounced. This is because the Shannon index places less emphasis on rare species that are only present in a few pitfall samples.

3.3 Plant protection products applied and residue analysis

The application pattern of plant protection products (PPPs) was obtained through direct contact with local producers (Figure 10). As a result, there is a degree of uncertainty associated with this information. Additionally, it should be noted that detailed application data was only available for vineyards, with no corresponding information for olive groves.

A total of nine different products were applied before the study period. In the north, six different products were applied, but only two were detected on the arthropod samples (Table 4), while in the south, seven different products were applied, and 4 were detected. The herbicide Flazasulfuron was the only substance detected with a record of application. The fungicides Fosetyl-Al, Cymoxanil, Metalaxil-M and Trifloxystrobin were applied but not detected.

In the southern vineyards, the compound with the highest concentration detected was Flazasulfuron (herbicide), followed by the fungicides Azoxystrobin, Folpet (and its metabolite Phthalimide) and Dimethomorph. In comparison, lower Folpet values were detected in vineyards in the northern zone. Despite having similar concentrations of Folpet, Olive groves show a different (detected) PPP

composition, with the interesting reading of Fipronil, a European banned insecticide. Northern olives showed a low reading of Tebuconazole (fungicide) and Fipronil, while olives from the south showed a much higher reading of Fipronil (0.84 mg/kg) and Tebuconazole (Table 4). Except for Flazasulfuron and Fipronil, all the compounds detected were fungicides, expected due to the preventive applications against mildew. Despite detecting the arthropod samples, samples from the bat faeces showed no significant detection of PPPs (with a minimum limit detection of 0.01 mg/kg).
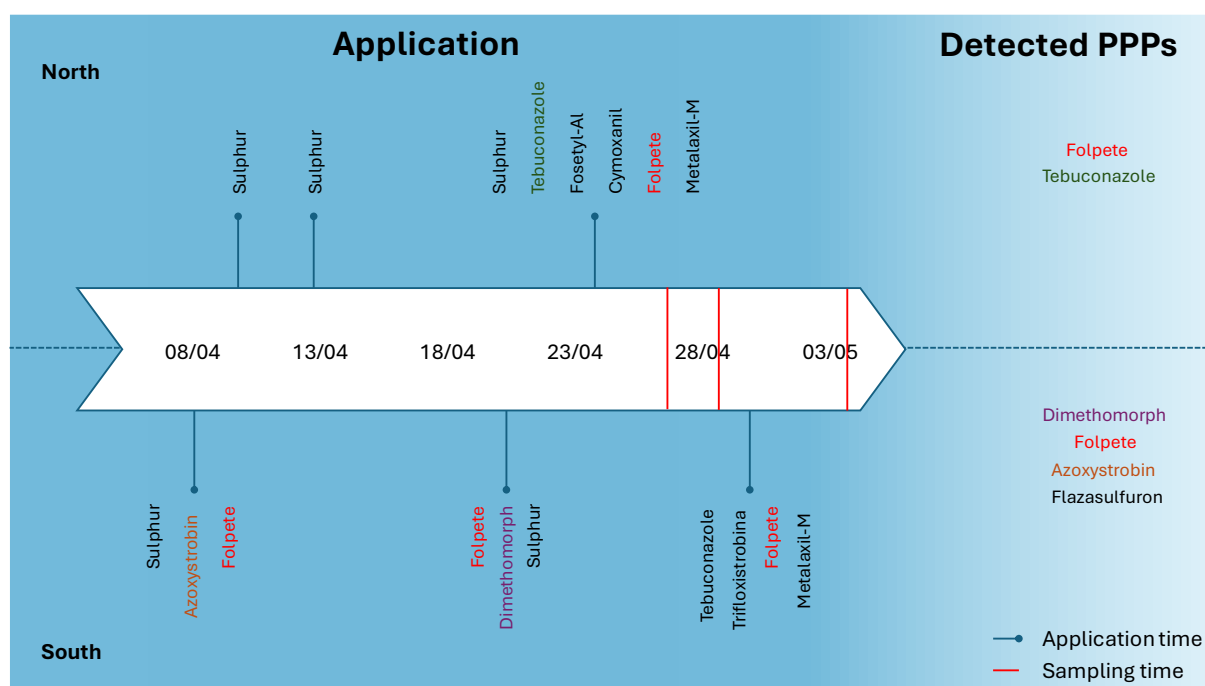
Figure 9. The timeframe of PPPs application in the North and South vineyards. The detected PPPs are on the right side. The timeframe of the application of PPPs is noted by the perpendicular blue lines; this information was given by the farmers. It must be noted that because each location (North, South) is comprised of three different farm plots, the application times when similar products were applied to different fields are noted.

Table 4. Summary table of the PPP detected through the chemical screening (in mg/kg).

	DIMETHOMORPH	FIPRONIL	FOLPET	PHTHALIMIDE	TEBUCONAZOL	AZOXYSTROBIN	FLAZASULFURON
OLIVE NORTH		0,018	0,073	0,036	0,049		
VINEYARD NORTH			0,053	0,026	0,015		
OLIVE SOUTH		0,84	0,085	0,042	0,49		
VINEYARD SOUTH	0,045		0,089	0,044		0,1	0,26
SUCTION		0,14		0,071		0,061	

Table 5, Summary table of the usage, half-life, mammal toxicity and EU status of the detected PPPs All of the information was taken from EFSA (European Food Safety Authority, 2024). Relevant to mention that the toxicity values do not take into account toxicity for bats.

	USAGE	DT50 (IN SOIL)	TOXICITY FOR MAMMALS	EU STATUS
DIMETHOMORPH	Fungicide	10 - 61 days	Oral LC50 > 3900 mg/kg bw, Dermal LC50 > 2000 mg/kg bw inhalation LC50 > 4,24 mg/L	Not approved (20/05/2024)
FIPRONIL	Insecticide	128 - 308 days	Not available, Banned for bee lethality	Not approved (2013)
FOLPET	Fungicide	0.2 -3.8 days	Oral LD50 > 2,000 mg/kg bw	Approved
TEBUCONAZOL	Fungicide	77 days	Oral LD50 > 1700 mg/kg bw Dermal LD50 > 5000 mg/kg bw Inhalation LC 50 > 371mg/m3	Approved
AZOXYSTROBIN	Fungicide	262 days	Oral LD50 >5000 mg/kg bw	Approved
FLAZASULFURON	Herbicide	3 - 22 days	Oral LD50 > 4694 mg/kg bw Dermal LD50 > 2000mg/kg bw Inhalation LC50 >6,17mg/kg bw	Approved

4. Discussion

Even though it is unclear if bats are protected from PPPs application by the existing environmental risk assessment (ERA) scheme, this is likely to change (Hernández-Jerez et al., 2019). So, it is of paramount importance when advancing with a bat ERA scheme to understand how bats use their habitat, what they eat and how they could be exposed to these compounds. In this line, this study positions itself as a first approach to understanding the habitat use by bats in this type of agricultural landscape, and to infer their potential exposure to PPPs.

4.1 Influence of Landscape Complexity on Bat Activity and Species Richness in Agricultural Areas

The presence of various bat species in agricultural landscapes, as observed in this study, aligns with findings from other regions and studies. For instance, *Pipistrellus pipistrellus* and *Pipistrellus kuhlii* are highly adaptable and commonly inhabit areas close to human activities, including agricultural landscapes. Their high abundance in vineyards and olive groves in this study is consistent with previous research indicating their preference for foraging in such environments due to the availability of insect prey attracted by crop vegetation and irrigation (Russo and Jones, 2003; Park, 2015). Additionally, detecting species such as *Hypsugo savii* and *Eptesicus isabellinus* in these habitats further supports their known habitat flexibility and adaptability to varied landscapes, including agricultural areas (Flaquer et al., 2009). The presence of *Miniopterus schreibersii*, although less frequent in agricultural settings, indicates that some individuals may still forage in these areas, possibly utilizing forest patches and hedgerows for roosting and commuting (Rodríguez-San Pedro et al., 2019).

The results underscore the significant impact of landscape complexity on bat activity in open agricultural areas. Bat activity and richness were higher in the most complex landscape, and the changes in landscape complexity accounted for 47.2% of the variation in bat activity and species richness. Previous studies have also highlighted the importance of landscape complexity in supporting bat diversity and activity. In fact, similar findings were observed by Tortosa et al. (2023), where bat species richness and total activity were higher in vineyards and pine plantations within diverse landscapes. Frey-Ehrenbold et al. (2013) demonstrated that heterogeneous landscapes with a mix of crop types and natural habitats provide better foraging opportunities and roosting sites for bats. Similarly, Lentini et al. (2012) found that bat activity and species richness were higher in agricultural areas with higher structural diversity, including the presence of hedgerows and forest patches. Furthermore, Froidevaux et al. (2019) and Tortosa et al. (2023) emphasised the importance of hedgerows, observing that bat activity decreases with increasing distance from the nearest hedgerow. They also highlighted the significance of hedgerow management, noting that an increase in bat diversity was observed when hedgerows were not trimmed for extended periods. An acoustic survey in central Chilean vineyards reported similar results, showing increased bat activity and species richness with a higher percentage of native vegetation cover, landscape composition accounted for more than 50% of the total variability in bat activity (Rodríguez-San Pedro et al., 2019).

However, it is important to note that the presence of bats in agricultural landscapes does not necessarily indicate optimal habitat conditions. Bats may be exposed to various threats, including pesticide exposure, which could have sublethal or chronic effects on their health and reproductive success. Further studies are needed to comprehensively assess these risks and implement conservation measures that enhance habitat quality and mitigate potential hazards in agricultural settings.

Similar results were found when analysing species richness of invertebrates across landscape gradients in the Mediterranean region. More heterogeneous landscapes exhibited higher biodiversity, whereas more homogeneous open areas showed lower diversity (Herrera et al., 2016). In the case of bats, the primary factor influencing activity and species richness appears to be larger-scale heterogeneity (Jiménez-Navarro et al., 2023).

The difference in community composition and activity between vineyards and olive groves was small, with the exploitation type explaining only 4.3 % of the total variance. Even though the community composition of bats may differ from olive groves and vineyards, it appears that it is not the main factor in explaining the variance in species composition in the study region. This could be attributed to the landscape of the study area. Vineyards predominate both in the north and in the south and they are typically interspersed with small plots of olives. The olive plots are likely too small and too close to the vineyards to observe a significant difference. The big difference between the North and South locations, as mentioned in section 2.1, is that the vineyard patches in the south were generally smaller than in the north, and they were interspersed with different natural/semi-natural structures and forest patches.

4.2 Assessment of PPP Exposure Risks for Bats in Agricultural Areas

No traces of PPPs were found in the faeces of *Miniopterus schreibersii*, this could be explained by the fact that the sampling was likely conducted too early for the bats to be very active. Additionally, *Miniopterus schreibersii* are known to not frequently forage in agricultural areas, also confirmed by the Audiomoth data (Vincent, et al., 2011).

However, other bat species might be at risk. For example, *Pipistrellus pipistrellus*, the most commonly recorded species, primarily feeds on Diptera (Barlow, 1997). Diptera was the most common order found in the suction samples, which contained PPPs with detected levels of Fipronil (0.14 mg/kg), Phthalimide (0.071 mg/kg), and Azoxystrobin (0.061 mg/kg). Even though direct exposure could not be proven due to the lack of blood or faecal analysis for this species, potential exposure must still be considered.

The presence of Fipronil is concerning, as it was banned from European agricultural use in 2013 (European Food Safety Authority, 2024) but is still being used in the region. The hypothesis of trace residues can be dismissed since Fipronil's half-life (DT$_{50}$ – Table 5) is less than a year. If the last application occurred in 2013, it is unlikely that traces would be detectable today, especially at levels that would expose insects (0.84 mg/kg in olive south).

When looking at the toxicity values of the detected products for mammals (Table 5), it is possible that toxicity via inhalation could be more hazardous (lower LC50 values) than oral or dermal toxicity.

Inhalation and dermal exposure are not so common unless PPP applications occur at dusk or night (in some areas, but not in this area), making oral exposure the most common. However, despite the very high LC50 values, we should not exclude other effects due to chronic exposure, especially at the sublethal level. So, further studies are necessary to assess the potential of exposure to PPs.

Further complicating the issue is the varied sensitivity within the same group of animal species to PPPs, which might be relevant for bats. Even within similar environments, different bat species may exhibit varied levels of bioaccumulation and sensitivity to toxins. For instance, a study by Bayat et al. (2014) demonstrated that insectivorous bats can accumulate significant levels of pesticides, impacting their immune response and overall health. This implies that *Pipistrellus pipistrellus* and other species frequenting agricultural areas could face sublethal effects that are not immediately apparent but could have long-term consequences on their populations. Therefore, comprehensive monitoring and targeted research are crucial to understanding the extent of PPP exposure and its impact on bat communities. This could involve more frequent and varied sampling times to capture a broader activity range of bats and employ more sensitive detection methods for PPP residues.

4.3 Recommendations for Further Research on Bat Exposure to Plant Protection Products

This study should be followed up with a larger project to better understand and assess the exposure of bats to plant protection products. A key limitation of this study is the lack of direct evidence of exposure, either through direct sampling of bat blood, tissue or through positive chemical analysis of their faeces. However, the only known roost close to the study area belonged to *Miniopterus schreibersii*, which, as confirmed by the results, is not very prevalent in agricultural areas. The setting up of bat boxes in the study area (in different habitat types) would allow different bat species to move in and use the surrounding area, making the sampling much easier. It would just require opening the box to have access to bats or faeces, without having to rely on the tedious task of catching bats in open area or find roosting sites. In the case of faeces collection, not only PPP residue analysis could be done, but the study of their diet, via metabarcoding, should be performed to assess the diet and to confirm that bats feed on agricultural areas and confirm that they feed on the contaminated prey. Moreover, with the use of bat boxes, bats could be sampled for blood or small membrane disks to be used for chemical residue or biological analysis. Membrane disk approach was proven to be effective in detecting metal bioaccumulation in bat, however its possible use on pesticide remains unknown (Mina et al., 2019)

It would be suggested to select an area in the Bairrada region with the installation of boxes in several areas along a gradient of landscape complexity. Schwegler Bat Box attached to them could be placed in the surrounding of vineyards and olive groves. In case of faeces sampling the procedure is minimally invasive for the bats, as those boxes can be open from the bottom with minimal disruption for the bat roosting on the top of it. These bat boxes need to be cleaned at least once per year, ensuring a minimum of one sampling per year. It should be noted that bats are much more active during the summer, which is likely the preferred sampling time for the study. Therefore, extra care should be taken to avoid

disturbing late-born pups and lactating females. The wooden pool could stay there for multiple years allowing for a continuous monitoring of bats, diet and their exposure. Implementing such project would allow long-term monitoring to understand the temporal patterns of PPP exposure and its possible effects on bat health and populations. Furthermore, as the effects of PPPs on bat health are mostly not known, I think that including behavioural studies, such as change in foraging pattern and reproductive success could be a relevant addition to the project. If such a project is successful, it would give powerful evidence that could help revise regulation on PPP usage in order to better protect bats.

5. Conclusion

To conclude, bat activity and species richness were closely linked to landscape complexity, with more complex landscapes exhibiting higher levels of both metrics. In this study, the type of agricultural exploitation—vineyard versus olive grove—had little impact on species composition and activity. Although no PPPs were detected in bat faeces, significant quantities were found in prey items, indicating that the possibility of exposure remains. To better assess bat exposure to PPPs, further work could include the use of bat boxes in agricultural areas and sampling later in summer.

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7. Annex I

Table 6. Table of the species codes with their respective scientific name and common name.

SPECIES CODE	SCIENTIFIC NAME	COMMON NAME
EPTISASER	*Eptesicus isabellinus/Eptesicus serotinus*	Meridional serotine/Common serotine
HYPSAV	*Hypsugo savii*	Savi's pipistrelle
MINSCHPIPPYG	*Miniopterus schreibersii/Pipistrellus pygmaeus*	Common bent-wing bat/Soprano pipistrelle
MYODAU	*Myotis daubentonii*	Daubenton's myotis
MYOMYO	*Myotis myotis*	Mouse-eared myotis
MYONAT	*Myotis nattereri*	Natterer's myotis
NYCLAS	*Nyctalus lasiopterus*	Giant noctule
NYCLEI	*Nyctalus leisleri*	Lesser noctule
PIPKUH	*Pipistrellus kuhlii*	Kuhl's pipistrelle
PIPPIP	*Pipistrellus pipistrellus*	Common pipistrelle
PLE	*Plecotus spp.*	Long-eared bat
RHIFER	*Rhinolophus ferrumequinum*	Greater horseshoe bat
TADTEN	*Tadarida teniotis*	European free-tailed bat