

**Universidade de Évora – Escola de Ciências e Tecnologia**

Mestrado em Biologia da Conservação

Dissertação

**Influence of landscape characteristics on the occupation of bat boxes in vineyards in the Alentejo region**

Sofia Moinhos Ferreira da Silva

Orientador(es) / João Tiago Marques

Isabel Alexandra Ramos

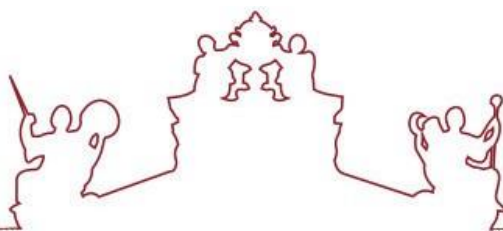
Évora 2024

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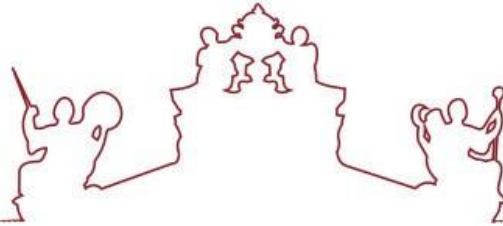
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A dissertação foi objeto de apreciação e discussão pública pelo seguinte júri nomeado pelo Diretor da Escola de Ciências e Tecnologia:

Presidente | Paulo Sá-Sousa (Universidade de Évora)

Vogais | João Tiago Marques (Universidade de Évora) (Orientador)

Rui Lourenço (Universidade de Évora) (Arguente)

## ACKNOWLEDGEMENTS

Quero agradecer com muito carinho:

À Professora Doutora Isabel Ramos, minha co-orientadora, quem fez a apresentação dos possíveis temas de dissertação de mestrado relacionados com as necessidades do Programa de Sustentabilidade dos Vinhos do Alentejo (PSVA), pela ajuda a fazer a ponte com os o PSVA e com os proprietários das herdades com caixas abrigo para morcegos instaladas ou com interesse na sua instalação junto às vinhas.

Ao Programa de Sustentabilidade dos Vinhos do Alentejo, especialmente ao Eng.º João Barroso, gestor do PSVA, pela sua disponibilidade para reunir comigo para esclarecimento de dúvidas e planeamento do contacto feito com os proprietários das herdades.

Aos proprietários e todos os colaboradores da Casa Relvas (Herdade da Pimenta e Herdade de São Miguel), da Herdade de Coelheiros, da Herdade do Esporão, da Herdade de Pinheiros (Fundação Eugénio de Almeida), da Herdade dos Lagos, da Herdade da Malhadinha Nova, e Herdade do Rocim por toda a ajuda no trabalho de campo (instalação e monitorização das caixas), pelo interesse em morcegos e nas caixas abrigo para morcegos e pela disponibilização de material (veículos, escadas etc.).

Ao professor Doutor Nuno de Sousa Neves por me ter ajudado a reativar a conta do ArcGis, algumas vezes!, e a resolver alguns obstáculos com o programa *ArcMap*® 10.3.1.

Ao professor Doutor Nuno Guiomar da Universidade de Évora, pela prontidão, paciência e eficácia com que me ajudou nos momentos mais difíceis da minha utilização com os Sistemas de Informação Geográfica.

Especialmente ao Professor Doutor Tiago Marques, pela incansável ajuda no delineamento da dissertação, no acompanhamento tanto nas saídas de campo, reuniões constantes, pela

motivação nas alturas mais difíceis, pelas inúmeras revisões de texto e pela amizade. Muito obrigada!

Aos meus amigos, que me ouviram, acompanharam e me motivaram nos piores momentos ao longo destes 2 anos.

A toda a equipa do Restaurante Tamuje, por me aturarem, por toda a amizade que me têm dado, ajuda e disponibilização de folgas para poder trabalhar na minha dissertação.

À Amália, ao Zé e toda a família Dias e Vedes por me acolherem e ajudarem em tudo o que eu preciso.

Ao Filipe Silva, por tudo! O meu melhor amigo, mesmo nos momentos mais difíceis que a vida traz. Obrigada pela paciência, motivação e ajuda, sem ti teria sido mais difícil terminar este meu desafio.

## "Influência das características da paisagem na ocupação de caixas de morcegos em vinhas na região do Alentejo"

### RESUMO

A conversão de habitats naturais em áreas de cultivo agrícola e silvícola tem sido a principal causa da perda de biodiversidade, tendo afetado mais de 50% da superfície terrestre. Entre as culturas frutícolas, a vinha ocupa a maior área cultivada em todo o mundo (sendo Portugal o 9º país com maior área), pelo que, ao afetar a qualidade do habitat, está mais suscetível à invasão por espécies exóticas. A traça europeia da videira é uma das pragas com mais impacto nas vinhas, sendo prática comum o controlo biológico através da montagem de caixas abrigo para morcegos com o objetivo de incentivar o estabelecimento de colónias de morcegos insectívoros. Apesar das características das caixas terem impacto na sua ocupação, a influência da paisagem tem sido pouco estudada. Neste estudo, pretendemos compreender que fatores (paisagísticos e características das caixas) são mais relevantes para a ocupação das caixas de morcegos em vinhas do Alentejo, Portugal. Um total de 64 caixas foram inspecionadas entre maio e agosto de 2021. As variáveis resposta foram modeladas com recurso à regressão logística. A ocupação das caixas foi menor nas caixas sem pintura (madeira) e pretas, e influenciada negativamente pela ocupação de outros animais. Ademais, o número de caixas vizinhas e a paisagem também pesaram na ocupação: proporção de água, área urbana, agricultura, floresta e distância média à floresta. Concluímos que tanto as características das caixas como as variáveis de paisagem influenciam a ocupação das caixas, no entanto, o fator determinante foi a ocupação por outros animais. Os resultados contribuem para o desenvolvimento de estratégias de gestão e de modelação que promovam a ocupação de caixas para morcegos em vinhas.

**Palavras-chave:** *Vitis vinifera L.*, *Lobesia botrana*, controlo biológico, aves, uso de solo

## “Influence of landscape characteristics on the occupation of bat boxes in vineyards in the Alentejo region”

### ABSTRACT

Land-use change has altered over 50% of the Earth's surface due to the conversion of wildlands to crops and timber plantations and is considered the leading cause of biodiversity loss. Among fruit crops, vineyards cover the largest cultivated area worldwide (Portugal being the 9<sup>th</sup> highest-ranking), and therefore, by affecting habitat quality, are more susceptible to species invasion. European grapevine moth is one of the most severe vineyard pests, which often results in stakeholders using biological control with bat box mounting to increase insectivorous bat populations. To improve bat box effectiveness, bat box traits and the surrounding landscape should be considered. However, there is a lack of information regarding landscape influence on bat box occupancy. In this study, I intend to understand which factors, both box traits and land cover (at three spatial scales), are more relevant to bat box occupancy in vineyards in Alentejo, Portugal. A total of 64 bat boxes were surveyed between May and August 2021 and box and landscape variables were evaluated using logistic regression modelling. Bat box occupancy was lower mainly in wooden and black boxes and influenced by other animal occupancy. Besides, the neighbouring boxes number and landscape variables also impacted bat box occupancy: proportion of water, urban area, agriculture, and average distance to forest patches. I conclude that both bat box traits and landscape characteristics influence box occupancy, but the crucial factor was bat box availability to bats. The results contribute to the development of management strategies that promote bat box occupancy in vineyards.

**Keywords:** *Vitis vinifera* L., *Lobesia botrana*, biological control, birds, land cover

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## 1. INTRODUCTION

The elevated and increasing numbers of the human population are causing profound changes in the global environment (Sage, 2020). Because human well-being depends directly on the availability of land for housing, industry, transportation networks, and the production of food and fibers (Sage, 2020), over 50% of the Earth's land cover has been directly altered through land-use change, largely via the conversion of natural habitats to cropland, pasture, and timber plantations (Foley *et al.*, 2005; Turner *et al.*, 2007; Hooke *et al.*, 2012). Thus, due to its very large scale, land cover change is considered the leading cause of biodiversity loss, even when compared to other often cited threats such as climate change, which, among other reasons, is caused by the elevated concentration of atmospheric CO<sub>2</sub> (Sala *et al.*, 2000; Vermaat *et al.*, 2017).

Biodiversity loss has a strong negative influence on ecological interactions, functions, structural complexity, co-dependencies, and mechanisms of resilience that characterize living systems and their ability to provide ecosystem services (ESs) (Díaz *et al.*, 2013; Dunn *et al.*, 2009; Sage, 2020; Valiente-Banuet *et al.*, 2015). The ESs are the benefits and resources that natural ecosystems and their ecological interactions provide to humanity (Ghanem & Voigt, 2012). The animal species adapted and occurring in agricultural landscapes provide several ESs such as pest control, pollination, seed dispersal, nutrient cycling, and resilience to environmental stressors (Bakış *et al.*, 2021; Garfinkel & Johnson, 2015; Whelan *et al.*, 2008; Williams *et al.*, 2018). However, the higher or lower animal population numbers in farmland, and the conservation of biodiversity, ecosystems, and goods and services they provide, will depend on how farmers manage agricultural landscapes (Fischer *et al.*, 2008; Phalan *et al.*, 2011; Viers *et al.*, 2013). Recently, several environmentally friendly farming practices have been applied, which enable agricultural production while preserving biodiversity and ecological processes, thus serving as alternatives to conventional agriculture (Kok *et al.*, 2018).

The most common practices are organic agriculture, conservation agriculture (Tittonell, 2014), agroforestry (van Noordwijk & Brussaard, 2014) and permaculture (Pretty, 2008).

One of the most relevant agricultural crops is the vineyard (*Vitis vinifera L.*), which covers, among fruit crops, the largest cultivated area, and the highest global revenues (International Organisation of Vine and Wine, 2021; Vivier & Pretorius, 2002). Vineyards cover a worldwide total surface area of 7.38 million ha, 3.3 million ha in the European Union, and 194 000 ha in Portugal. The area covered by vineyards in Portugal accounts for 2.7% of the total worldwide area, ranking the country as the 9<sup>th</sup> highest-ranking in terms of vineyard planted area (International Organisation of Vine and Wine, 2021). Besides land cover conversion and consequent biodiversity loss, vineyard installation has enduring effects on habitat quality and may interfere with freshwater elements (Hannah *et al.*, 2013). Usually, vineyard plantation involves the removal of native vegetation, followed by deep tillage (that affects the soil characteristics such as soil temperature, evapotranspiration, water infiltration and soil water conservation (Busari *et al.*, 2015)), and several annual applications of pesticides and fertilizers (Coll *et al.*, 2011; Coulouma *et al.*, 2006). One consequence of these impacts is that vineyards are more susceptible to imbalances in food chains, and therefore to invasion by alien species or species that were not previously present, since they have low habitat value (Hilty *et al.*, 2006; Hilty & Merenlender, 2004).

Among the many arthropod species that usually invade and can damage vineyards, causing significant yield losses, are four main species (Baroja *et al.*, 2019): the European grapevine moth (*Lobesia botrana*), the Grape berry moth (*Eupoecilia ambiguella*), the Leaf rolling tortrix (*Sparganothis pilleriana*) and the Spotted wing drosophila (*Drosophila suzukii*) (Ioriatti *et al.*, 2015). The most severe pest is the European grapevine moth, *Lobesia botrana* (Gonçalves *et al.*, 2013), that can have up to four generations per year (Harari *et al.*, 2007; Pavan *et al.*, 2014; Roditakis & Karandinos, 2001) and mostly affects central and southern

European countries (Altamira *et al.*, 2021). The larvae of *L. botrana* feed on grapevine inflorescences and green grapes (Pavan *et al.*, 2014), which increases its susceptibility to infectious diseases caused by phytopathogenic micro-organisms, resulting in higher damage severity on grapevines (Cozzi *et al.*, 2006). One of the most common practices to control insect pests is the spray of insecticides but they negatively affect environment and trophic chains (Geiger *et al.*, 2010), and their continuous use can lead to pest resistance to biochemical products (Boyer *et al.*, 2012). Thus, the scientific community and governmental agencies have been recommending the use of alternative, less invasive and more environmentally friendly methods of pest control (Cozzi *et al.*, 2006). Alternative techniques of pest management could include natural insecticides, biological control and integrated pest management strategies (Kogan, 1998; Thiery, 2011), like the use of insect pheromones (mating disruptors) (Ioriatti *et al.*, 2011), autocidal control (release of sterile males), and auxiliary macro organisms (parasitoids and predators) (Walter, 2003). Although grape moths control with auxiliary fauna can employ a large range of arthropod predators (spiders, harvestmen, true bugs, lacewings and syrphids) (Thiéry *et al.*, 2018), birds and bats are also major controllers of insect pests, as has been observed in several case studies (Maas *et al.*, 2013; Van Bael *et al.*, 2008).

Between all potential natural enemies contributing to limiting the development of agricultural pests, bats are frequently mentioned as efficient predators (Kunz *et al.*, 2011; McCracken *et al.*, 2012). Bats are flying mammals of the order Chiroptera, the second most diverse mammalian group (> 1350 species) with remarkable levels of abundance, morphological and ecological diversity (Kasso & Balakrishnan, 2013), and that exploit a great variety of habitats (occurring in all geographic regions apart from the poles (Russo *et al.*, 2018)), roosting structures, foraging techniques, and feeding habits (Patterson *et al.*, 2003). Foraging strategies differ both among and within bat species (Denzinger & Schnitzler, 2013) allowing for consumption of a broad variety of prey, ranging from insects (including several

arthropods harmful to human health or economic activities), nectar and fruit, to seeds, frogs, fish, small mammals, and even blood (Kunz *et al.*, 2011; Russo *et al.*, 2018). Their flying distance from roost to foraging areas can range from  $< 1$  km up to  $\geq 20$  km (Ghanem & Voigt, 2012; Marques *et al.*, 2004; Sahley *et al.*, 1993), serving as extreme example the *Tadarida brasiliensis* that can cover distances of more than 100 km each night, which is more than the normal annual movements of many species (Davis *et al.*, 1962; T. C. Williams *et al.*, 1973). Bats have long been postulated to play important ecological roles as prey and predator, in arthropod suppression, seed dispersal, pollination, material and nutrient distribution, and recycling (Kunz *et al.*, 2011), which make them, providers of all four categories of ESs (provisioning, regulating, supporting and cultural services), as listed by the Millennium Ecosystem Assessment (Ghanem & Voigt, 2012).

In Europe most bats are insectivorous (Puig-Montserrat *et al.*, 2015) and they have been considered important contributors to insect pest control in agricultural landscapes (Boyles *et al.*, 2011; Cleveland *et al.*, 2006; Ghanem & Voigt, 2012; Kunz *et al.*, 2011), including in vineyards (Aizpurua *et al.*, 2018; Baroja *et al.*, 2019; Russo *et al.*, 2018). Insectivorous bats are nocturnal and have a flight height of 5–10 m above the vines, which matches both the activity pattern and flying behaviour of *L. botrana* adult individuals (Thiéry *et al.*, 2018). Moreover, bats' wide flight range allows biological control to extend over several hectares. For example, *Pipistrellus pipistrellus*, a common and insectivorous microchiropteran in Europe, can fly every night almost 5 km (Racey & Swift 1985). Insectivorous bats can eat a large quantity of insects per night: individuals from the largest European bat species, *Nyctalus noctula*, consume, on average, 2.5g of insects each night (ranging from 0.5 to 8.2g) or 9% of their weight, while bats from the smallest European bat genus, *Pipistrellus spp.*, consume 0.4g, or 12% of body mass (ranging from 0.1 to 1.3g) (Moiseienko & Vlaschenko, 2021). Another bat biological trait that is an advantage for vineyard pest control is their long-life spans. For their body size, bats



live longer than any other mammal of similar body weight (Bourliere, 1958; Jürgens & Prothero, 1987; Wilkinson & South, 2002), which can be a long-term investment for farmers. One of the most prominent consequences of bat pest control is the insecticide cost reduction (Kunz *et al.*, 2011). Although it is difficult to quantify the monetary value of this ESs, Cleveland *et al.* (2006) estimated bat economic contribution in cotton agroecosystems in southern Texas (USA) to be worth of 12 to 173 dollars per hectare each year, whereas Boyles *et al.* (2011) assessed the overall economic bat contribution to USA agroecosystems of between 3.7 and 53 million dollars per year.

Despite their crucial role as ESs providers, bats are under severe threats (Ghanem & Voigt, 2012). According to the IUCN Red List (IUCN, 2020), nearly 16% of all bat species are classified as ‘threatened’. Bat populations are declining due to hunting for sport, meat, to control them as crop pests or capture for medicinal purposes, however bat populations are also suffering the negative impact from indirect poisoning due to excessive use of pesticides, loss and fragmentation of natural habitats, decrease in roost availability, deforestation, house renovations, and more recently, by emerging infectious diseases such as the White-nose syndrome (WNS) and by collisions with wind turbines (Ghanem & Voigt, 2012; O’Shea *et al.*, 2016). A significant drop in population size can put bat ES and their recovery at risk even when bats persist at low densities (Ghanem & Voigt, 2012), since most species produce only one or two offspring each year (Wilkinson & South, 2002). To prevent insectivorous bat species decline and to integrate them into pest management practices, the establishment of new populations must be promoted (Baroja *et al.*, 2019) with the help of multiple techniques: reduction of agricultural pesticides use and conversion to organic farming practices (Wickramasinghe *et al.*, 2003); development of disease mitigation strategies (e. g. WNS) (Ghanem & Voigt, 2012); natural roost protection initiatives and installation of artificial roosts (Alcalde *et al.*, 2017; Baroja *et al.*, 2019).

The installation of artificial roosts and bat boxes can be an important way of protecting bats, as they mimic natural crevices and cavities (Riccucci, 2014). Artificial structures can provide roosts that serve as nursery colony sites, hibernation sites or night roosts (Fenton, 1997). In the specific case of bat boxes, there are several designs, but those consist on two main typologies: flat boxes, that replicate naturally occurring crevices (Pschonny *et al.*, 2022; Rueegger, 2016), and voluminous boxes mimicking woodpecker-like nesting holes (Boye & Dietz, 2005). Regardless of the bat box typology, to improve its effectiveness (occupancy and bat mortality risk reduction), certain elements must be taken into account (Pschonny *et al.*, 2022), namely the internal temperature, which is influenced by several factors (i.e. bat box orientation, mounting, sun exposure, colour, design, construction material, and the number of occupants) (Fontaine *et al.*, 2021; Lourenço & Palmeirim, 2004). The boxes' effectiveness may also be impacted by the installation structure; pole-mounted boxes are usually occupied more quickly and with greater numbers than tree-mounted boxes (Flaquer *et al.*, 2006; White, 2004) that may lack sun exposure, usually have obstructed flight path to the entrance due to vegetation, or may be less readily found by bats (Flaquer *et al.*, 2006; Ruczyński *et al.*, 2011). Bat boxes should be also deployed in clusters to facilitate roost switching, which is important to avoid parasites or predators (Ruczyński & Bogdanowicz, 2008; Russo *et al.*, 2005), social behaviour and to allow local selection of boxes comprising favourable conditions (Rueegger, 2016). Rueegger (2016) suggested that box densities should range from two to eight boxes per 10 ha, and Pschonny *et al.* (2022) recommend installing box groups including flat, colony and voluminous boxes with small entrances, and a combination of bird and bat boxes to offer enough roost variety for the preferences of different bat species. Another important attribute is the height at which they are installed, since the higher they are, the greater is the occupation frequency (Ruczyński & Bogdanowicz, 2005). Agnelli *et al.* (2011) and Ruczyński & Bogdanowicz (2005) reported that bats preferred boxes at mounting heights higher than 4 m

and natural cavities between 8 and 30 m, as these heights offer safer roosts. Bat-box occupancy rates are also influenced by bat box orientation, with south or east-facing boxes being ideal in a northern temperate regions (Long *et al.*, 2006), and by box age as they take time to become occupied (Agnelli *et al.*, 2011; Chambers *et al.*, 2015; Griffiths *et al.*, 2017).

In addition to the box attributes, it has been suggested that, to increase bat box occupancy, both box and its installation characteristics should be adjusted to the surrounding landscape (López-Baucells *et al.*, 2017; Rueegger, 2017). However, most studies only consider bat box traits (Kerth *et al.*, 2001) and there is a remarkable lack of specific information regarding the influence of the surrounding landscape composition on box occupancy (Boughey *et al.*, 2011; Rueegger, 2016) despite the existence of some research. In Netherlands, Limpens & Kapteyn (1991) have indicated that most bat species, in their summer habitat, prefer to move in linear landscape elements such as hedgerows, tree lanes, wood edges, canals, etc. instead of crossing open areas. López-Baucells *et al.* (2017) evaluated how surrounding landscape structure and composition affect bat box occupancy in Barcelona Provincial Council's network of natural parks and provided strong evidence to suggest that landscape composition should be considered when using bat boxes for conservation to increase their success. Their data showed that forest cover has a positive effect on bat-box occupation rates, especially for tree-dwelling bats, while urban cover tends to have a negative impact (López-Baucells *et al.*, 2017). Contrarily, it has also been shown that distance to small urban areas is an important parameter for some species like *M. schreibersii*, which favoured areas close to small villages probably to exploit insect swarms that concentrate around streetlamps (Rainho & Palmeirim, 2011). Medinas *et al.* (2012) assessed the role of landscape in bat road kills in southern Portugal and observed a positive relationship between bat presence and dense *montados* (agro-silvo-pastoral systems), proximity to streams with riparian gallery and water reservoirs, as they provide shelter, high availability of arthropods, serve as riparian corridors and are a water source, a

scarcity resource in the region.

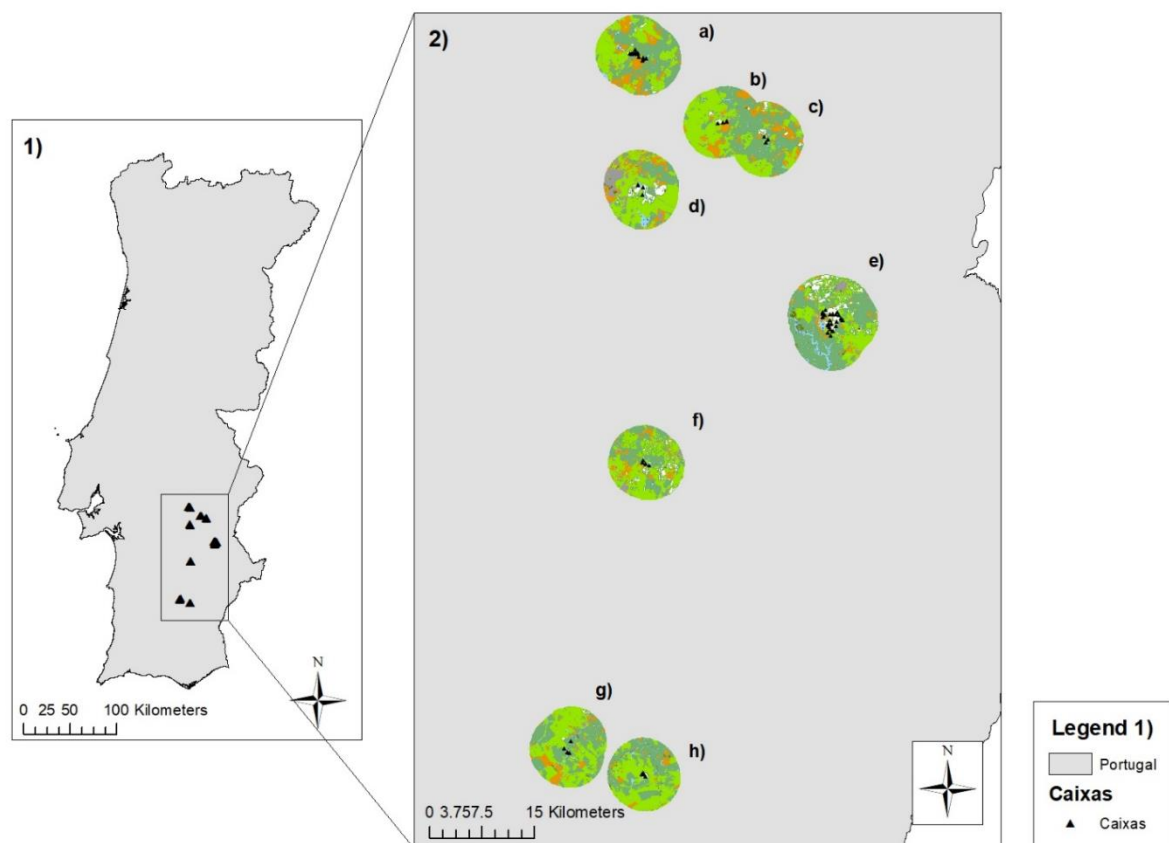
The presence and activity of bats are known to be influenced by land cover, although, due to the scarcity of studies in agroecosystems, it is important to study the influence of different landscape variables in different climates to improve the efficacy of bat conservation strategies around the world, such as the installation of artificial roosts.

In this study I intend to understand which factors, both landscape and box-specific characteristics, are more relevant for bat box occupancy in vineyards in the Alentejo region, Portugal. I investigated box occupancy as a function of land cover at a landscape level and bat box attributes. Therefore, the general objectives of the present study are: (1) To investigate which land cover variables influence the occupancy of bat-boxes installed in vineyards in Alentejo region; (2) To understand whether landscape variables or box trait variables are more important to bat box occupation. The tested hypotheses are the following: (1) The landscape variables will be more important in bat box occupancy than the bat box variables; (2) Bat box occupancy is greater in vineyards where the surrounding land cover has a greater proportion and/or shorter distances to water and forest areas; (3) Boxes with a higher density of neighbouring boxes are occupied more frequently.

## 2. MATERIALS AND METHODS

### 2.1. STUDY AREA

The present study was conducted on eight farms located in Alentejo, six in Évora (Lat.: 38°34'N; Lon.: 07°54'W), and two in Beja district (Lat.: 38°01'N; Lon.: 07°52'W), southern Portugal (**Figure 1**). Alentejo region has a typical Mediterranean-continental Climate (IPMA, 2022a) which, according to the Köppen classification, is characterized by hot, dry summers and cool, wet winters (Britannica, 2020). The average daily air temperature is around 16.5°C, with minimums reaching -3.2°C in the winter and maximums of 45.4 °C in summer (IPMA, 2022c, 2022b). The average total precipitation amount is 570 mm (IPMA, 2022c, 2022b) where the lowest values are observed in the Guadiana Valley (<500 mm) (CIMAC, 2017). Central



**Figure 1** 1) Portugal map and study area. 2) States and box location with buffers around: a) Herdade Coelhoiros; b) Herdade Pimenta; c) Herdade São Miguel; d) Fundação EA; e) Herdade do Esporão; f) Herdade do Rocim; g) Herdade da Malhadinha Nova; and h) Herdade dos Lagos.

and Southern Alentejo regions are dominated by plains with small hills, with an average altitude of 304 m, ranging from 24 m to 653 m (INE, 2013). The landscape is composed by agriculture (64%), agroforestry systems dominated by cork and holm oaks – (“Montado”, 22%), urban areas (6%), pastureland (4%), water surface (3%) and shrubland (1%) (INE, 2020). “Montado” refers to an agro-silvo-pastoral system that consists of tree stands of evergreen cork (*Quercus suber*) and/or holm (*Q. rotundifolia*) oaks intermixed with extensive agricultural areas (Pinto-Correia *et al.*, 2011). The less-represented land cover types include meadows, pastures and fallows, eucalyptus plantations, vineyards, pine groves, shrubs, and urban areas (Medinas *et al.*, 2012).

The vineyards selected for this study were chosen from a subset of vine farms participating in a partnership between the University of Évora and Alentejo Wines Sustainability Program (PSVA), to enhance bat presence in vineyards and, in turn, to improve natural pest control. The eight vineyard farms used for this study are: Casa Relvas with two areas (Herdade da Pimenta and Herdade de São Miguel), Herdade de Coelheiros, Herdade do Esporão, Herdade de Pinheiros (Fundação Eugénio de Almeida), Herdade dos Lagos, Herdade da Malhadinha Nova, and Herdade do Rocim. Vineyard sizes vary between 25 ha and 702 ha, with a mean of 152 ha per farm.

The farms have different numbers of bat boxes installed, varying between four, in five vineyards, and 42, in Herdade do Esporão. The longest distance between farms was approximately 105 km. Most of the studied vineyards are surrounded by “Montado” landscape, and some have different crops such as olive groves and/or walnut trees.

## 2.2. FIELD METHODS AND DATA COLLECTION

I surveyed for bat occupancy a total of 79 bat boxes that were installed between 2010 and 2021. The most recently installed boxes (in Herdade de São Miguel and Herdade da Pimenta) had 120 days since installation ( $n = 7$ ), the oldest had 3892 days of installation ( $n =$

9), and the average bat-box age was 1158 days. The installed bat boxes are of three typologies (Schwegler 2FN (n = 39); Schwegler 2F (n = 12) and; wood made (n = 28)) and colours (white (n = 47); black (n = 21); wood (n = 7); and four with no data due to survey problems) (**Figure 2**). Most of the boxes were installed on wood poles (n = 59) and some on trees (n = 20), like *Eucalyptus sp.* (n=6), *Pinus sp.* (n=1), *Populus sp.* (n=7) and *Quercus ilex* (n=6). The boxes installed on trees were located below the canopy to avoid branches interfering with bat flight behaviour. The boxes were installed at a height between 2.9 m and 5.7 m, with an average of 4.5 m (n = 32). All boxes were approximately oriented south or southeast.



**Figure 2** Box typologies: 15.6% Schwegler 2F (left); 59.4% Schwegler 2FN (center); 25% wooden box (right). The sample (n = 64) consisted of 9.4% black and 6.2% white Schwegler 2F boxes, 20.3% black and 39.1% white Schwegler 2FN boxes, and 14.1% white and 10.9% unpainted wooden boxes.

Bat-box surveying was performed once in each vineyard between May and August 2021, during breeding season. On each box survey I recorded the following data: presence or absence of bats; presence or absence of bat traces (stools); bat species identification, when present; number of individuals; and the presence of other animals. A box was considered occupied when a bat was present or when indirect evidence of bat box use was obtained (the accumulation of droppings since the last box cleaning happened around a year before). When

bat was present, a pipe inspection camera was used to photograph the bats, in order to identify the species and count the number of individuals. However, the number of individuals and bat species identification was not considered as response variables in the statistical analyses, due to its uncertainty, being only considered the presence or traces of bats (1/0).

During bat-box surveying, although the number of boxes was higher ( $n = 79$ ), it was only possible to sample 75 boxes, since four boxes were not found ( $n = 1$ ) or unreachable ( $n = 3$ ) due to overgrown vegetation. Moreover, due to the pair installation of 22 boxes of the same typology and colour on the same pole, that I pooled as a single observation during data collection (the sum of both box observations), the data set size was reduced from 75 to 64 observation.

### 2.3. HABITAT MEASUREMENTS

To analyze the relationship between bat box occupancy and box traits with the surrounding landscape, two land cover data maps were used: Land cover and Occupancy Map (COS2018) produced by DGT (Direção-Geral do Território, 2020) and the “Sub-parcelário IFAP” (iSIP, 2021).

The first stage of the landscape variables extraction was the preparation of a land cover base map. As verified by field observations, the COS2018 map does not represent all water surface areas and, it was necessary to complement it with the support of the IFAP map. Thus, water surface polygons from both maps (COS2018 and IFAP) were selected, extracted, and merged resulting in a layer representing exclusively water surface land cover. The resulting layer was then merged with the initial COS2018 map to get a more detailed map. To assess the relationship between surrounding land cover and bat box occupancy, land cover was analyzed at three different spatial scales. So, three concentric buffers were defined around each bat box location to characterize the local landscape (0.5 km and 1 km) and the broader landscape (5 km). The buffer distances were defined as an approximation of the reported foraging areas for



the Kuhl's bat (*Pipistrellus kuhlii* s.l.), the only species observed during data collection, which, under natural conditions, is thought to have a maximum movement range of about 5 km (Hukov *et al.*, 2020). The COS2018 and IFAP land cover feature classes were reclassified into simpler categories (**Table 1**), to reduce the number of variables and simplify *ArcMap*® 10.3.1 habitat measurements, while maintaining the categories relevant to the present research. As such, the land cover present in the bat box surroundings for all buffers are a) Urban areas; b) Vineyards; c) Other crops/Agriculture; d) Pastures; e) Forests; f) Scrublands; g) Open spaces and h) Water surface (**Table 1**). The extracted variables for each type of land cover present in the buffers were: area; proportion; edge length (perimeter); minimum distance from the box; average distance from the box; and number of nearby bat boxes. The measurements extracted for each land cover were chosen based on previous studies that emphasize the importance of these variables in bat box occupancy (area; proportion; edge length; minimum and average distance from the box and number of nearby boxes). The final phase of the landscape variables extraction was to summarize the values extracted for each polygon according to its land cover classification, which resulted in three tables (one per distance buffer), two with 42 variables (for 0.5 km and 1km buffer tables) and one with 47 variables (for the 5 km buffer table), since it included 5 measurements for an additional land cover (shrubland). All spatial analyses were carried out using *ArcMap*® 10.3.1 Geographic Information System Software (Esri, 2015).

#### 2.4. STATISTICAL ANALYSIS

To understand the influence of landscape composition and box traits on bat-box occupation, the statistical analyses were performed using *RSutio Software* (R version 4.2.3 (2023-03-15 ucrt) - "Shortstop Beagle").

In a first phase I did an exploratory data analysis (EDA). In data cleaning and reparation, adjustments were made to the variables, such as pooling the bat presence ("Presenca") with the bat traces variable ("Vest\_morc") into a single variable ("pres\_tot" as

**Table 1** Dissolved land cover categories and description.

Land Use	Dissolved land use categories	Description
Urban areas	Building environment	Area of land devoted to human use. This category includes building environments, commercial, industrial, and touristic zones, infrastructure, road and rail network, service zones, gardens, and equipment.
	Industry, commerce and agricultural facilities	
	Infrastructures	
	Transports	
	Inert material extraction, waste disposal and construction sites	
	Equipment	
Agriculture	Parks and gardens	Agricultural land that is used for annual and perennial crops, protected agriculture, and nurseries.
	Temporary crops (rainfed, irrigated and rice paddies)	
	Permanent crops (olive groves and orchards)	
	Heterogeneous agricultural areas	
Vineyards	Protected agriculture and nurseries	Areas covered in vines for both table and wine grapes. All vineyard plots that have at least 50% of their area taken up by vines fall under this category. Contains regions where vines predominate over other types of perennial crops like orchards and olive groves.
	Permanent crops (Vineyards)	
Pastureland	Pastures	Areas with or without human intervention covered in vegetation mainly of the herbaceous type, whether cultivated (sown) or natural (spontaneous), that occupy more than or equal to 25% of the surface and are not part of a farm rotation system.
Forest	Agroforestry areas	The consociation (vertical association on the same plot) of temporary crops, pastures (improved or poor spontaneous), and/or permanent crops with a degree of cover greater than or equal to 10%. Includes a variety of trees, including Cork oak, Holm oak, other oaks, stone pine, other species, Cork oak mixed with Holm oak, and other mixtures.
	Hardwood forests	Angiosperm tree species dominance
	Coniferous forests	Gymnospermous tree species dominance
Scrublands	Scrublands	Natural areas of spontaneous vegetation, either sparse or very dense, with a shrub cover of at least 25%, such as gorse, heather, or brambles. Includes abandoned olive groves if less than 45 trees per ha.
Open spaces	Bare rock	Natural areas with minimal or no vegetation, including bare rock, beaches and sandy areas and sparse vegetation where the surface area of shrub and herbaceous vegetation is less than 25%.
	Sparse vegetation	
Water	Wetlands	Inland or coastal areas, temporarily or permanently covered by fresh, salt or brackish, flowing or stagnant water, which include saltmarshes, reed beds, halophytic reed beds and intertidal zones.
	Surface water bodies	Freshwater surfaces that include natural, heavily modified and artificial watercourses and flats; saltwater surfaces that include oceans, and/or brackish water surfaces that include coastal lagoons and river mouths.
	Landscape Element: Riparian Galleries (IFAP)	Linear formation of woody tree species associated with watercourse edges, which can coexist with woody shrub species.
	Linear Element: Water Line (IFAP)	Temporary or permanent watercourse that allows surface water to flow within the same catchment area.
	Water bodies (IFAP)	Areas affected by natural and artificial watercourses, including dams, ponds and irrigation canals or pipes and watercourses.
	Wetlands (IFAP)	These include apaulic zones (reed beds), peat bogs, salt marshes, lagoon or riverine protection areas and coastal and estuarine intertidal zones.
	Riparian Galleries in Forest Areas (IFAP)	Formation of native woody tree or shrub species, long and narrow, along watercourse margins. The riparian gallery must have a minimum surface area of 0.1 hectares, a minimum length of 25 metres and a width of between 5 and 12 metres from the edge of the water line.

the response variable), days of bat box installation (“ndias\_inst”) log transformation, factorizing categorical variables, and eliminating irrelevant variables. The percentage of missing data (NAs) of each explanatory variable was calculated and the variables with NAs > 70% were excluded from further analyses to reduce risk of erroneous conclusions (for the 0.5km and 1 km buffer: shrubland proportion (prop\_mat), edge (orlm\_mat), minimum (distmin\_mat) and average distance (distave\_mat); and for 5km buffer: open land proportion (prop\_esp\_desc), edge (orlm\_esp\_desc), minimum (distmin\_esp\_desc) and average distance (distave\_esp\_desc).

After, a correlation analysis was calculated with a correlation matrix of all candidates’ numerical explanatory variables to check for variable collinearity. For each pair of independent variables showing high collinearity ( $|r| > 0,7$ ) (Dormann *et al.*, 2013), only the most biologically meaningful variables was retained for further analysis. The area (“Area\_”), proportion (“prop\_”), edge (“orlm\_”), minimum distance (“distmin\_”) and average distance (“distave\_”) of all land cover types were highly correlated, resulting in the elimination of the area, edge, and minimum distance variables while the proportion and average distance variables were preserved for future analyses. The minimum distance (“distmin\_”) variables were eliminated because the values were the same for all three buffers, and because the average distance better represents the different distances of the polygons to the box in the different buffers. This resulted in a decrease in the number of predictor variables from 42 to 18 (0.5 km and 1 km buffer) and 47 to 20 (5 km buffer) (**Table 2**). The visualization of the data was done with histograms and boxplots to help identify patterns and relationships in the data and provide insights into potential data quality issues.

In a second phase, to model the relationship between the response variable “presence of bats” (sighting or indirect evidence of bat occupancy) and the predictor variables, I applied a generalized linear mixed effect model (GLMM). I used *glm* with the R-packages *glmulti*

(Calcagno & de Mazancourt, 2010) to model selection, and *lme4* (Bates *et al.*, 2015) with the *glmer* function, to fit and analyze the model. Initially, since bat boxes were grouped spatially by vineyard estate, the “Herdade” was used as a random variable effect. However, while running *glmulti* function with all variables, besides the presence of the warning “*boundary (singular) fit: see help ('isSingular')*”, which generally indicates that one or more predictors in the model are linearly dependent on other predictors and that may be an infinite number of solutions that fit the data, the number of observations contemplated in the candidate models was lower than the observed in reality, which indicates that the observations were eliminated due to the presence of NAs.

To circumvent these problems, I first removed the variables with missing values (**Table 2**) and then, replaced the GLMM fitting function by a multiple binomial logistic regression, only with main effects, with the function *glm* (Dobson, 2002) from *stats* package (R Core Team, 2023).

To select the best candidate model, a null model was initially generated for comparison with the created models. Then, since box traits have an influence on bat box occupancy by bats, a *glmulti* function was run only with the box characteristic variables (Colour – “Cor”; type – “Tipologia”; days since of installation – “Ndias\_inst”; and presence or traces of other animals – “Vest\_faun”). The colour and box type variables were run separately with the other box traits variables because, despite not being correlated (p-value = 5.68e-06 of Fisher's Exact Test for Count Data), I think they should have been counted as a single variable to simplify the data and the statistical analysis. Separately, the *glmulti* function was run only with landscape-related variables (urban area proportion - “prop\_ter\_art”; agriculture proportion - “prop\_agr”; vineyard proportion - “prop\_vin”; pastureland proportion - “prop\_pas”; forest proportion - “prop\_flo”; water proportion - “prop\_ag”; and shrubland proportion (just for 5km buffer) – “prop\_mat”). The choice of the best candidate models was made by ranking the models using Akaike's

**Table 2** Description and summary statistics of explanatory variables of the three buffers. The variables with missing values > 0 were not included in the statistical analysis. The table was obtained with the resource of the *vtable* R package.

Variable (unit)	Description	Missing values			Range (min - max)			Mean		
		500m	1000m	5000m	500m	1000m	5000m	500m	1000m	5000m
Tipologia	Box type	0.00	0.00	0.00	-	-	-	-	-	-
Cor (categoric)	Box color	0.00	0.00	0.00	-	-	-	-	-	-
Durac_inst (days)	Days since installation	0.00	0.00	0.00	120 - 3892	120 - 3892	120 - 3892	1158.28	1158.28	1158.28
ncaix_buf (numeric)	Number of neighboring boxes	0.00	0.00	0.00	0 - 6	0 - 10	2 - 28	1.69	4.83	15.69
dias_inst (days)	Days of installation (log)	0.00	0.00	0.00	4.79 - 8.27	4.79 - 8.27	4.79 - 8.27	6.62	6.62	6.62
Vest_faun (binary)	Other animal presence or traces	0.00	0.00	0.00	0; 1	0; 1	0; 1	0.55	0.55	0.55
prop_ter_art (proportion)	Urban area proportion	0.00	0.00	0.00	0 - 0.08	0 - 0.05	0 - 0.14	0.01	0.01	0.02
prop_vin (proportion)	Vineyard proportion	0.00	0.00	0.00	0 - 0.94	0 - 0.88	0 - 0.26	0.37	0.30	0.10
prop_agr (proportion)	Agriculture proportion	0.00	0.00	0.00	0 - 0.60	0.01 - 0.72	0.19 - 0.53	0.22	0.23	0.32
prop_past (proportion)	Pasture land proportion	0.00	0.00	0.00	0 - 0.51	0 - 0.41	0.05 - 0.20	0.11	0.12	0.10
prop_flo (proportion)	Forest proportion	0.00	0.00	0.00	0 - 0.90	0 - 0.75	0.17 - 0.60	0.24	0.29	0.41
prop_mat (proportion)	Shrubland proportion	0.00	0.00	0.00	-	-	0 - 0.02	-	-	0.01
prop_ag (proportion)	Water proportion'	0.00	0.00	0.00	0.003 - 0.26	0 - 0.22	0.01 - 0.08	0.05	0.05	0.04
distave_ter_art (m)	Urban area average distance	34.00	11.00	0.00	0.58 - 480.67	184.70 - 994.86	2227.06 - 3888.57	323.81	615.34	3375.68
distave_vin (m)	Vineyards average distance	2.00	0.00	0.00	0 - 479.46	0 - 984.56	75.41 - 3630.37	93.85	264.26	2688.66
distave_agr (m)	Agriculture average distance	7.00	0.00	0.00	3.67 - 464.85	24.32 - 854.19	2331.92 - 3851.04	211.82	500.21	3311.22
distave_past (m)	Pasture land average distance	17.00	4.00	0.00	0 - 487.51	193.04 - 955.50	2717.65 - 3743.12	220.44	561.69	3086.05
distave_flo (m)	Forest average distance	9.00	0.00	0.00	86.34 - 447.06	193.25 - 904.74	2809.91 - 3572.19	241.88	572.83	3199.11
distave_mat (m)	Shrubland average distance	0.00	0.00	21.00	-	-	3126.61 - 4288.63	-	-	3613.32
distave_ag (m)	Water average distance	0.00	0.00	0.00	78.26 - 437.88	301.47 - 757.54	0 - 3716.64	257.87	600.72	3316.37

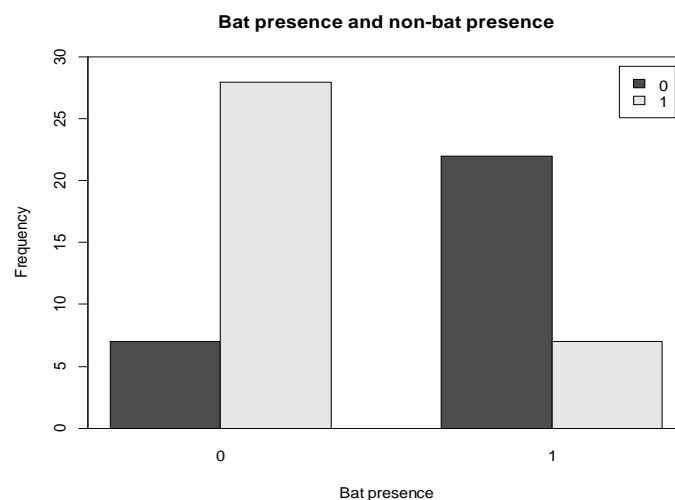
Information Criterion (AICc), that is particularly suited for small sample sizes (Akaike, 1974), to identify the top model ( $\Delta AICc > 2$ ) (Burnham & Anderson, 2002) and by assessing the goodness of fit of the competing statistical models with the resort of the Likelihood-ratio test with a one-way ANOVA. The resulting models were analyzed for the presence of collinear variables using the variance inflation factor (VIF) of each model.

Finally, the best candidate models resulting from the two analyses were compared with each other and the variables from the two best-fitting models from each analysis was added into a third analysis that included the most relevant landscape and bat box variables. The same model selection as described above was applied to the resulting candidate models to choose the final best model.

### 3. RESULTS

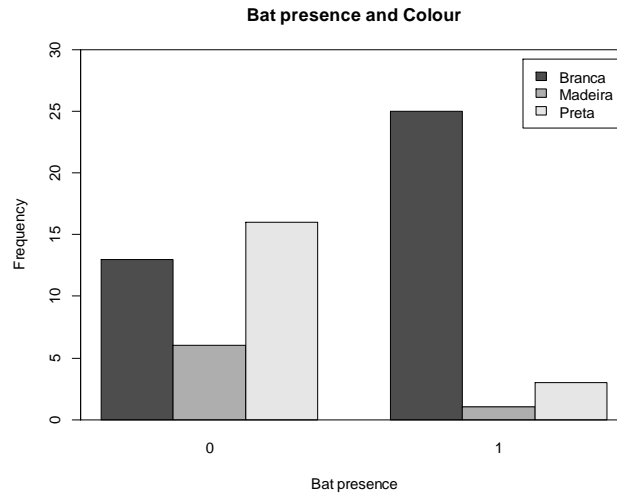
#### 3.1. BAT BOX OCCUPANCY

A total of 45.3 % bat boxes (n = 29 of 64) were occupied by bats during the study period (**Figure 3**): 16 bat boxes (55.2 %) hosted more than one bat – between two and 15 individuals -, three boxes had only one bat (10.3%), and in 10 boxes I only found bat traces (34.5%). In the only occasion that I was able to capture bats from the boxes, the only bat species found was the Kuhl's pipistrelle (*Pipistrellus kuhlii*), but bats from other *Pipistrellus* species were very likely present (*Pipistrellus pygmaeus* and/or *Pipistrellus pipistrellus*). Of the 64 bat boxes, 7 were empty (10.9%) and 35 (54.7%) were occupied by other fauna groups (**Figure 3**): 88.6% were birds (n = 31), but also Hymenoptera, like wasps (n = 2; 5.7%) or ant nests (n = 1; 2.9%), and other unidentified insects (n = 1; 2.9%). I detected seven boxes that had both bat presence and the presence of other fauna, mostly unoccupied bird nests.



**Figure 3** Bat presence vs. other fauna presence (“non-bat presence”)

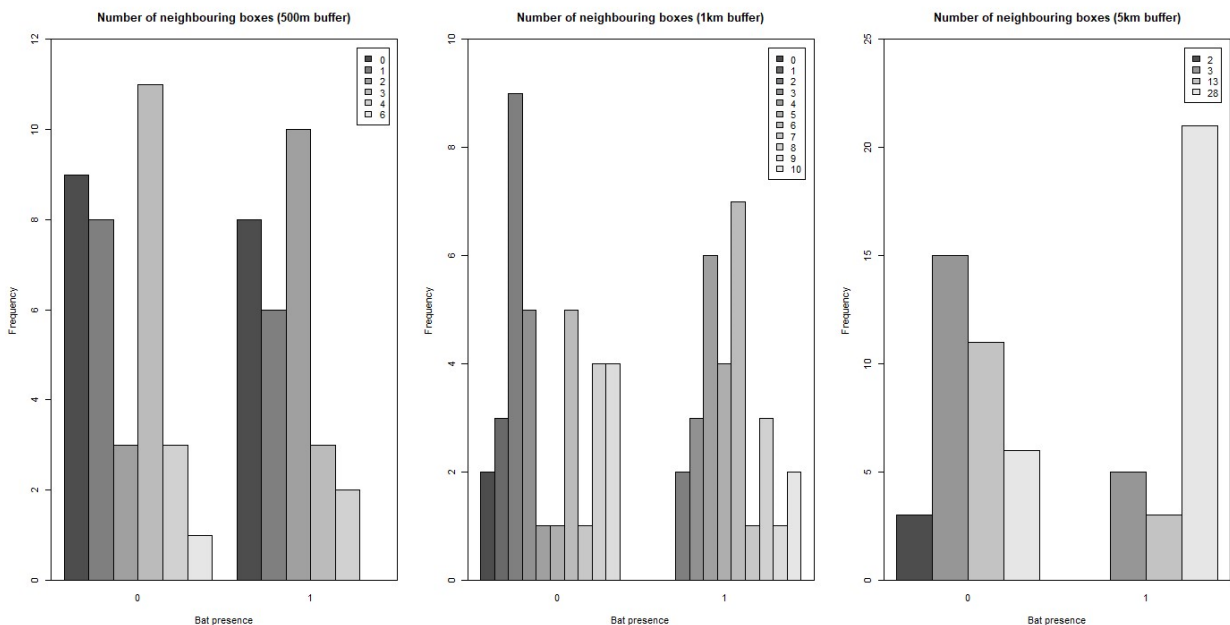
Bat box occupancies were found more frequently in Schwegler 2FN boxes (48.3% of the 29 bat presences) (13 painted white and 1 black), followed by wooden boxes (34.5%) (9 white and 1 colourless), with Schwegler 2F boxes being the less occupied (17.2%) (3 white and 2 black) – **Figure 4**.



**Figure 4** Number of bat presences by colour. The left bar corresponds to white bat boxes (“Branca”), the middle to wood (“Madeira”), and the right bar to black (“Preta”) bat boxes

The highest number of occupied boxes was found in boxes with 943 days since installation (n = 10), followed by the oldest boxes (n = 9), both installed in Herdade do Esporão.

The number of neighbouring boxes for each sampled bat box (**Figure 5**), within the 500 m buffer, varied between 0 and 6, with 1.6 boxes on average. Within the 1 km buffer, the number of neighbouring boxes varied between 0 and 10, and for the 5 km buffer, between 2



**Figure 5** Number of bat presence per number of neighbouring boxes.



and 28, with an average of 15.7 boxes. For the 500 m buffer, the highest number of occupied boxes ( $n = 10$ ) had 2 neighbouring boxes, in the 1 km buffer the boxes with 6 neighbouring boxes were the most occupied ( $n = 7$ ) and in the 5 km buffer, 21 occupied boxes had the highest number of neighbouring boxes ( $n = 28$ ).

Regarding occupied bat box land cover surroundings, in the 500 m (**Appendix I**) and 1 km buffer (**Appendix II**), the vineyard was the most prevalent land cover, with an average land cover percentage of 43% for the 500m buffer (min: 0 – max: 0.93) and 35% for the 1 km buffer (min: 0.08 – max: 0.81), and the closest land cover, with a mean distance of 83 m (min: 0 – max: 479.5 m) and 198.5 m (min: 0 – max: 731 m), respectively. On the contrary, urban land was the least represented land cover and the furthest from the boxes, in both 500 m and 1 km buffer, with 1% (min: 0 – max: 0.08) and a mean distance of 300 meters (min: 0.58 – max: 480.67 m) for the 500 m buffer, and; 1% (min: 0 - max: 0.05) and a mean distance of 686 m (min: 184 – max: 947 m) for the 1 km buffer. In the 5 km buffer (**Appendix III**), the most frequent land cover was forest, occupying 41% of the area (min: 0.18 - max: 0.50), while scrubland was the least represented, with an average of 0.8 % of the area occupied (min: 0 – max: 0.019). Shrubland was also the one with greater distances from de boxes (mean = 3556 m; min = 3127; max = 4289), while vineyards (mean = 3060 m; min = 178; max = 3630) and pastureland (mean = 3033 m; min = 2718; max = 3702) were the closest on average.

### 3.2. MODEL SELECTION (500 M, 1 KM, AND 5 KM)

#### 3.2.1. BAT BOX TRAIT MODELS

In the first stage of model selection, where the fitting function only considered bat box traits (Bat box typology - "Tipologia", colour - "Cor", number of neighbouring boxes - "Ncaix\_buf", box time since installation (in days) - "dias\_inst", and presence or traces of other fauna - "Vest\_faun"), of all possible candidate models generated, four models had good support ( $\Delta AICc < 2$ ) for each three buffer analyses. For the 500 m buffer, the best-fitted model

(modelo\_500\_caixa\_2) included the variables colour and other species presence (“Cor” + “Vest\_faun”), and for both the 1 km and 5 km buffer the chosen best models (modelo\_1000\_caixa\_2 and modelo\_5000\_caixa\_2\_2) included the variables colour, number of neighbouring boxes and other animal presence (“Cor + Ncaix\_buf + Vest\_faun”). The first five candidate models with a  $\Delta AICc < 2$  are shown in **Table 3**.

**Table 3** The five models with the highest support ( $\Delta AICc < 2$ ) from the bat box traits’ analyses carried out on the 3 buffers. The best-fitted models are presented in bold. Below each distance buffer line are the candidate variables used to build the best fitted models.

Model	AICc	Weights	Model name
<b>500 m</b>			
<i>Cor + dias_inst + ncaix_buf + Vest_faun</i>			
<b>pres_tot ~ 1 + Cor + Vest_faun</b>	<b>52.7180</b>	<b>0.5690</b>	<b>modelo_500_caixa_2</b>
<i>Tipologia + dias_inst + ncaix_buf + Vest_faun</i>			
pres_tot ~ 1 + Tipologia + dias_inst + Vest_faun	59.4797	0.4251	modelo_500_caixa_1
pres_tot ~ 1 + dias_inst + Vest_faun	60.2429	0.2902	modelo_500_caixa_1_s_tipol
pres_tot ~ 1 + Tipologia + dias_inst + ncaix_buf + Vest_faun	61.2086	0.1791	modelo_500_caixa_1_3
<b>1 km</b>			
<i>Tipologia + dias_inst + ncaix_buf + Vest_faun</i>			
pres_tot ~ 1 + dias_inst + Vest_faun	60.2428	0.4169	modelo_1000_caixa_1
pres_tot ~ 1 + dias_inst + Ncaix_buf + Vest_faun	60.9780	0.2887	modelo_1000_caixa_1_2
pres_tot ~ 1 + Tipologia + dias_inst + Vest_faun	61.7755	0.1938	modelo_1000_caixa_1_3
<i>Cor + dias_inst + ncaix_buf + Vest_faun</i>			
<b>pres_tot ~ 1 + Cor + Ncaix_buf + Vest_faun</b>	<b>50.0169</b>	<b>0.6057</b>	<b>modelo_1000_caixa_2</b>
<b>5 km</b>			
<i>Tipologia + dias_inst + ncaix_buf + Vest_faun</i>			
pres_tot ~ 1 + Vest_faun + dias_inst + Ncaix_buf	57.6466	0.4861	modelo_5000_caixa_1
pres_tot ~ 1 + Tipologia + Vest_faun + dias_inst + Ncaix_buf	59.3089	0.2117	modelo_5000_caixa_1_2
<i>Cor + dias_inst + ncaix_buf + Vest_faun</i>			
<b>pres_tot ~ 1 + Cor + Vest_faun + Ncaix_buf</b>	<b>38.8401</b>	<b>0.6155</b>	<b>modelo_5000_caixa_2_2</b>
pres_tot ~ 1 + Cor + Vest_faun + dias_inst + Ncaix_buf	39.7887	0.3830	modelo_5000_caixa_2

### 3.2.2. LAND COVER MODELS

In the second stage of model selection, which only used land cover variables in the formulas (average distance to urban area – “distave\_ter\_art”, agriculture – “distave\_agr”, vineyard – “distave\_vin”, forest – “distave\_flo”, pastureland – “distave\_past” and water surface – “distave\_ag”; and proportion of urban area - “prop\_ter\_art”, agriculture - “prop\_agr”, vineyard - “prop\_vin”, forest - “prop\_flo”, pastureland “prop\_past”, srubland – “prop\_mat”, and water surface - “prop\_ag”), the *glmulti* results yielded 5 candidate models for the 500 m

buffer, 16 models (where the first 3 were analysed) in the 1 km buffer, and 3 models in the 5km buffer analysis. Among the candidate models, the chosen models (**Table 4**) contained the variables: forest and water proportion ("prop\_flo + prop\_ag" - modelo\_500\_uso\_1) in the 500 m buffer; average distance to agriculture and forest and, proportion of urban area, forest, and water surface ("distave\_agr + distave\_flo + prop\_ter\_art + prop\_flo + prop\_ag" - modelo\_1000\_uso\_4), and also average distance to forest, and proportion of urban area, agriculture, forest and water surface ("distave\_flo + prop\_ter\_art + prop\_agr + prop\_flo + prop\_ag" - modelo\_1000\_uso\_5) for the 1km buffer; and vineyard and water surface proportion ("prop\_vin + prop\_ag" - modelo\_5000\_uso\_1) for the 5 km buffer.

**Table 4** The five models with the highest support ( $\Delta AICc < 2$ ) from the land cover analyses carried out on the 3 buffers. The best-fitted models are presented in bold. Below each distance buffer line are the candidate variables used to build the best fitted models.

Model	AICc	Weights	Model name
<b>500 m</b>			
<i>distave_ag + prop_ter_art + prop_agr + prop_past + prop_vin + prop_flo + prop_ag</i>			
<b>pres_tot ~ 1 + prop_flo + prop_ag</b>	<b>90.4570</b>	<b>0.1103</b>	<b>modelo_500_uso_1</b>
pres_tot ~ 1 + distave_ag + prop_flo + prop_ag	91.7199	0.0587	modelo_500_uso_5
pres_tot ~ 1 + prop_past + prop_flo + prop_ag	92.0356	0.0501	modelo_500_uso_2
pres_tot ~ 1 + prop_ter_art + prop_flo + prop_ag	92.3736	0.0423	modelo_500_uso_3
pres_tot ~ 1 + prop_ag	92.4311	0.0411	modelo_500_uso_4
<b>1 km</b>			
<i>distave_vin + distave_agr + distave_flo + distave_ag + prop_ter_art + prop_agr + prop_past + prop_vin + prop_flo + prop_ag</i>			
<b>pres_tot ~ 1 + distave_agr + distave_flo + prop_ter_art + prop_flo + prop_ag</b>	<b>90.2139</b>	<b>0.0430</b>	<b>modelo_1000_uso_4</b>
<b>pres_tot ~ 1 + distave_flo + prop_ter_art + prop_agr + prop_flo + prop_ag</b>	<b>90.4779</b>	<b>0.0377</b>	<b>modelo_1000_uso_5</b>
pres_tot ~ 1 + distave_agr + distave_flo + prop_ter_art + prop_flo	90.4792	0.0376	modelo_1000_uso_6
pres_tot ~ 1 + distave_flo + prop_ter_art + prop_flo + prop_ag	90.7474	0.0329	modelo_1000_uso_7
pres_tot ~ 1 + distave_flo + prop_ter_art + prop_agr + prop_past + prop_vin	90.8429	0.0314	modelo_1000_uso_8
<b>5 km</b>			
<i>distave_ter_art + distave_agr + distave_past + distave_vin + distave_flo + distave_ag + prop_ter_art + prop_agr + prop_past + prop_vin + prop_flo + prop_ag + prop_mat</i>			
<b>pres_tot ~ 1 + prop_vin + prop_ag</b>	<b>76.9116</b>	<b>0.0554</b>	<b>modelo_5000_uso_1</b>
pres_tot ~ 1 + distave_ag + prop_vin + prop_ag	77.5282	0.0407	modelo_5000_uso_3
pres_tot ~ 1 + prop_ter_art + prop_vin + prop_ag	78.6124	0.0237	modelo_5000_uso_2

### 3.2.3. BOX TRAIT MODELS + LAND COVER MODELS

In the last step, where the chosen model variables from the previous two analyses were

included, the best-fitted models (**Table 5**) included the variables: colour (“Cor”), other animal presence (“Vest\_faun”), water proportion (“prop\_ag”) and forest proportion (“prop\_flo”) for the 500 m buffer (modelo\_500\_caixa\_uso\_5); colour (“Cor”), other animal presence (“Vest\_faun”), forest average distance (“distave\_flo”), proportion of urban area (“prop\_ter\_art”), agriculture (“prop\_agr”), forest (“prop\_flo”) and water (“prop\_ag”) for the 1 km buffer (modelo\_1000\_caixa\_uso\_10\_1); and, colour (“Cor”), other animal presence (“Vest\_faun”), number of neighbouring boxes (“Ncaix\_buf”) and water proportion (“prop\_ag”) for the 5 km buffer (modelo\_5000\_caixa\_uso\_1).

**Table 5** The five models with the highest support ( $\Delta AICc < 2$ ) from bat box traits and land cover analyses carried out on the 3 buffers. The best fitted models are presented in bold. Below each distance buffer line are the candidate variables used to build the best fitted models

Model	AICc	Weights	Model name
<b>500 m</b>			
(modelo_500_caixa_1 + modelo_500_uso_1) = (Cor + Vest_faun) + (distave_ag + prop_ag + prop_flo)			
<b>pres_tot ~ 1 + Cor + Vest_faun + prop_ag + prop_flo</b>	<b>44.5355</b>	<b>0.6210</b>	<b>modelo_500_caixa_uso_5</b>
pres_tot ~ 1 + Cor + Vest_faun + distave_ag + prop_ag + prop_flo	44.9240	0.2681	modelo_500_caixa_uso_10
pres_tot ~ 1 + Cor + Vest_faun + distave_ag + prop_ag	45.5733	0.1938	modelo_500_caixa_uso_10_1
pres_tot ~ 1 + Cor + Vest_faun + prop_ag	45.6167	0.3617	modelo_500_caixa_uso_5_1
<b>1 km</b>			
(modelo_1000_caixa_2) + (modelo_1000_uso_4) = (Cor + Ncaix_buf + Vest_faun) + (distave_agr + distave_flo + prop_ter_art + prop_flo + prop_ag)			
pres_tot ~ 1 + Cor + Vest_faun + distave_flo + prop_ter_art + prop_flo + prop_ag	48.5957	0.1129	modelo_1000_caixa_uso_5_1
pres_tot ~ 1 + Cor + Ncaix_buf + Vest_faun + distave_flo + prop_ter_art + prop_flo + prop_ag	49.0732	0.0889	modelo_1000_caixa_uso_5_2
pres_tot ~ 1 + Cor + Ncaix_buf + Vest_faun + distave_flo + prop_ter_art + prop_flo	49.4950	0.0720	modelo_1000_caixa_uso_5_3
(modelo_1000_caixa_2) + (modelo_1000_uso_5) = (Cor + Ncaix_buf + Vest_faun) + (distave_flo + prop_ter_art + prop_agr + prop_flo + prop_ag)			
<b>pres_tot ~ 1 + Cor + Vest_faun + distave_flo + prop_ter_art + prop_agr + prop_flo + prop_ag</b>	<b>45.2558</b>	<b>0.2735</b>	<b>modelo_1000_caixa_uso_10_1</b>
pres_tot ~ 1 + Cor + Ncaix_buf + Vest_faun + distave_flo + prop_ter_art + prop_agr + prop_flo + prop_ag	47.1778	0.1046	modelo_1000_caixa_uso_10_2
<b>5 km</b>			
(modelo_5000_caixa_2_2 + modelo_5000_uso_1) = (Cor + Vest_faun + Ncaix_buf) + (prop_vin + prop_ag)			
<b>pres_tot ~ 1 + Cor + Vest_faun + Ncaix_buf + prop_ag</b>	<b>39.6403</b>	<b>0.3726</b>	<b>modelo_5000_caixa_uso_1</b>
pres_tot ~ 1 + Cor + Vest_faun + Ncaix_buf	39.7887	0.3460	modelo_5000_caixa_2_2

### 3.3. FINAL MODELS

After model selection, I fitted three logistic models (estimated using ML) to predict bat presence with colour, other animal presence, forest and water proportion (formula: pres\_tot ~

1 + Cor + Vest\_faun + prop\_ag + prop\_flo) for the 500 m buffer; colour, other animal presence, urban areas, agriculture, forest and water proportion, plus forest average distance (formula:  $pres\_tot \sim 1 + Cor + Vest\_faun + distave\_flo + prop\_ter\_art + prop\_agr + prop\_flo + prop\_ag$ ) for the 1km buffer; and with colour, other fauna traces, number of neighbouring boxes and water proportion (formula:  $pres\_tot \sim 1 + Cor + Vest\_faun + Ncaix\_buf + prop\_ag$ ) for the 5km buffer. The parameters were obtained by fitting the models on a standardized version of the dataset. The models are presented in **Table 6**.

**Table 6** The 500m model ( $pres\_tot \sim 1 + Cor + Vest\_faun + prop\_ag + prop\_flo$ ) - first, 1km ( $pres\_tot \sim 1 + Cor + Vest\_faun + distave\_flo + prop\_ter\_art + prop\_agr + prop\_flo + prop\_ag$ ) – second, and 5 km model ( $pres\_tot \sim 1 + Cor + Vest\_faun + Ncaix\_buf + prop\_ag$ ) – third model.

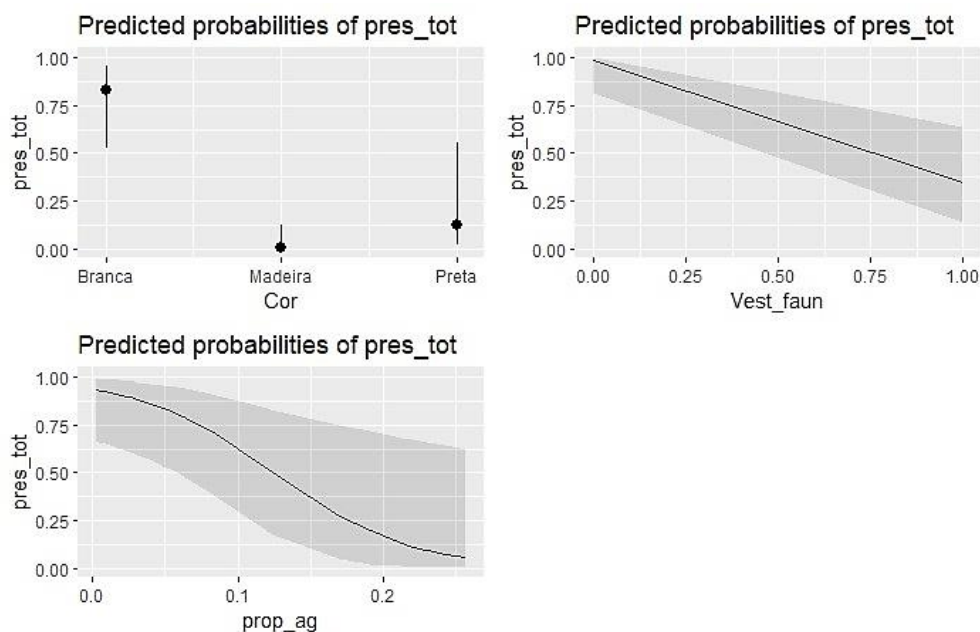
Predictors	pres_tot			Odds Ratios	pres_tot			Odds Ratios	pres_tot		
	Odds Ratios	CI	p		CI	p	CI		p		
(Intercept)	492.98	28.06 – 44935.56	<b>0.001</b>	3310120245664.30	223011.11 – 3307188797625041787086826884.00	<b>0.017</b>	58.13	1.48 – 13289.20	0.072		
Cor [Madeira]	0.00	0.00 – 0.03	<b>0.001</b>	0.00	0.00 – 0.20	<b>0.039</b>	0.01	0.00 – 0.22	<b>0.018</b>		
Cor [Preta]	0.03	0.00 – 0.29	<b>0.013</b>	0.00	0.00 – 0.09	<b>0.005</b>	0.00	0.00 – 0.05	<b>0.005</b>		
Vest faun	0.01	0.00 – 0.08	<b>0.001</b>	0.00	0.00 – 0.08	<b>0.015</b>	0.01	0.00 – 0.14	<b>0.006</b>		
prop ag	0.00	0.00 – 0.00	<b>0.016</b>	0.00	0.00 – 0.00	0.062	0.00	0.00 – 79.64	0.101		
prop flo	0.03	0.00 – 2.80	0.179	0.00	0.00 – 0.00	<b>0.021</b>					
distave flo				0.98	0.96 – 1.00	<b>0.046</b>					
prop ter art				0.00	0.00 – 0.00	<b>0.030</b>					
prop agr				0.00	0.00 – 0.21	<b>0.043</b>					
Ncaix buf							1.40	1.13 – 1.99	<b>0.012</b>		
Observations	64			64			64				
R <sup>2</sup> Tjur	0.688			0.761			0.721				
AICc	46.096			46.772			42.301				

The 95% Confidence Intervals (CIs) and p-values were computed using a Wald z-distribution approximation. The residuals of the final model(s) can be found in **Appendix IV (Fig 15 – 23 and Table 10)**. For all three buffers (500m, 1km, and 5km), the models' explanatory power was substantial (Tjur's R<sup>2</sup> = 0.69 for 500m buffer; Tjur's R<sup>2</sup> = 0.76 for 1km buffer; and Tjur's R<sup>2</sup> = 0.72 for 5km buffer) and the Pseudo R<sup>2</sup> values (**Table 7**) indicated a good model fit. Bat box occupancy was negatively influenced, at all spatial scales, by box colour (“Cor” - wooden and black), other fauna occupancy (“Vest\_faun”), and water proportion

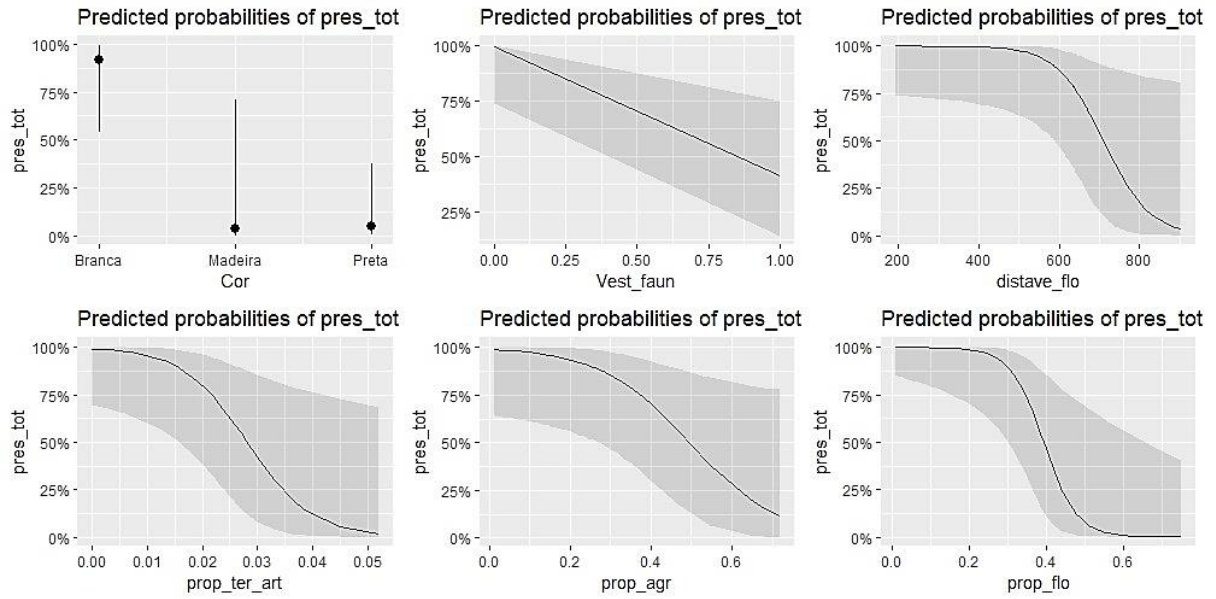
**Table 7** Pseudo R<sup>2</sup> for the three buffer models.

Pseudo R <sup>2</sup> for Logistic regression	500m	1km	5km
Hosmer and Lemeshow R <sup>2</sup>	0.63	0.711	0.673
Cox and Snell R <sup>2</sup>	0.58	0.625	0.604
Nagelkerke R <sup>2</sup>	0.776	0.835	0.808

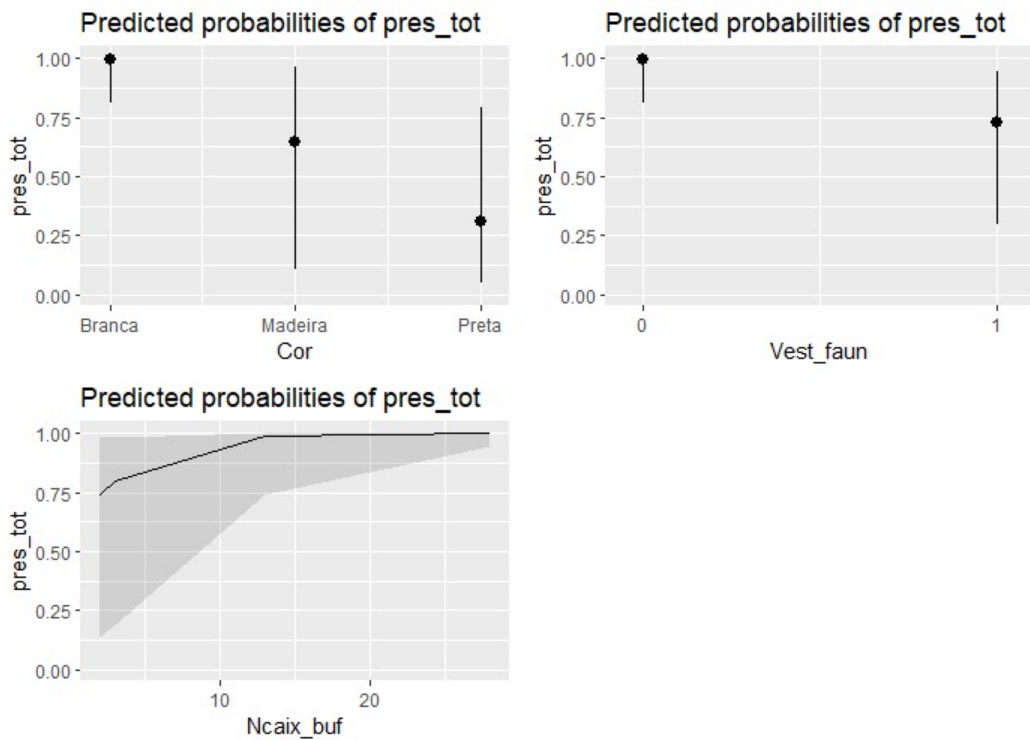
(“prop\_ag”). The effect of forest proportion, in the 500 m buffer was statistically non-significant and negative. For the 1km buffer, box occupancy was also negatively influenced by distance to the nearest patch of forest (“distave\_flo”), proportion of forest (“prop\_flo”), urban areas (“prop\_ter\_art”), and agriculture (“prop\_agr”). With respect to the 5km buffer, the occupancy of the boxes was positively affected by the number of boxes in the neighborhood (“Ncaix\_buf”). The predicted effects of the variables included in the models are presented in (Figure 6, 7 and 8) and the prediction summary in matrix form is present in the Tables 8 and 9.



**Figure 6** Predicted probabilities of bat presence (pres\_tot) in the 500m buffer for the significant variables: Colour (Cor), other animal occupation (Vest\_faun), and water proportion (prop\_ag).



**Figure 7** Predicted probabilities of bat presence (pres\_tot) in the 1km buffer for the significant variables: Colour (Cor), other animal occupancy (Vest\_faun), forest average distance (distave\_flo), urban area proportion (prop\_ter\_art), agriculture proportion (prop\_agr), forest proportion (prop\_flo).



**Figure 8** Predicted probabilities of bat presence (pres\_tot) in the 5km buffer for Colour (Cor), other animal traces (Vest\_faun), and number of neighbouring boxes (Ncaix\_buf).

**Table 7** Confusion Matrix for the 500m, 1km and 5km buffer models.

	500m		1km		5km	
Prediction	Reference					
	0	1	0	1	0	1
0	33	3	35	2	34	5
1	2	26	0	27	1	24

**Table 9** Confusion Matrix Statistics

	Models		
	500m	1km	5km
Accuracy :	0.9219	0.9688	0.9062
95% CI :	(0.827, 0.9741)	(0.8916, 0.9962)	(0.807, 0.9648)
No Information Rate :	0.5469	0.5469	0.5469
P-Value [Acc > NIR] :	5.54E-11	2.41E-14	4.63E-10
Kappa :	0.8419	0.9366	0.8086
Mcnemar's Test P-Value :	1	0.4795	0.2207
Sensitivity :	0.8966	0.9310	0.8276
Specificity :	0.9429	1.0000	0.9714
Pos Pred Value :	0.9286	1.0000	0.9600
Neg Pred Value :	0.9167	0.9459	0.8718
Prevalence :	0.4531	0.4531	0.4531
Detection Rate :	0.4062	0.4219	0.375
Detection Prevalence :	0.4375	0.4219	0.3906
Balanced Accuracy :	0.9197	0.9655	0.8995
Positive Class :	1	1	1



## 4. DISCUSSION

In this study, I aimed to understand which landscape factors influence bat box occupancy, and which both landscape and box-specific variables are more relevant for bat box occupancy, at three spatial scales, in the vineyards of Alentejo region, Portugal.

Based on the results, this study demonstrates that both box traits and landscape-level variables influence bat box occupancy. Nevertheless, the number of landscape-level variables in each model changed markedly; the 1km buffer model was the only that showed the influence of a greater number of landscape variables (forest average distance and proportion, urban areas, agriculture, and forest proportion) while in the other two models, the predominant independent variables were the bat boxes characteristics (colour, other animal traces and the number of neighbouring bat boxes).

### 4.1. BAT BOX TRAITS

#### 4.1.1. COLOUR

Bat box occupancy in the vineyards showed a strong relation to box colour (“Cor”). Bats occupied more frequently white bat boxes than wooden or black boxes. This negative link was observed in the three spatial scale models (500m, 1km and 5km). The effect of bat box colour has been intensively studied by several authors and proven to influence bat box occupancy (e.g., Lourenço & Palmeirim, 2004), so it would be expected that the colour variable would be included in the models. The internal temperature of the artificial roosts needs to be high enough for the individual bats and maternity colonies to colonize bat boxes. However, the high internal temperatures of bat boxes may also cause heat stress on bats. Bideguren *et al.* (2019) explored which bat box features might cause bat mortality in Ebro Delta Natural Park, Catalunya, and they found that overheating events, inside temperatures higher than 40 °C, were more often recorded in black cement boxes (Schwengler 2F) than in the other bat box types. As such, they do not recommend the use of black boxes in south-facing sites in hot regions. It

is worth noting that all the black boxes in the data set were either 2F or 2FN, and there was even a 2F box that had 7 dead adult bats and a dead chick. These recent reports apparently challenge Lourenço and Palmeirim (2004) who claimed that, to provide artificial roosts for maternity colonies, “the best option are black boxes even in Mediterranean areas”. However, the bat box type differs between the referred study and the present study; the black boxes surveyed in the current research are smaller and non-ventilated (Schwellenger 2F and 2N) compared to the Bat Conservation International (BCI) typology installed by Lourenço and Palmeirim (2004). These differences in size and ventilation may be important for regulating extremely high temperatures inside the boxes but it is worth further caution because of the present trend of higher summer temperatures in the Alentejo. Furthermore, although in spring the boxes are attractive to female bats to roost and form maternity colonies, during the summer, when temperatures rise sharply, they can suffer from overheating, turning the boxes, a common conservation practice, into an ecological trap (Crawford & O’Keefe, 2021).

#### 4.1.2. NUMBER OF NEIGHBOURING BOXES

Our results also indicate that in the wider spatial scale, the 5km buffer model, the number of neighbouring boxes (“N\_caixbuf”) influences positively bat box occupancy. The response curve of the number of neighbouring boxes shows that estimated bat box occupancy is higher when the number of neighbouring boxes is approximately 15 boxes per buffer (**Figure 5**). In fact, it has been shown that higher densities of boxes at the landscape scale positively influence box occupancy (Ciechanowski, 2005; Mering & Chambers, 2014) as this allows bats to frequently switch suitable roosts (Willis & Brigham, 2004), which is a necessary behaviour to avoid parasites or predators (Ruczyński & Bogdanowicz, 2008; Russo *et al.*, 2005).

#### 4.1.3. OTHER ANIMAL OCCUPANCY

Bat box occupancy by other animals (“Vest\_faun”) was related with lower box occupancy by bats across the models of the three spatial scales. Although bats occurred

simultaneously with a non-bat species in 10.94% of all bat boxes, according to the models, the probability of bat presence in the boxes can drop by more than 50% when other animals are present (bird nests and/or wasps). The lower box occupancy by bats due to the box being used by other animals has also been recorded in other studies (Dodds & Bilston, 2013; Mering & Chambers, 2014; Pschonny *et al.*, 2022). In bat box surveys, between May and August, the most frequent case of use by other animals was by bird nests either in use or recently abandoned. A report from Dodds and Bilston (2013) indicate how the competition between birds and bats can influence box occupancy. They conducted an experiment to determine if Natterer's bat *Myotis nattereri* and brown long-eared bat *Plecotus auritus* exhibited an occupation preference between different bat box types and found a seasonal variation in box occupation by bats due to the impact of bird competition: despite the provision of bird boxes, in the pre and post bird nesting period (May-June) birds routinely occupied 1FS and 2FN boxes.

## 4.2. LAND COVER

Regarding land cover influence on bat box occupation, from all the considered land cover variables, the *Pipistrelle spp.* bats occupied more frequently bat boxes where the proportion of urban areas, forest, agriculture (for the 1km buffer model), and water areas (for the 500m buffer model) was lower. However, bat occupation was affected negatively by box average distance to forest patches, which mean that bat box occupation by bat was higher when the average distance to forest was lower.

### 4.2.1. SURFACE WATER PROPORTION

As mentioned before, bat box occupancy was negatively influenced by water proportion (“prop\_ag”) in 500m buffer model and was not selected in the final models of the 1km and 5km, which does not confirm my expectations (a higher proportion of water would increase bat occupancy). These expectations are based on existing literature which describes the importance of water-related habitats (Maslonek, 2009; Russo *et al.*, 2005) for bat survival, especially in

Mediterranean regions where the water sources are very scarce (Rainho, 2007). Maslonek (2009) affirms that wetlands of all sizes, even small ones, created in a landscape with an overall lack of wetlands may be particularly important for all species of bats as they can be vital foraging areas for bat species. However, water bodies are also important for birdlife, which, as mentioned before, increases the competition for boxes that are closest and have a greater proportion of water in their surroundings and inhibits bats from occupying the boxes. So, the relation between bat occupancy and water variables may be biased.

#### 4.2.2. FOREST PROPORTION AND AVERAGE DISTANCE

Interestingly, although bat box occupancy by bats decreased as the proportion of forest (“prop\_flo”) increased (negatively significant in the 1km buffer, not significant in 500m, and not contemplated in the 5km buffer), bats occupied the boxes at a shorter distance from the forest patches (< 500m). It would be expected that bat occupancy would increase in areas with a higher proportion of forest, as previously concluded in other studies (López-Baucells *et al.*, 2017; Rainho, 2007). Perhaps, the findings of the present study differ from the literature because of the high level of bird occupancy. Forest birds prefer to forage close to the forest patches, usually not using the open vineyard areas away from the trees. Thus, boxes closer to the forest patches were more often occupied by birds. Because of bats’ avoidance behaviour to occupied boxes by birds, the relation between the two variables may be inverted. Moreover, the simplification of the land cover representation may have had an impact on the results, for example, the forest land cover category includes *Montados* and eucalyptus plantations, which are very different crops and have a different influence on bat activity (Cruz *et al.*, 2016).

#### 4.2.3. URBAN AREA PROPORTION

The impact of urbanized areas (“prop\_ter\_art”) on bat box occupancy by bats has been studied, and contrasting conclusions have been reported: Russo and Ancillotto (2015) point to the consequences of urbanization for bats: habitat loss and fragmentation, road mortality, high

density of domestic predators, anthropogenic noise, artificial lighting and direct human interference, while others, like Medinas *et al.* (2012), affirmed that *Pipistrellus spp.*, often occupies urban environments since they are tolerant to noise and artificial lighting. In accordance, López-Baucells *et al.* (2017) stated that urban cover areas around bat boxes tends to have a negative impact on bat box occupation rates, since buildings often offer optimum roosting sites for house-dwelling bats, and it is possible that only large bat boxes will tempt these bats to move away from buildings (Flaquer *et al.*, 2014; Tuttle *et al.*, 2013). Similarly, this study concludes that higher urbanized areas proportion around vineyards tend to decrease bat box occupation by *Pipistrellus spp.* bats, which is in line with the expectations.

#### 4.2.4. AGRICULTURE PROPORTION

In line with the other land cover variables, bat occupancy decreased as the proportion of agricultural land (“prop\_agr”) increased. This result is in line with the expectations, since homogenous and structurally simpler habitats and farmed areas are associated with low bat activity and low number of recorded bat species (Rainho, 2007).

Although the objective of this study was to characterize the impact of land cover on the occupation of bat boxes, the results were certainly influenced by the occupation of other animals, particularly birds, and therefore conclusions about bat preferences for various land cover must take bird occupation into account. Nevertheless, this study still contributes to increase bat box effectiveness by the recommendation of bat box installation practices. Our findings indicate that managers should mount white boxes, as they are less susceptible to high temperature peaks (Griffiths *et al.*, 2017), and together or near nest-boxes for birds, to reduce bat box occupancy by birds. Moreover, regarding bird occupancy, it is crucial to install unsuitable bat boxes for birds, and to clean bat boxes frequently to prevent occupation by birds and other animals. It is also recommendable to install bat boxes by groups (cluster of 15 boxes per 5km radius) which will increase bat box occupancy, at least for the *Pipistrellus spp.*

Considering land cover surroundings, it is possible to recommend bat box installation at around 500 metres or less from forest areas, to avoid locations with high proportion of urban areas and agriculture land cover within a 1 km radius. Also, although there was a negative correlation between the increase in the proportion of water and forest, and the occupation of bat boxes, due to the occupation of the boxes by birds, it is known that water and forest are important elements for the presence of bats (Heim et al., 2015; Hendel et al., 2023; Rainho, 2007; Stahlschmidt et al., 2012). It is therefore possible to recommend, despite contradicting the results of this study, installing bat boxes where there is a higher proportion of water and forest within a 1 km radius. Simultaneously, vineyard landowners should also implement conservation management strategies involving the creation and management of woodland and water elements to benefit bat populations.

Despite of its relevance, the present study had some limitations that may have influenced the results. For this study to be more meaningful the data set size should have been larger (more boxes), principally in a landscape scale study, since the number of independent variables is high. Moreover, the number of surveys to each box should have been higher throughout the year and during bat shelter-seeking season. Checking bat box occupancy once per year may be insufficient, due to the seasonal variation in species activity patterns - some species are extremely roost-faithful, others regularly (even daily) change roosts (Fenton, 1997) -, and does not consider if the box had been occupied prior to the surveying data. This potential disadvantage was, however, circumvented by pooling the data of the bat box occupancy upon inspection with the presence of bat signs that indicate a recent presence (droppings). Also, the results only gain final validity after gathering data about the real tendencies of the indicators for several years (Bakış *et al.*, 2021). Because I had to survey bat-boxes of different types and colours, the resulting models must include these bat-box trait variables and integrate this information with the landscape variables on the final models. A modelling approach focused

only on the landscape would need a sample of the standardization of the type and colour of bat boxes, i.e. all boxes of the same type and colour. Considering the land cover map used to extract the landscape variables the feature class reclassification could also result in variables that are too simplified and that do not specify land uses that may be relevant to the bat species under research. One example, are the forest variables (“prop\_flo” and “distave\_flo”) that included *montados* and other types of exotic cultures such as eucalyptus plantations, which although with very low representativeness in the study area, accommodate a very low insect abundance and diversity compared to *montados* (Zahn *et al.*, 2010). Instead, the land cover measurements should have been obtained through the interpretation of aerial photos complemented by ground validation and with the inclusion of detailed cartography of both built and natural linear structures (Medinas *et al.*, 2012). Identifying patterns between bat box occupancy and the surrounding land cover may also be hindered by the opportunistic and generalist behaviour of *Pipistrellus spp.* These species are often difficult to create reliable models and to understand their preference. In addition, the estates where the bat boxes were installed are located in different regions, up to 100 km apart, where climatic variables vary, so it would have been advisable to record some important climatic/meteorological variables (rainfall gradients, temperature, elevation, topography, or landform) which are frequently important predictors of faunal responses in land mosaics (Bennett *et al.*, 2006) when analyzing the landscape at large scales, and which could affect the occupation of the box at the time of monitoring.

Our results prompt additional questions that should be answered with more data. For example, does prior bat box cleaning increase occupancy by bats? Furthermore, does the installation of bird boxes in bat box vicinity increase the occupancy of these boxes by bats? Or is there any anti-bird bat box that exclude bird use? It would also be important to study land cover influence on bat box occupancy with the same bat box typology and routine cleaning to minimize the influence of the boxes and to remove the influence of bird competition on bat

occupancy.

Therefore, more work is still necessary to fully understand which box trait and landscape predictors most influence bat box occupancy and efficacy. By studying the influence of land cover on bat box occupation, it will be possible to improve bat conservation initiatives in agriculture land, increase the degree of biodiversity and ecological resilience of the system and thereby reduce pesticide costs and pest impact in grape production.



## 5. CONCLUSION

The present study aimed to study the influence of the different land cover types and bat box traits, at three spatial scales (500m, 1km, 5km), in bat box occupancy in the context of vineyards in Alentejo region. As such, the general objectives of the current study were: (1) To investigate which land cover variables influence the occupancy of bat-boxes installed in vineyards in Alentejo region; (2) To understand whether landscape variables or box trait variables are more important to bat box occupation. The tested hypotheses are the following: (1) The landscape variables will be more important in bat box occupancy than the bat box variables; (2) Bat box occupancy is greater in vineyards where the surrounding land cover has a greater proportion and/or shorter distances to water and forest areas; (3) Boxes with a higher density of neighbouring boxes are occupied more frequently. To answer these questions, 79 bat boxes were sampled in eight wine-producing estates in Beja and Évora district, and three multiple binomial logistic regressions were made, one for each spatial scale, in the statistical analysis.

Regarding land cover influence on bat box occupancy, in the 500m buffer model, the only land cover with bat box occupancy impact was the proportion of water, and even then, the effect of increasing the water proportion decreased bat box occupancy, which goes against my initial beliefs, as bats are known to depend on water elements to drink and feed. The 1km buffer analysis was the one that included the greatest number of variables related to land cover, however, the forest proportion results did not meet the expectations given bats' habitat preferences, as it had a negative impact on the occupation of the boxes by bats. All the other contemplated land cover variables (forest proximity, urban area proportion and agriculture proportion) had a positive effect on bat box occupation, which was expected. Furthermore, in the occupancy model for the 5km buffer, no land cover had an impact, which was also an unexpected result. Still, and although only observed in the 5 km buffer analysis results, another

factor with a positive influence on the presence of bats in the boxes was the installation of bat boxes by groups. It was observed that the occupancy of the boxes increased when 15 boxes within a 5km radius were installed.

On the contrary, bat box traits had a more consistent influence through the three spatial buffers. In all three models, the negative impact of colour (black and wood) and the presence of other animals on box occupancy were verified. The results regarding the influence of bat box occupation by other animals deserves special attention, as it may be, due to competition between bats and other animals (mostly birds), a factor that impacts on the response of bat box occupancy by bats.

Thus, this study was able to answer both initial questions, by the description of which land cover types influenced bat box occupation (specially water proportion and forest proximity and proportion), despite being conditioned by the presence of other animals in the bat boxes, and by finding that bat box traits also influence their occupancy.

Obtaining these results is relevant for giving practical instructions to landowners that installed or plan to install bat boxes in their vineyards, particularly in relation to bat box typology and colour, its best location in the surrounding landscape and bat box density. Vineyard landowners should choose flat bat boxes with small entrances and install them in association with bird boxes to prevent bird use. Also, bat boxes should be white, to avoid overheating and consequent bat death, and installed in groups (ideally 15 boxes within a 5 km radius) to promote roost switching. Regarding bat box location, bat boxes must be placed where water proportion (within 500 m radius), and forest (specially *montados*) proportion and proximity (within 1 km radius) is higher. In addition, also within a 1 km radius, bat boxes may be placed in areas where the urban cover and agriculture proportion is lower. Besides, landowners should also implement conservation management strategies involving the creation

and management of woodland and water landscape elements to benefit bat populations.

By following these suggestions, there is a greater likelihood that the boxes will be occupied by bats, which will enable farmers to better benefit from auxiliary biological pest control, lessen the impact of pests on grape production and its associated costs, and in the end, increase the agricultural system biodiversity and its ecosystem services. Furthermore, studying the significance of distinct land cover on bat box occupancy by different bat species supports the development of spatially habitat suitability models that can help predict where boxes have the most potential to maximize conservation effectiveness to the target species and the make-up of the surrounding landscape. Moreover, this will improve bat box management and give decision-makers more assurance that bat boxes will help as a natural pest management tool.

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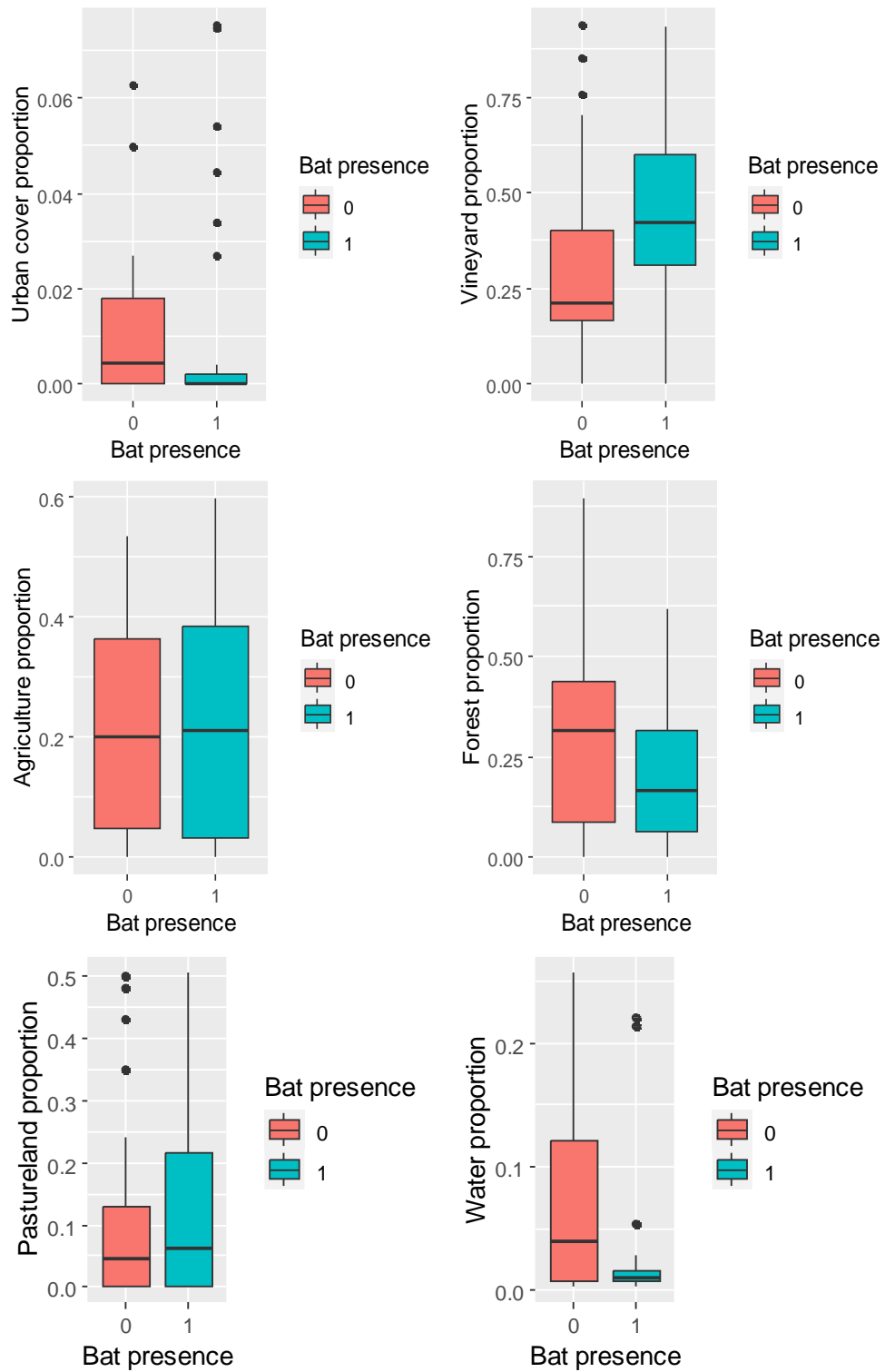
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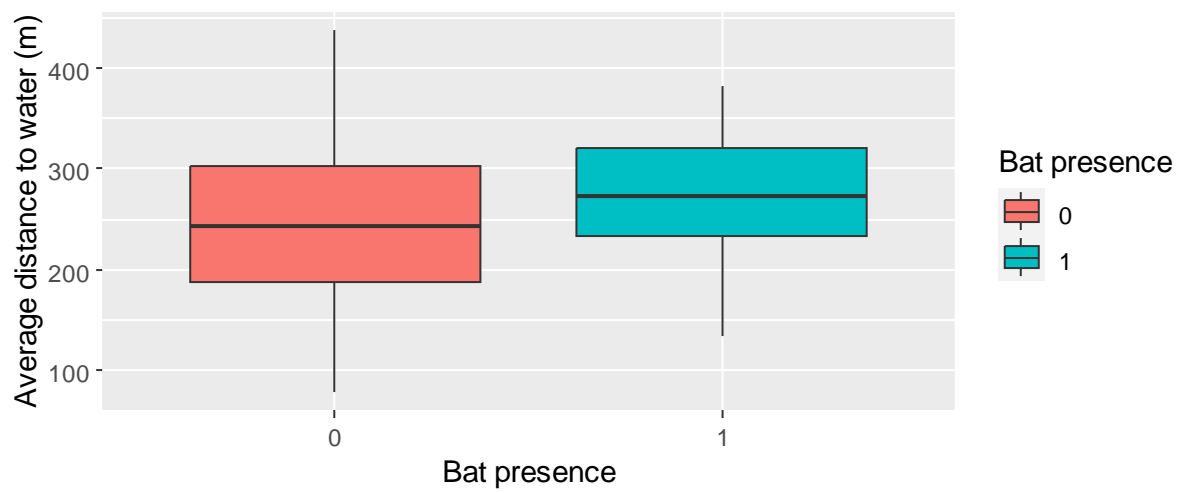


## 7. APPENDIX I

### 7.1. LANDSCAPE OVERALL RESULTS (500M BUFFER)



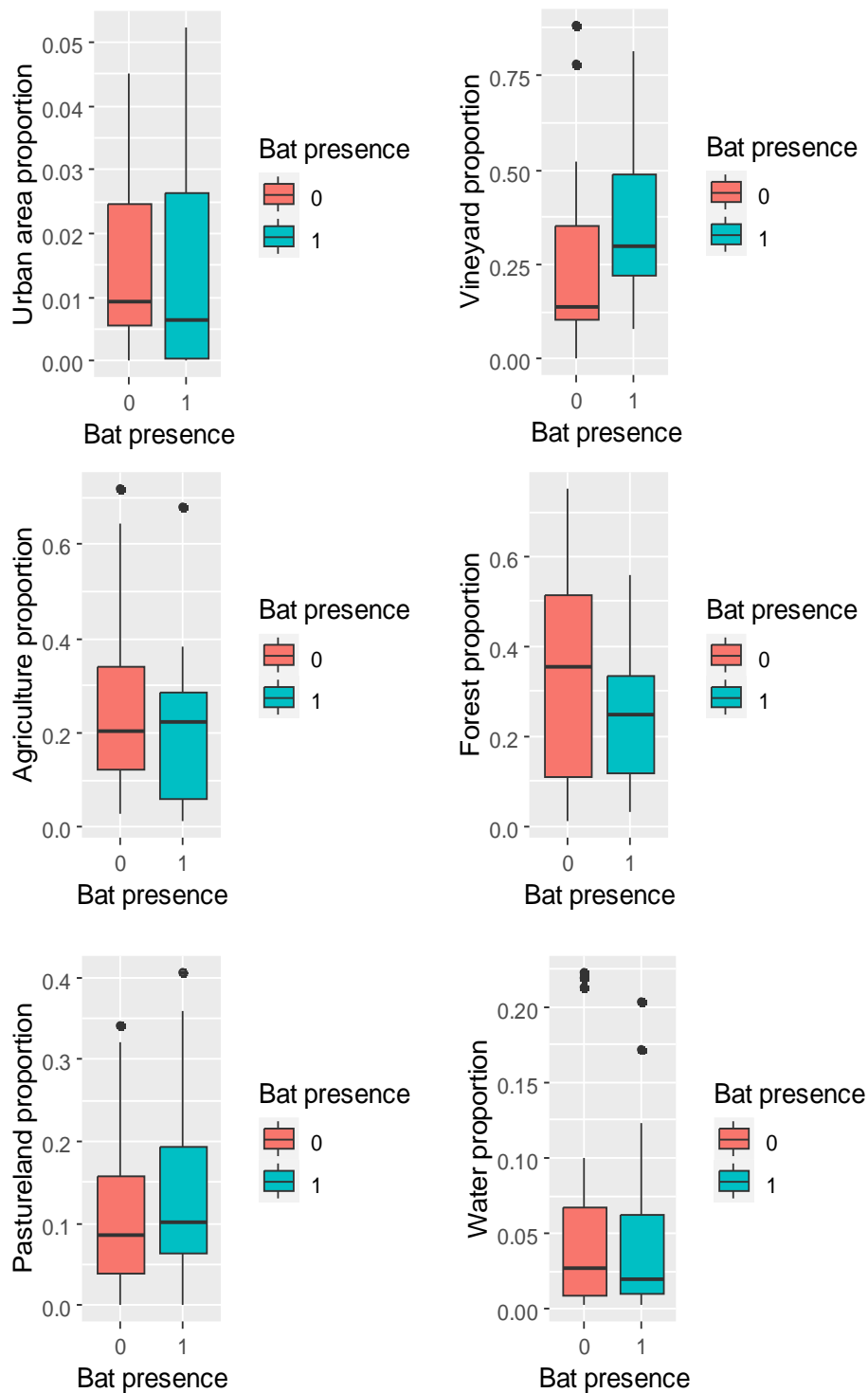
**Figure 9** Bat presence for the analysed landscape independent variables (proportion) for the 500m buffer.



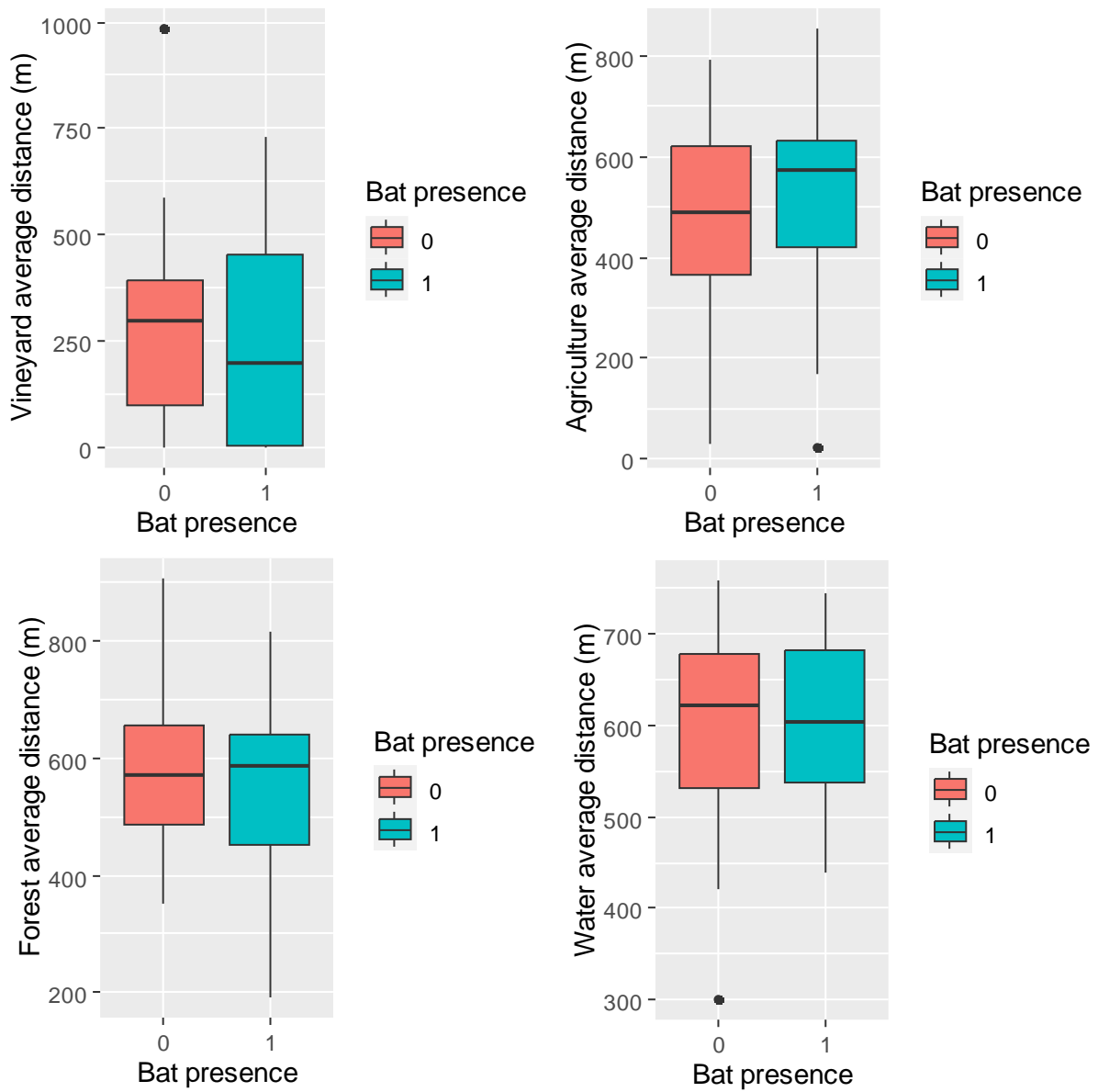
**Figure 10** Bat presence in function of the average distance to water for the 500m buffer.

## 8. APPENDIX II

### 8.1. LANDSCAPE OVERALL RESULTS (1KM BUFFER)



**Figure 11** Box plots for bat presence in relation to landscape independent variables (proportion) for the 1km buffer.



**Figure 12** Boxplots for bat presence in relation to landscape independent variables (average distance) - 1km buffer.

9. APPENDIX III

9.1. LANDSCAPE OVERALL RESULTS (5KM BUFFER)

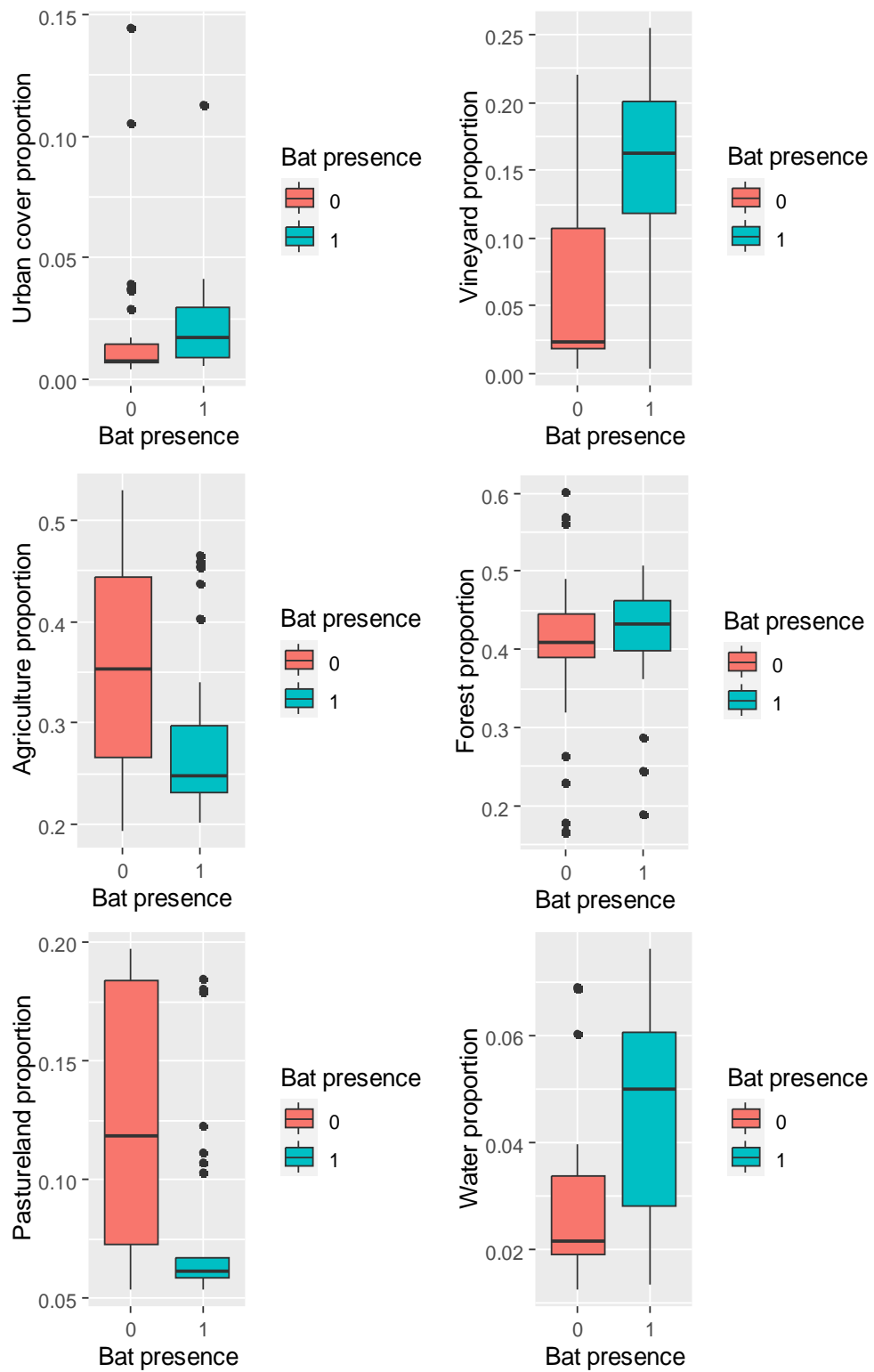
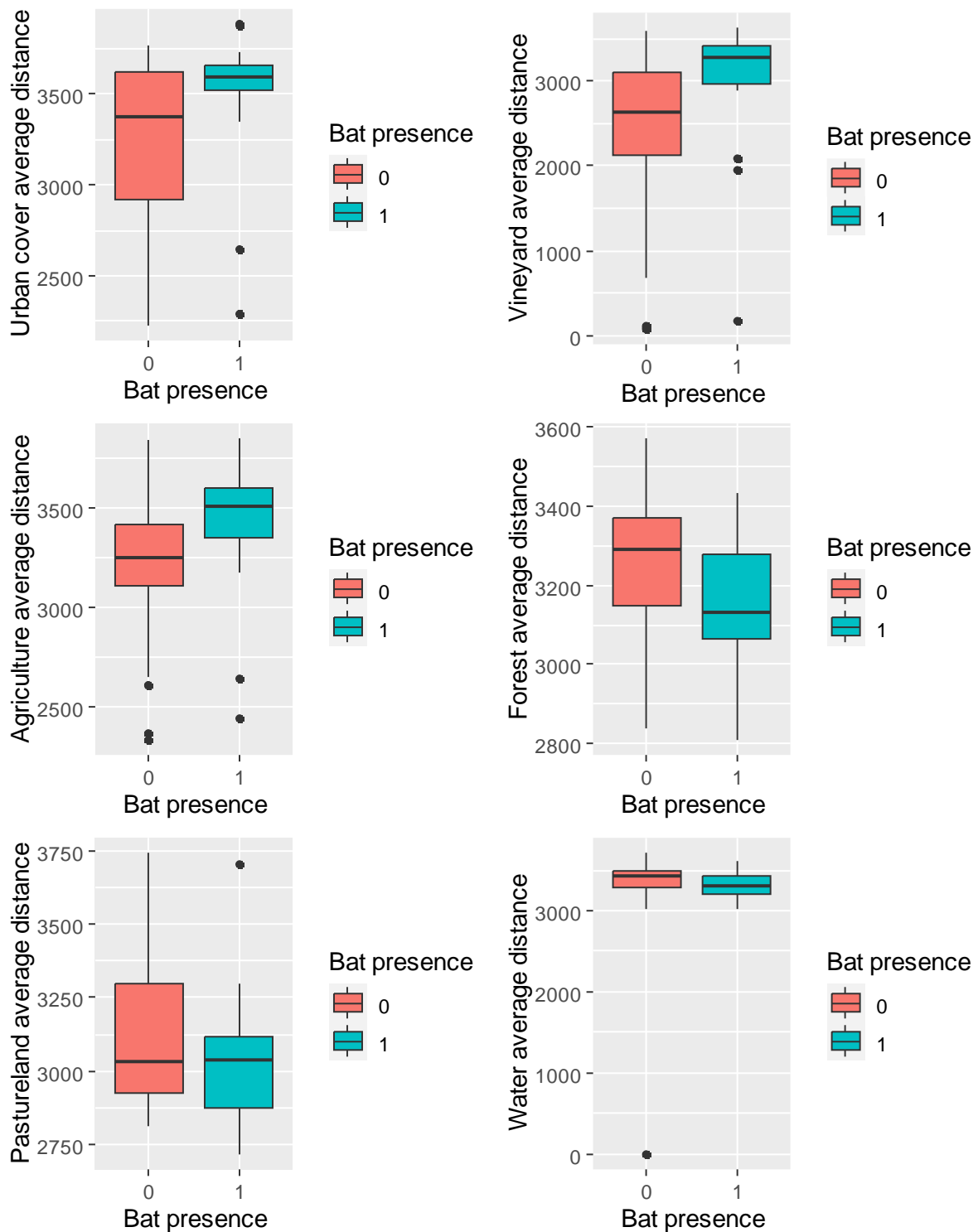


Figure 13 Bat presence in relation to landscape independent variables (proportion) for the 5km buffer.



**Figure 14** Bat presence in relation to average distance to land cover variables for the 5km buffer.

10. APPENDIX IV

10.1. FINAL MODEL (RESIDUALS)

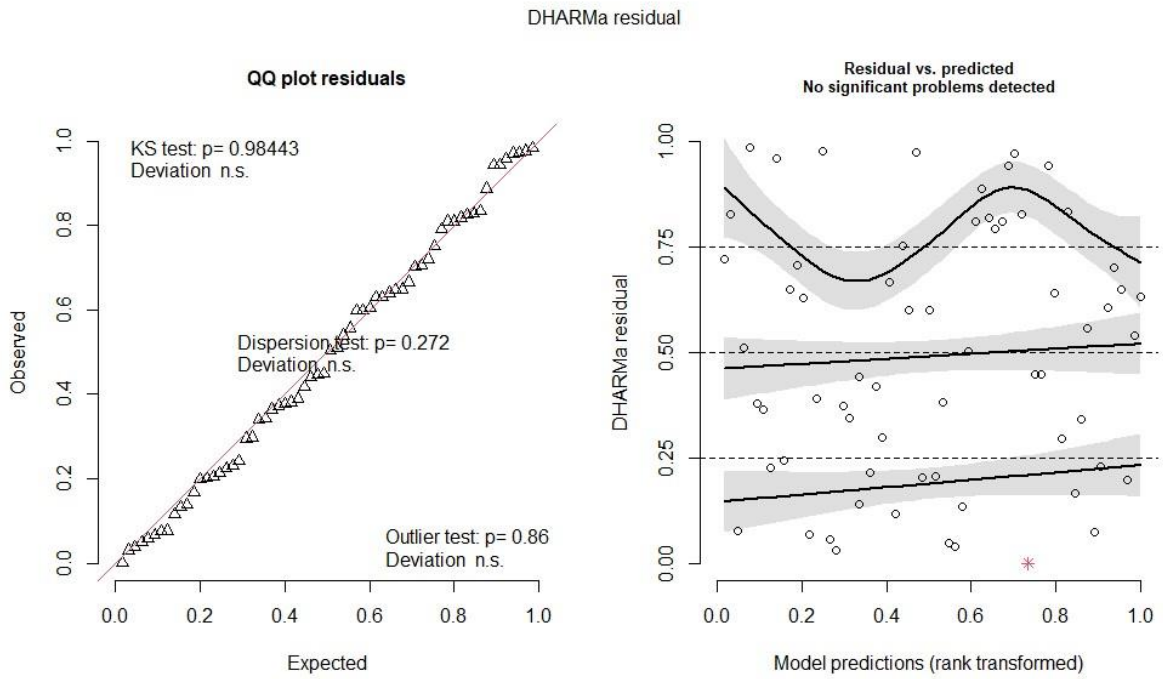


Figure 15 DHARMA residuals of 500m buffer model.

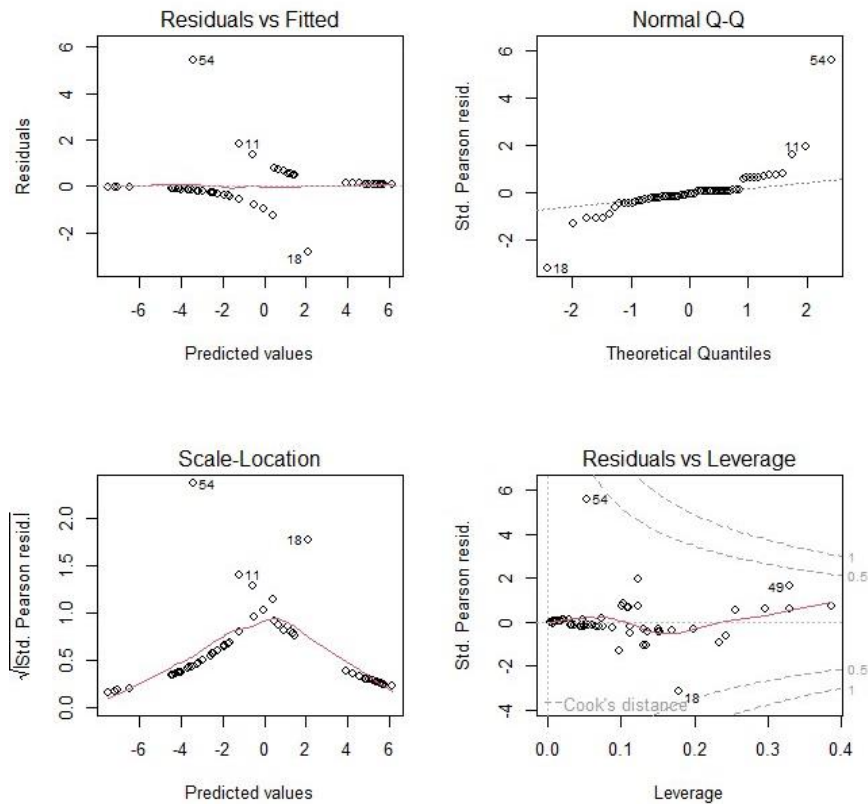


Figure 16 Residuals for the 500m buffer model.

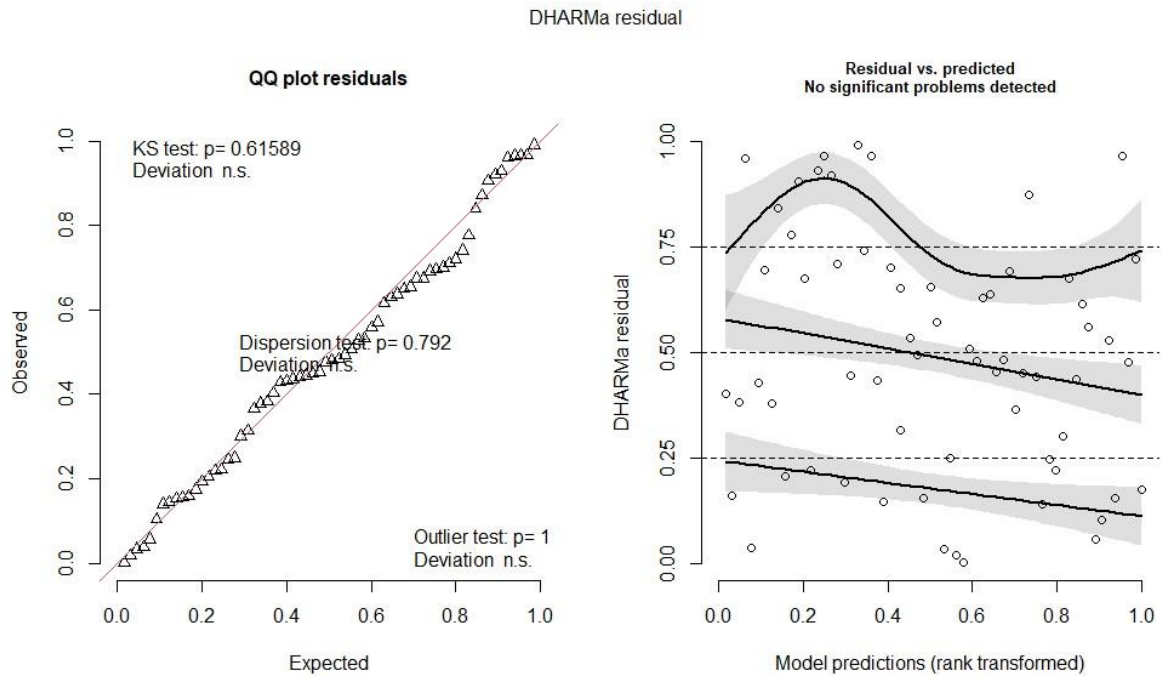


Figure 17 DHARMA residuals of 1km buffer model.

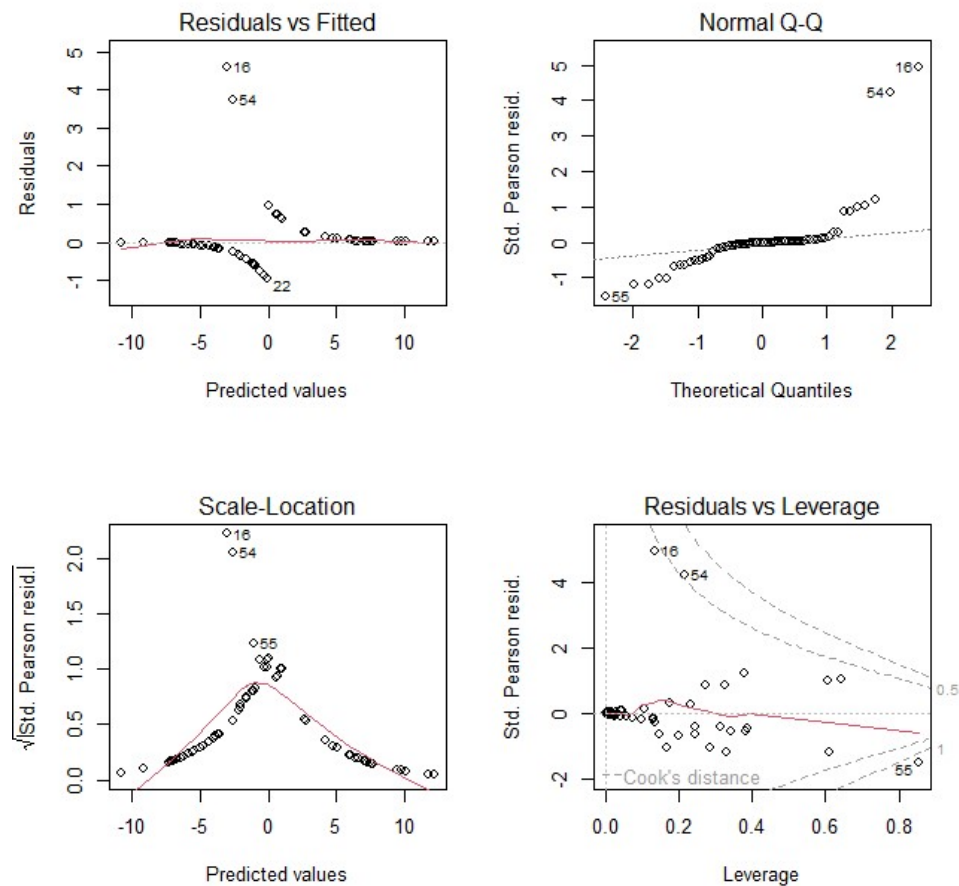
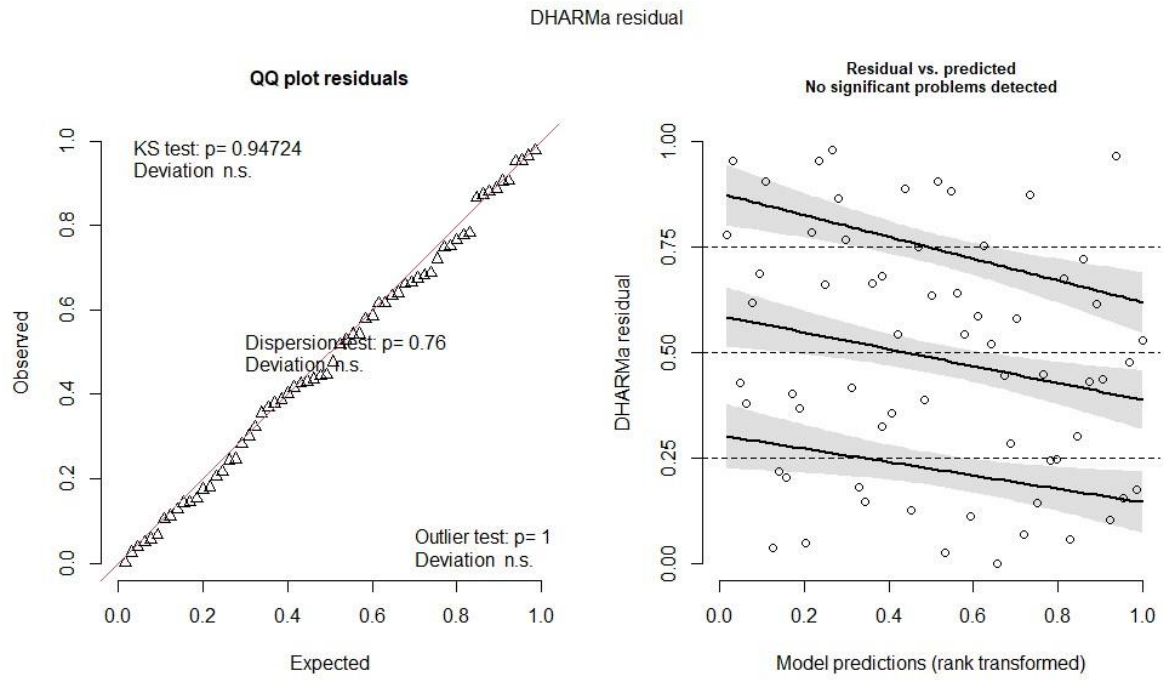
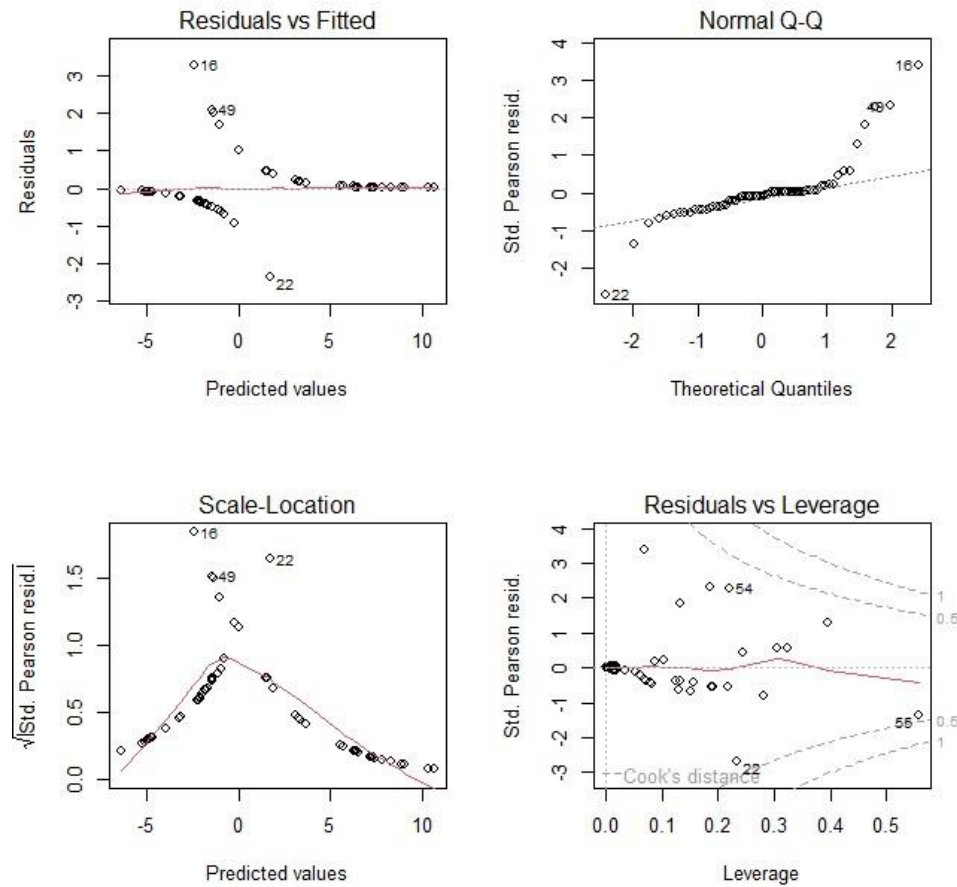


Figure 18 Residuals for the 1km buffer model

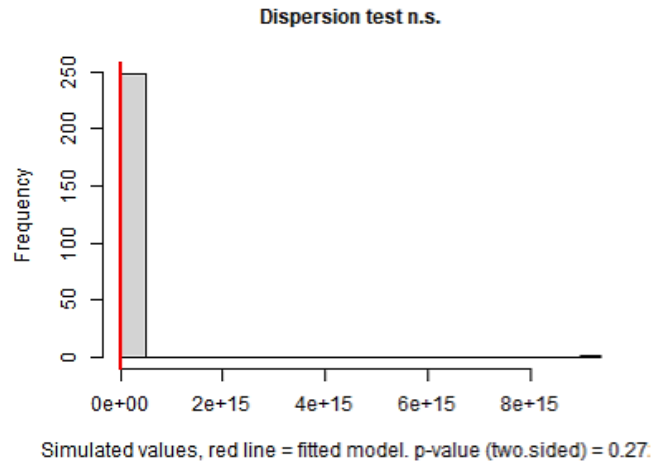




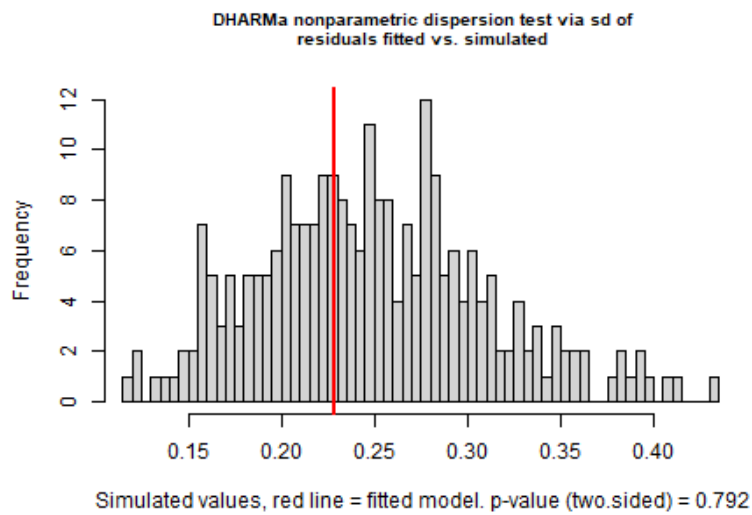
**Figure 19** 5km buffer model DHARMA residuals.



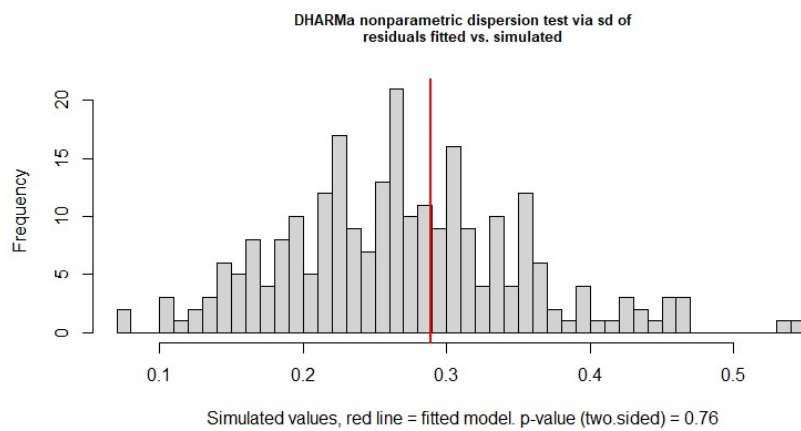
**Figure 20** Residuals for the 5km buffer model.



**Figure 21** DHARMA nonparametric dispersion test via sd of residuals fitted vs. simulated (500m).



**Figure 22** DHARMA nonparametric dispersion test via sd of residuals fitted vs. simulated (1km)



**Figure 23** DHARMA nonparametric dispersion test via sd of residuals fitted vs. simulated (5km)

**Table 11** DHARMA nonparametric dispersion test via sd of residuals fitted vs. simulated.

Data	500m model	1km model	5km model
Dispersion	0.272	0.792	0.76
Alternative hypothesis	two.sided	two.sided	two.sided