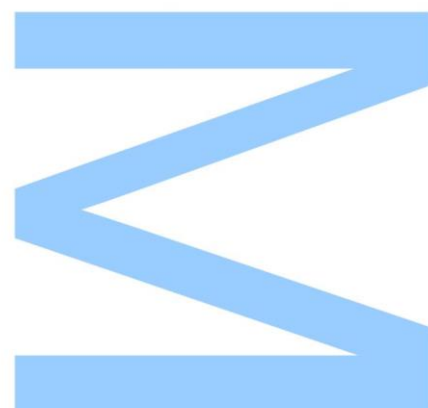




The effect of landscape history on bat species richness and habitat use in a future reservoir area



Inês Garcia de Brito Jorge

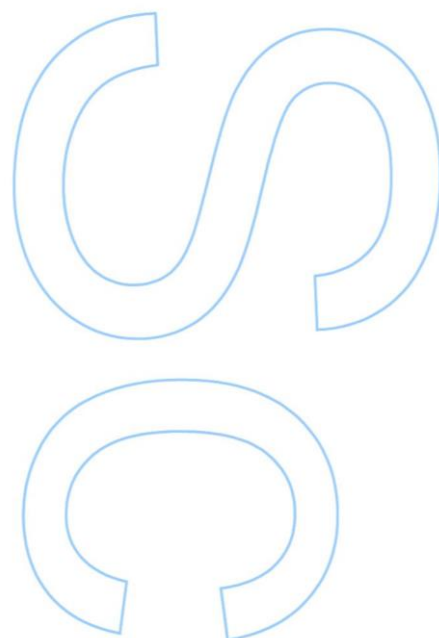
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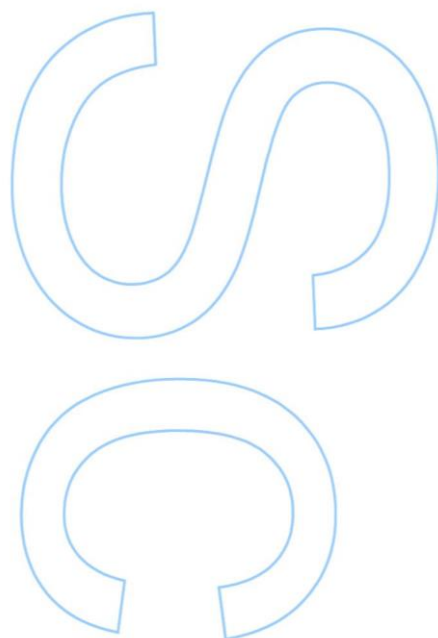
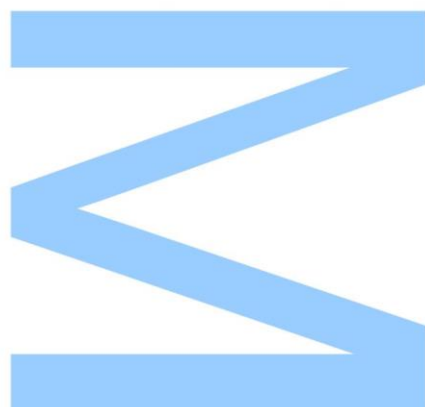




Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, ____/____/____



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Abstract

Landscape alteration and fragmentation are among the main drivers of current biodiversity loss. The building of a large dam and its reservoir in the Sabor river may cause a huge impact on the landscape, flooding numerous habitats, transforming a diverse and complex landscape into a homogenous wide reservoir and causing irreversible damages to local species. Consequently, these changes can affect the permanence of bat species in this ecosystem.

In this study, three sets of GLMM were made in order to (1) understand which are the drivers of bat species richness in the Sabor valley, (2) which habitats support greater bat species diversity and (3) how does landscape structure affect the occurrence of bat species.

The results showed that from the tested eco-geographical variables, the area of water bodies, number of patches of closed areas and the mean slope are drivers of bat species diversity in the study area. Also, besides the area of water bodies, small urban areas and native forests have positive effect on bat species richness, while arable lands have negative effect. On landscape structure, results showed that an intermediate level of number of patches (fragmentation) combined with patch richness of both closed and open areas influence positively bat species diversity. Taking these results into consideration, conservation measures on bat species richness can be designed for maintaining bat species diversity in the Sabor valley.

Keywords: bats, reservoir, fragmentation, homogenous, drivers, habitats, landscape structure

Resumo

A alteração e a fragmentação da paisagem estão entre as principais causas da perda de biodiversidade atual. A construção de uma grande barragem e do seu reservatório no rio Sabor pode causar um enorme impacto sobre a paisagem, submergindo vários habitats, transformando uma paisagem diversificada e complexa num grande corpo de água homogéneo e causando danos irreversíveis nas espécies de fauna e flora locais. Consequentemente, essas mudanças podem afetar a permanência de várias espécies de morcegos neste ecossistema.

Neste estudo, três conjuntos de GLMM foram desenvolvidos com o intuito de (1) compreender quais as variáveis ambientais que promovem a diversidade de morcegos no vale do Sabor, (2) quais os habitats que proporcionam uma maior diversidade de morcegos e (3) de que forma pode a estrutura da paisagem afetar a diversidade de morcegos.

Os resultados mostraram que a partir das variáveis eco-geográficas testadas (EGV's), as que promovem a diversidade de morcegos são a área de corpos de água, o número de manchas de parcelas de áreas fechadas e o declive médio. Mostraram também que, além da área de corpos de água, pequenas áreas urbanas e florestas nativas têm um efeito positivo na diversidade de morcegos, enquanto terras aráveis têm um efeito negativo. Relativamente à estrutura da paisagem, os resultados mostraram que um nível intermédio de número de parcelas (fragmentação), aliado à riqueza de parcelas de áreas abertas e fechadas, influenciam positivamente a diversidade de espécies de morcegos. Tendo estes resultados em consideração, poderão ser desenvolvidas medidas de conservação para manter a diversidade de morcegos no vale do Sabor.

Palavras-chave: morcegos, fragmentação, homogéneo, promotores, habitats, estrutura da paisagem

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1 | Introduction

Biodiversity decline in the last century is an acknowledged fact of great concern. Climate change, habitat loss and the introduction of exotic species are some of the main causes associated to this environmental problem (Fahrig 2003, Pereira, Leadley *et al.* 2010, Pereira, Navarro *et al.* 2012). Pereira, Navarro *et al.* (2012) identified habitat change as one of the main factors responsible for the decrease of global biodiversity. They classified habitat change into three categories: conversion of natural to humanized habitats, intensification of human use of human-dominated habitats and recovery of natural vegetation in areas of agricultural abandonment. With natural habitats changing to humanized environments, species permanence will mainly depend on the remnant patches of the original habitats remain isolated in a fragmented landscape, and the quality and connectivity between those patches (Krauss, Bommarco *et al.* 2010). Natural habitats remnants, such as forest patches, have major importance for biodiversity, since they are great food sources, provide shelter and may act as stepping stones or corridors for several animal species (Harvey, Guindon *et al.* 2000).

Primary forests are being lost worldwide at a rate of 15 million ha per year, mainly to agricultural use (ca. 60%) (Donald 2004): in Europe, 45% of the total land area is currently used for agriculture (FAO 2003, Rounsevell, Reginster *et al.* 2006). As a response to the need of freshwater for fields' irrigation, as well as for domestic consumption and as an alternative energy source, today, there are over 45,000 large dams (>15 meters height (McAllister, Craig *et al.* 2001)) around the world (Pereira, Navarro *et al.* 2012).

The conversion of forested areas into agriculture has been subject to a number of studies (Matson, Parton *et al.* 1997, Norris, Asase *et al.* 2010), but the impact of man-made structures like roads, industrial complexes and reservoirs is still a recent topic on environmental conservation (see Forester and Machlist (1996), Trombulak and Frissell (2000), McAllister, Craig *et al.* (2001)).

Despite the advantages of the use of hydropower, there are several acknowledged impacts of this type of energy production on the environment. The construction of big dams causes major irreversible impacts as the loss of ripicolous galleries and landscape homogenization. The disappearance of riparian areas implies the decline of

plants' biomass and insects' abundance, while the reservoir transforms the landscape into a wide uniform mass of water (McAllister, Craig *et al.* 2001).

In the northeast of Portugal, a large dam was built in the Sabor River valley. This valley is recognized by its rich biodiversity, which justified its inclusion on the Natura 2000 network (PTCON0021), as it is rich in endemic plant species, a corridor for several mammals as otters and wolves and a crucial habitat for numerous birds (Freitas and Horta 2003, Jackson 2011). Regardless of the acknowledged environmental loss, the project was accepted under the justification of the energy and local development benefits. The Baixo Sabor dam is over 120m high and its reservoir will have a total area of around 2730 ha (EDP 2002), flooding an important part of the Sabor River valley (ca. 50Km), together with the lower reaches of some of its main tributaries (EDP 2002, Jackson 2011).

The high biodiversity of the Sabor valley relates to its biogeographical location, lying in the transition zone between the Atlantic and the Mediterranean climate, gathering favourable conditions for species associated to either. Bats are one of the taxon where this is visible, since from the 25 species occurring in mainland Portugal, at least 17 species are known to be present in this area (Amorim, Alves *et al.* 2013, Amorim, Carvalho *et al.* 2014). Riparian habitats are of extreme importance for bat communities not only as foraging grounds (Russo and Jones 2003) but also as commuting routes (Verboom, Boonman *et al.* 1999). Moreover, due to the orography of the study area it is highly likely that the flooding of large areas of rocky habitats will affect important colonies of crevice-dwelling bat species.

Jones, Jacobs *et al.* (2009) discussed the importance of bats as bioindicators, concluding that bats' sensitivity to human-induced changes to habitats makes them great bioindicators. Bats are widely distributed and have slow reproductive rates, meaning that they take a long time to recover from population declines and through the monitoring of their populations information on the impact of these alterations can be obtained (Jones, Jacobs *et al.* 2009, Amorim, Carvalho *et al.* 2014).

A study on the effect of forest fragmentation on bats was carried by Cosson, Pons *et al.* (1999), in an area flooded by a dam in French Guiana, where a previously forest-dominated matrix led to several hundred land-bridge islands. By establishing capture stations before and after the flooding, Cosson *et al.* came to the conclusion that the main cause of the decline in diversity and abundance in frugivorous bats was the forest patches' (the land-bridge islands) isolation in the new water matrix, either because

these patches have lower ecological quality than similar patches on the original forest or because fragmentation difficult the movement between patches. Likewise, Rebelo and Rainho (2009) carried out a study on the largest reservoir in Europe, the Alqueva dam, in Alentejo (Southern Portugal). This construction has resulted on the deforestation and flooding of an area of 250km², changing the entire landscape; by surveying the bat activity with the use of ultra-sound detectors before and after the flooding, they concluded that bat activity had declined over the reservoir area, but had increased in the surrounding areas. Their results also confirmed the importance of riparian habitats as foraging areas. Given the scale of Sabor valley, and despite having a smaller dimension than the Alqueva reservoir, the ecological impact of this reservoir can bring irreversible changes to bat species richness.

Despite bats sensitivity to habitat change and their potential as bioindicators, no compensation measures were statistically designed yet. Thus, the landscape alteration caused by the Sabor dam arises as an excellent opportunity to use data collected prior to its construction to understand how bat species richness is spatially shaped in the Sabor valley.

1.1 | Objectives

The landscape changes predicted to occur in the Sabor valley may affect the survival or permanence of bats in this ecosystem. To develop appropriate conservation and protection measures that efficiently promote the development of bat communities and diversity, it is essential to understand how they are distributed in the Sabor valley.

The main aim of the present study is to understand what supports bat species richness in a Mediterranean fragmented landscape and how this diversity is shaped by different landscape features. These results can be used to design compensation measures that promote bat species diversity in the region.

Hence, the specific objectives of this study were: a) to understand which are the drivers of bat species diversity in the Sabor valley; b) which habitats support greater bat species richness; c) how does landscape structure affect the occurrence of bat species.

2 | Methods

2.1 | Study area

The Sabor River has its headwaters in Spain and flows south, crossing the northeastern Portuguese region of Trás-os-Montes through approximately 120km until it reaches the Douro River (Fig. 1). It is surrounded by a mountainous matrix, where flattened areas by the river and uplands contrast with deep valleys and steep rocky slopes. The altimetry of the Sabor valley varies between 110m and 1190m. Located at the transition between the Mediterranean and the Euro-Atlantic biogeographic regions, where the climate is characterized by short and extremely hot summers average temperature ca. 17.5°C (www.ipma.pt)) and cold and humid winters (average temperature ca. 7.8°C (www.ipma.pt)) (Costa, Aguiar *et al.* 1998); the mean annual temperature is approximately 12.7°C (www.ipma.pt).

These orographic and climatic characteristics reflect on the landscape and ecosystems of the study area: sparse areas of perennial oaks (*Quercus suber*, *Quercus ilex*) and juniper (*Juniperus oxycedrus*) forests and native shrubland, interrupted by fruit farm and extensive agriculture patches; also, the riparian gallery follows the river through a major part of its course. During the last decades the human activities mainly associated to agriculture have shaped the region into a highly fragmented mosaic, where urban areas located in the surrounding plateau together with the difficult access to the river valley resulted in a mix of highly natural areas and extremely transformed and humanized ones, with great value to several endangered species (ICNB). The importance of this valley for biodiversity conservation has been recognized through its inclusion as a Special Protected Area in Natura 2000 Network.

The study area was delimited by considering ICNB (2010) guidelines for monitoring plans of dams, in which a buffer of 5Km around the quota of maximum load is suggested. There are two reservoirs predicted to be created by respective dams – distancing 3km and 12km of the river mouth. The main one, the Baixo Sabor Dam, is going to reach the quota of 239m, flooding an approximate area of 2822ha with maximum length of 50Km. Moreover, the lower reaches of Maçãs and Angueira Rivers, two tributaries of Sabor, will also be affected, since the flooding area will extend over 50km of the Sabor River valley.

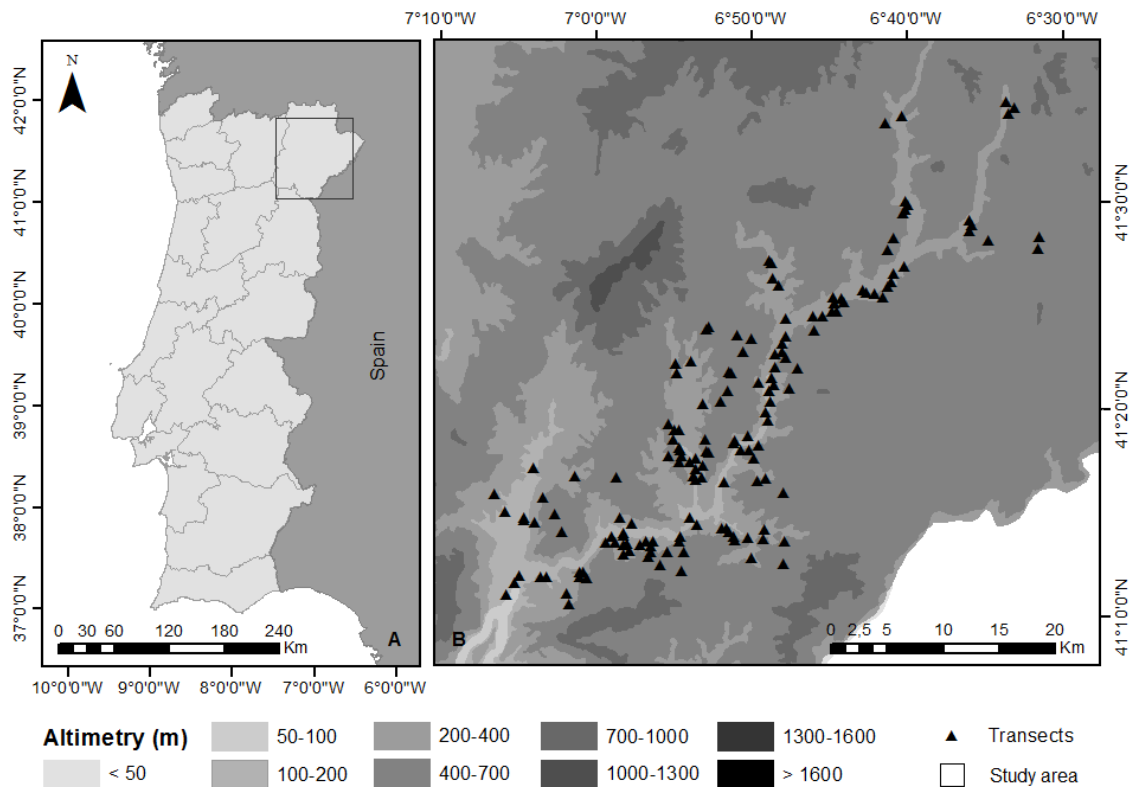


Figure 1 - Location of the study area in the northeast of Portugal (A) and location of the conducted transect along the Sabor valley (B)

2.2 | Ultra-sound survey

Data on bat activity was collected from June to October 2011 and from May to September 2012. A previous random selection of the sampling sites and posterior adjustment to include representative types of habitats (by visiting the field) was made. At each selected site, a walking transect was conducted: transects' duration was standardized to 15 minutes and walked at a constant low pace (ca. 2km.h^{-1}).

Bats were sampled using a bat detector (D1000X Ultrasound Detector, Pettersson Elektronik AB, Uppsala, Sweden). The acoustic recording started 45 minutes after sunset - this way, both early and later emergence bat species have time to reach foraging sites - and lasted for 3 hours to ensure that the sampling timing coincided with peak of bat activity (Russo and Jones 2003, Wickramasinghe, Harris *et al.* 2003)

To avoid biases in the bat activity analyses, surveys were only performed when climatic conditions were favourable - temperatures above 10°C , no rain, fog or strong winds (Russo and Jones 2003, Rainho 2007). Also, no lights were used during the acoustic surveys to minimize disturbance.

2.3 | Ultra-sound analyses

A bat detector is a device that allows the detection and recording of the bat ultra-sound calls. However, it does not enable the identification of individuals, only provides information on bat activity and allows some level of species identification. Recorded vocalizations were analysed using sound-analysis software BatSound Pro 3.31 (Pettersson Elektronik AB). In order to measure call parameters, this programme generates oscillograms, allowing the measurement of temporal variables, as well as power spectrums to record frequencies. Thereby, species identification was made using published data on bat acoustics (Boonman, Dietz *et al.*, Schnitzler, Kalko *et al.* 1987, Kalko and Schnitzler 1989, Jones 1995, Waters, Rydell *et al.* 1995, Waters and Jones 1995, Barlow and Jones 1997, Ahlen and Baagøe 1999, Russo and Jones 1999, Parsons and Jones 2000, Siemers and Schnitzler 2000, Russo and Jones 2002, Pfalzer, Kusch *et al.* 2003, Martin, Boesch *et al.* 2004, Rainho, Amorim *et al.* 2011, Walters, Freeman *et al.* 2012). Due to similarity between the vocalization characteristics of some species, not all echolocation calls were able to be identified (Russo and Jones 2002); not identified calls were classified as unidentified species.

2.4 | Variable selection

A set of ecological meaningful variables were chosen to determine what were the main drivers of bat species diversity in the study area. Therefore, a set of eco-geographical variables (EGV's) was chosen and divided into five categories: climatic, orographic, land cover, landscape configuration and distance variables. These type of variables have already been acknowledged to delimit bat occurrence and diversity (Ulrich, Sachanowicz *et al.* 2007).

From a set of climatic variables made available by Santos (2010), 18 variables were chosen (Table 1). For each of the selected variables was then calculated its minimum, maximum, mean and median - using ArcGIS 10.1 (ESRI, INC.) software - resulting in a total of seventy-two variables. A preliminary assessment of the correlation between these variables revealed that strong correlations between several variables (Pearson correlations were assessed and variables were considered correlated whenever $r > 0.7$ (Dormann, Elith *et al.* 2013); in order to eliminate the correlations, a Principal Component Analysis (PCA) was made.

Table 1 - Description of the climatic variables adapted from Santos (2010)

Abbreviation	Description
anom_io_hum	Ombrothermic index anomaly in humid year
anom_io_dry	Ombrothermic index anomaly in dry year
Ic	Continental index, or annual thermal amplitude
Io	Annual ombrothermic index
io_hum	Equivalent ombrothermic index for humid year
io_dry	Equivalent ombrothermic index for dry year
ios2	Ombrothermic index of the warmest bimonth of the summer quarter
ios3	Ombrothermic index of the summer quarter
ios4	Ombrothermic index of the summer quarter plus the previous month
It	Thermicity index
M	Mean maximum temperature of the coldest month
M	Mean minimum temperature of the coldest month
Pp	Positive precipitation (sum of monthly precipitation, relative to months with positive mean temperature)
pp_hum	Annual positive precipitation in humid year
pp_dry	Annual positive precipitation in dry year
Tmax	Mean temperature of the warmest month of the year
Tmin	Mean temperature of the coldest month of the year
Tp	Annual positive temperature (sum of the positive monthly mean temperatures, in Celsius degree x10)

The orographic variables were calculated from the Digital Elevation Map (DEM), using ArcGIS 10.1 (ESRI 2011) software to obtain the study area's altimetry and slope (divided into three different variables: areas of slopes higher than 20 degrees, 30 degrees and 40 degrees; see Table 2). Also, eastness and northness were calculated using the tool Raster Calculator from ArcGIS 10.1 (ESRI 2011) according to the Zar (1999):

$$Eastness = \sin \frac{aspect \times \pi}{180} \quad Northness = \cos \frac{aspect \times \pi}{180}$$

Similarly to the climatic variables, the minimum, maximum, mean and median were calculated for these variables resulting in a total of 19 variables.

Table 2 - Description of the orographic variables

Abbreviation	Description
altim	Altimetry
east	Eastness
north	Northness
slope	Slope
AreaSlope20	Area of slope bigger than 20°
AreaSlope30	Area of slope bigger than 30°
AreaSlope40	Area of slope bigger than 40°

For the land cover variables, the Land Cover Maps (Carta de Ocupação dos Solos – COS) from 1990 and 2007 were joined, using ArcGIS 10.1 software (ESRI 2011), to describe the transects' surrounding habitats (on a 500 meters buffer); the information from the two sources was always validated using CELPA (the Portuguese Association for the Paper Industry; www.celipa.pt) maps and Google Maps images. Nine types of habitats were chosen to reclassify the habitats: native forest, coniferous forest, eucalyptus groves, shrubland, fruit farm, arable land, urban areas, water bodies and riparian gallery. For each buffer, the proportion of each habitat type area was calculated (Table 3). Furthermore, habitat types were classified as closed or open areas according to vegetation density (Table 3).

Table 3 - Description of the land cover variables and its classification in open or closed areas

Abbreviation	Description	Vegetation density
AreaNF	Proportion of area of native forest	Closed areas
AreaUA	Proportion of area ou urban areas	Closed areas
AreaH2O	Proportion of area of water bodies	Open areas
AreaFF	Proportion of area of fruit farm	Open areas
AreaConif	Proportion of area of coniferous forest	Closed areas
AreaEuc	Proportion of area of eucalyptus groves	Closed areas
AreaShrub	Proportion of area of shrubland	Open areas
AreaAL	Proportion of area of arable land	Open areas
AreaRG	Proportion of area of riparian gallery	Closed areas

Considering the importance of the landscape configuration to bat navigation, four structural landscape variables were calculated for the closed and open areas, namely: number of patches, patch richness, edge density and mean patch area (Table 4).

These variables were calculated using R Statistics 3.1.0 (R Development Core Team 2010) and Fragstats 4 (McGarigal, Cushman *et al.* 2012) software.

Table 4 - Description of the landscape configuration variables

Variable	Description
ED_O	Edge density of open areas
ED_C	Edge density of closed areas
NP_O	Number of patches of open areas
NP_C	Number of patches of closed areas
MN_A_O	Mean area of open areas' patches
MN_A_C	Mean area of closed areas' patches
PR_O	Patch richness of open areas
PR_C	Patch richness of closed areas

Native forests, urban areas and steep slopes are ideal roosting habitats for some bat species (Kunz 1982); also, some species might prefer east or north facing slopes (Arlettaz 1999, Flaquer, Torre *et al.* 2006). As the distance to specific environmental resources might be crucial to the presence of some bat species, a set of distance variables was calculated using ArcGIS 10.1 (ESRI 2011) (see Table 5).

Table 5 - Description of the distance variables

Variable	Description
DistNF	Distance to native forest
DistUA	Distance to urban areas
DistSlope20	Distance to slope bigger than 20°
DistSlope30	Distance to slope bigger than 30°
DistSlope40	Distance to slope bigger than 40°
DistEast	Distance to east facing slopes
DistWest	Distance to west facing slopes
DistNorth	Distance to north facing slopes

2.5 | Statistical analyses

Generalised Linear Mixed Models (GLMM) were the selected method to model the drivers of bat activity. This class of models has been referred by some authors (McCulloch and Neuhaus 2005) as the best tool to deal with non-normal distributed data with random effects variables which introduce variation that might affect the response variable while not being explanatory.

GLMMs are a combination of Generalized Linear Models, which handle non-normal data distribution like binomial, and Linear Mixed Models, which integrate both fixed and random effects (Bolker, Brooks *et al.* 2009). GLMM are a generalization of a linear regression which models a response variable through a set of fixed effects (independent variables) linear combinations, with the addition of random effects. In this study, the year and the month of each transect were considered the random effects. The total number of species or species groups detected in each transect, a count variable, was the response variable. Three sets of GLMM were made in order to achieve different objectives: the first aiming to determine which ecologically meaningful variables are drivers of bat species richness; the second, to assess which habitats promote bat species richness, and the last set of GLMM was made to understand how landscape features affect bat species presence. All GLMM were calculated using the packages lme4 and MuMIn of R statistics.

Aikaike information criterion (AIC) is a model selection criterion which measures the relative quality of a set of models for the given data; the model with the lower AIC value represents the most informative model from the set (Johnson and Omland 2004). A series of univariate models were made with the variables chosen for each set of GLMM separately, to determine informative variables on bat species diversity; only AIC scores lower than the null model were considered for subsequent analyses. Subsequently, combinations of the variables with AIC scores lower than the null model were made to calculate multivariate GLMM. Also, in order to test specific effects of landscape on bat species richness, two sets of GLMM were made: the first with previously selected habitat variables, and the second with landscape structure variables.

The differences between the AIC value of a given model and the lowest AIC value in the totality of the models is known as ΔAIC and is essential for ranking the models according to information loss (Burnham, Anderson *et al.* 2011): ΔAIC values between 1 and 5 have substantial support, $5 > \Delta AIC \geq 10$ have relatively less support and ΔAIC

> 10 have basically no support (Burnham, Anderson *et al.* 2011). For this study, a threshold of $\Delta AIC \leq 5$ was fixed for multivariate model selection.

For the determination of variable importance, Akaike weights (AIC_w) were calculated, as they are indicative of the weight of evidence in favour of a model and are interpreted as the probability of a model being the best model for the data (Burnham, Anderson *et al.* 2011).

Finally, response curves were calculated for each of the relevant variables in order to understand how these variables relate to the obtained data.

3 | Results

3.1 | Ultra-sound analyses

A total of 155 transects were sampled corresponding to 38 hours and 45 minutes. As a result, 6929 bat passes were recorded of which approximately 34% were not possible to identify and were consequently discarded. Analyses of the remaining calls revealed the presence of a total of 18 classes of species and/or species group (Table 6 and Fig. 2).

Table 6 - List of identified species or species groups and number of registered contacts

Species/Species group	Abbreviation	Nº of contacts
<i>Barbastella barbastellus</i>	<i>Bbar</i>	10
<i>Eptesicus serotinus</i> or <i>Eptesicus isabelinus</i>	<i>Eser/Eisa</i>	45
<i>Eptesicus species</i> or <i>Nyctalus leisleri</i>	<i>Ept/Nlei</i>	66
<i>Myotis daubentonii</i>	<i>Mdau</i>	366
<i>Myotis escaleraei</i>	<i>Mesc</i>	2
<i>Myotis</i> large species (<i>Myotis myotis</i> or <i>Myotis blythii</i>)	<i>Myo. large spp</i>	2
<i>Myotis</i> small species (<i>Myotis daubentonii</i> , <i>Myotis escaleraei</i> , <i>Myotis emarginatus</i> , <i>Myotis nattereri</i>)	<i>Myo. small spp</i>	327
<i>Nyctalus lasiopterus</i> or <i>Nyctalus noctula</i>	<i>Nlas/noc</i>	7
<i>Nyctalus leisleri</i>	<i>Nlei</i>	134
<i>Nyctalus</i> species	<i>Nyc. spp</i>	24
<i>Hypsugo savii</i>	<i>Hsav</i>	32
<i>Pipistrellus kuhlii</i>	<i>Pkuh</i>	790
<i>Pipistrellus Pipistrellus</i>	<i>Ppip</i>	2124
<i>Plecotus</i> species	<i>Plec</i>	9
<i>Rhinolophus ferrumequinum</i>	<i>Rfer</i>	10
<i>Rhinolophus hipposideros</i>	<i>Rhip</i>	22
<i>Rhinolophus</i> species	<i>Rhino spp</i>	11
<i>Tadarida teniotis</i>	<i>Tten</i>	580
Total identified calls		4561

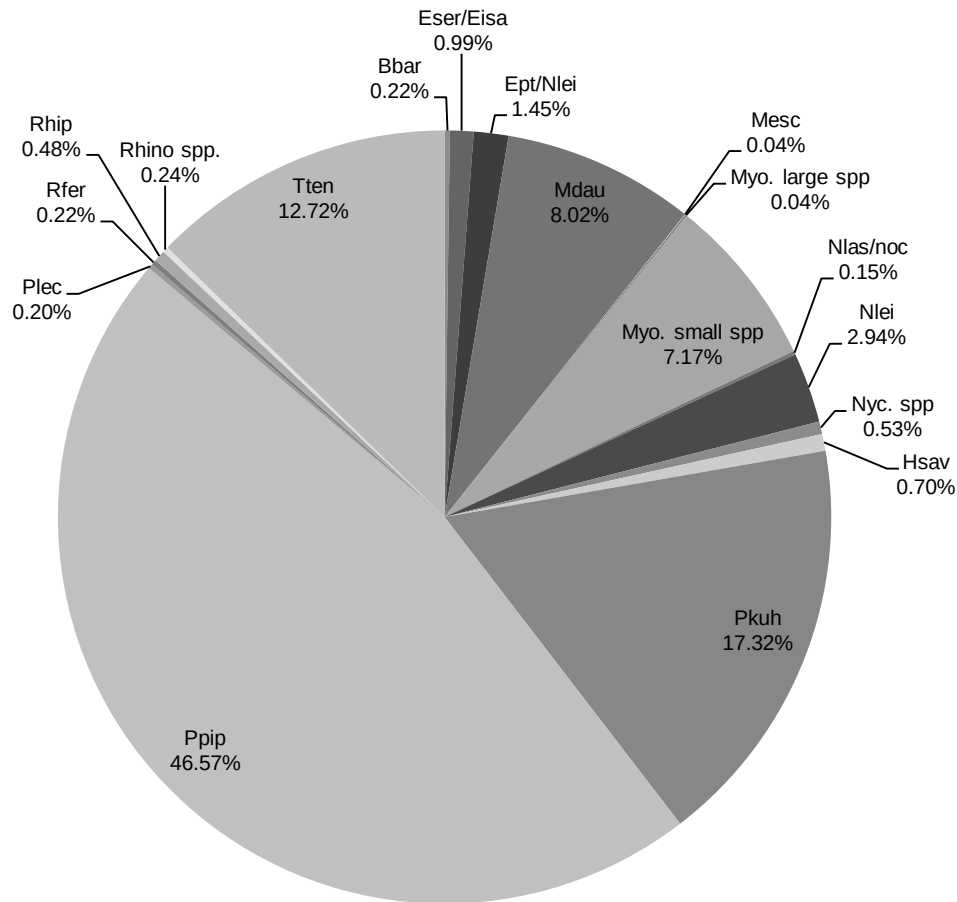


Figure 2 - Percentage of identified species' contacts in the total of conducted transects

As previously referred, some bat species have similar echolocation characteristics (Russo and Jones 2002), making the species identification a challenging task. Therefore, in some cases, it was only possible to identify the group of species to which the bat call belongs (e.g. "*Plecotus* species"). However, as it is possible to observe in Table 6, "*Myotis* small species" might be redundant, as it includes *Myotis daubentonii* and *Myotis escaleraei* species, which are two other defined classes; in this case, accounting for "*Myotis* small species" was only considered in transects whose *Myotis daubentonii* and *Myotis escaleraei* have not been identified, in order to avoid redundancy and information loss. The approach was similar for "*Nyctalus* species" in relation to the species group "*Nyctalus lasiopterus* or *Nyctalus noctula*" and "*Nyctalus leisleri*", for "*Rhinolophus* species" with respect to "*Rhinolophus ferrumequinum*" and "*Rhinolophus hipposideros*", and for "*Eptesicus* species or *Nyctalus leisleri*" regarding

“*Eptesicus serotinus* or *Eptesicus isabelinus*”, “*Nyctalus leisleri*” and “*Nyctalus* species”.

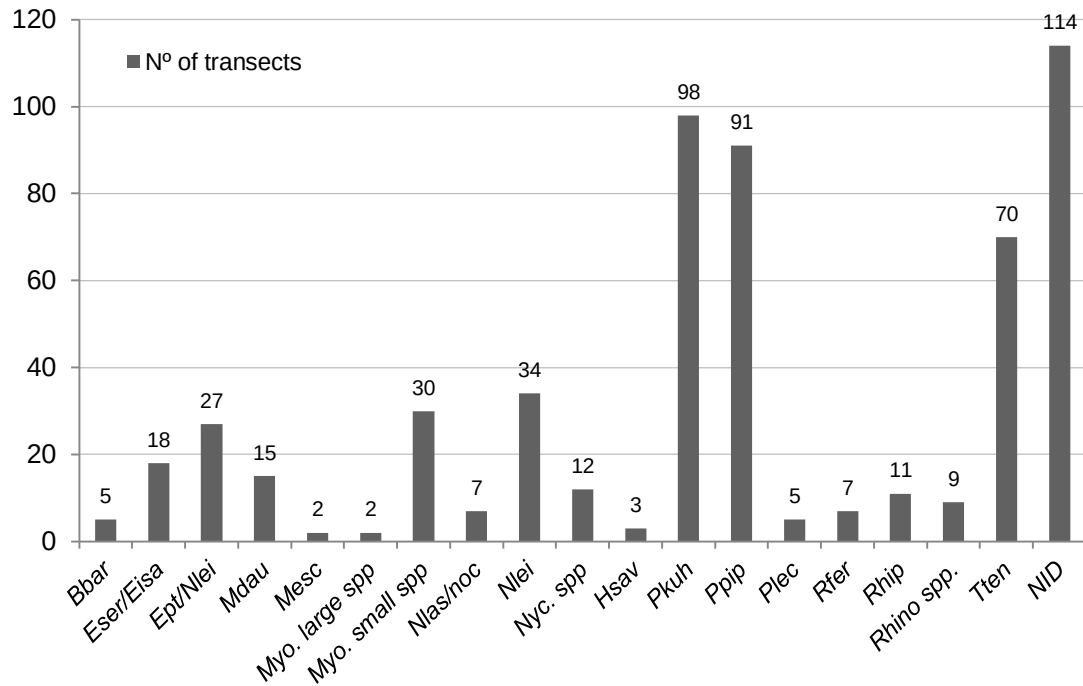


Figure 3 – Absolute frequency of identified species presence in transects (see table 6 for species abbreviations)

The species that have been detected over a higher number of transects were *Pipistrellus* species, *Pkuh* and *Ppip*, in over 90 transects, followed by *Tten*, present in approximately half of the 155 transects (Figure 3). Calls from *Ept/Nlei*, *Myo. small spp* and *Nlei* species were detected in between 17% and 22% of the transects, while other species were only found in less than 18 transects (Figure 3). In a total of 114 transects there were detected bat species whose identification was not possible.

3.2 | PCA

From the seventy-two climatic variables, two principal components resulted (PC1 and PC2; see Figure 4). The proportion of variance of PC1 and PC2 were 76.4% and 7.9%, respectively, resulting in a total of 84.3% of the variance explained by these two components. The variables which had the strongest influence in the PC1, but low influence on PC2, were the temperature related variables (tmin, tmax, M, m, it and tp

(see Table 1 for abbreviations) and the ombrothermic indexes. For the PC2, the ic and both anom_io_hum and anom_io_dry were the most explanatory variables.

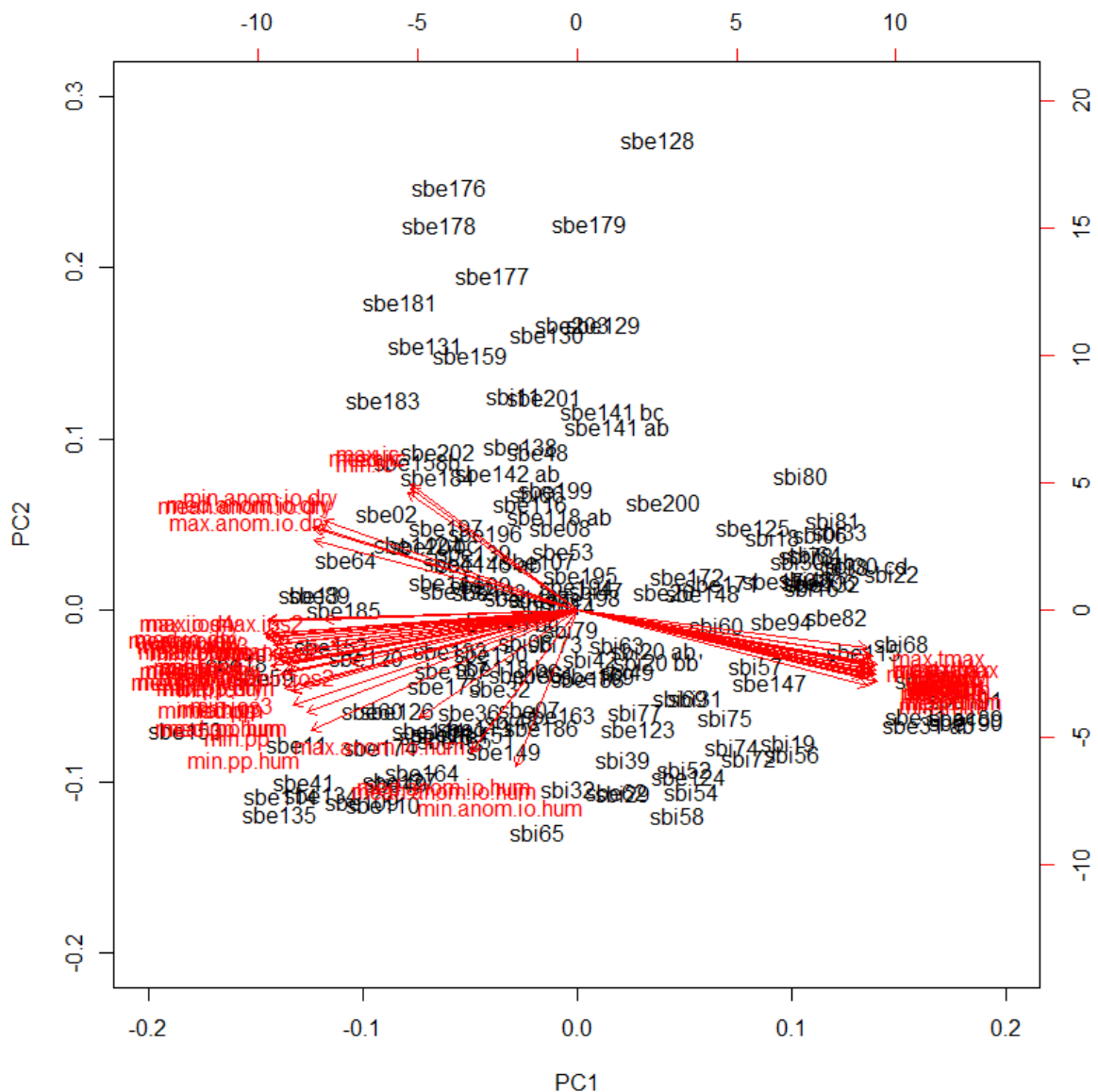


Figure 4 - Principal Components Analysis of climatic variables

3.3 | Drivers of bat species diversity

In the following table (Table 7) it is possible to see that from 23 variables tested with univariate GLMM, 16 have an AIC value lower than the null model; however, in order to obtain a parsimonious set of multivariate models, only three variables were selected: the **AreaH2O**, having the lower AIC from the set of tested variables and consequently being the most informative variable, followed by **NP_C** and **MeanSlope** (Table 7). All the remaining variables were discarded.

Table 7 - Univariate GLMM models on driver of bat species diversity, ranked by Aikake information criteria (AIC) and Aikake differences (Δ AIC). Each model is named by the variable tested (see Tables 2, 3 and 4 for variable abbreviations; in bold, the three models with Δ AIC $\leq$ 5)

Model	AIC	Δ AIC
AreaH2O	624.0961	0.0000
NP_C	645.7837	21.6876
MeanSlope	650.1756	26.0795
PR_C	657.1647	33.0686
AreaUA	657.6503	33.5542
AreaNF	657.9549	33.8588
AreaSlope40	658.8562	34.7601
AreaAL	660.4431	36.3470
MIN NORTH	660.6234	36.5273
MIN EAST	661.9744	37.8783
AreaRG	662.2363	38.1402
AreaEuc	662.4228	38.3267
MED ALTIM	664.4442	40.3481
PR_O	665.4076	41.3115
AreaConif	666.0261	41.9300
AreaFF	666.1327	42.0366
<u>Null</u>	666.4325	33.0686
PC1	667.8030	43.7069
AREA_MN_C	668.7958	44.6997
ED_C	670.3002	46.2041
PC2	671.6850	47.5889
AREA_MN_O	672.9076	48.8115
NP_O	672.9150	48.8189
ED_O	673.5959	49.4998

The results of the candidate models (Table 8) show that only D1 and D2 have a Δ AIC $\leq$ 5; according to its AICw, D1 and D2 explained the species richness recorded in up to 95% of the transects. Although the Δ AIC of the D3 model was higher than 5, this model explained only 4% of the species richness patterns in the study area. Considering all potentially informative variables, these three models together explained a total of 99% of the bat species richness in the survey.

The area of water bodies and the number of patches of closed areas were selected in both models D1 and D2, showing their relevance for predicting bat species richness (Table 9). All these three variables are positively related with bat species diversity.

Table 8 - Multivariate GLMM for drivers of bat species richness – candidate models. Reference for model identification (Ref.), Log-likelihood, Aikake information criteria (AIC), Aikake differences (Δ AIC), Akaike weights (AICw) and accumulated AIC weights; in bold, the two models with Δ AIC $\leq$ 5.

Ref.	Model	Log-likelihood	AIC	Δ AIC	AICw	Accumulated AICw
	<u>Null</u>	-329.2163	666.4325	51.4940	0.0000	
D1	AreaH2O + NP_C	-301.4693	614.9386	0.0000	0.7356	0.7356
D2	AreaH2O + MeanSlope + NP_C	-301.7016	617.4031	2.4646	0.2145	0.9501
D3	AreaH2O + MeanSlope	-304.3248	620.6497	5.7111	0.0423	0.9924
D4	AreaH2O	-307.0480	624.0961	9.1575	0.0076	1.0000
D5	MeanSlope + NP_C	-315.0680	642.1360	27.1974	0.0000	1.0000
D6	NP_C	-317.8918	645.7837	30.8451	0.0000	1.0000
D7	MeanSlope	-320.0878	650.1756	35.2370	0.0000	1.0000

Table 9 - Candidate models' variables importance. Summary showing the variable name (see Tables 2, 3 and 4 for abbreviations), its Importance, n° of containing models, variable slope estimate (Estimate), variable slope estimate standard error (St. Error), Z-test score (Z value) and the probability of Z-test fit ($P(>|z|)$) (- 0.01; * - 0.05; . - 0.1; - 1)**

Variable	Importance	N° containing models	Estimate	St. Error	Z value	P ($> z $)
AreaH2O	1.00	2	29.48298	5.71181	5.12	0.00000 ***
NP_C	1.00	2	0.09779	0.02561	3.79	0.00015 ***
MeanSlope	0.23	1	0.05325	0.02315	2.28	0.02256 *

As for the response curves of the three informative variables (Fig. 5), it is possible to observe that all are positively related to bat species richness. However, it is important to notice that with respect to the AreaH2O, only a few transects were conducted in sites with over 8% of the buffer area occupied by water bodies. The same applies to buffers where the number of patches was larger than 20.

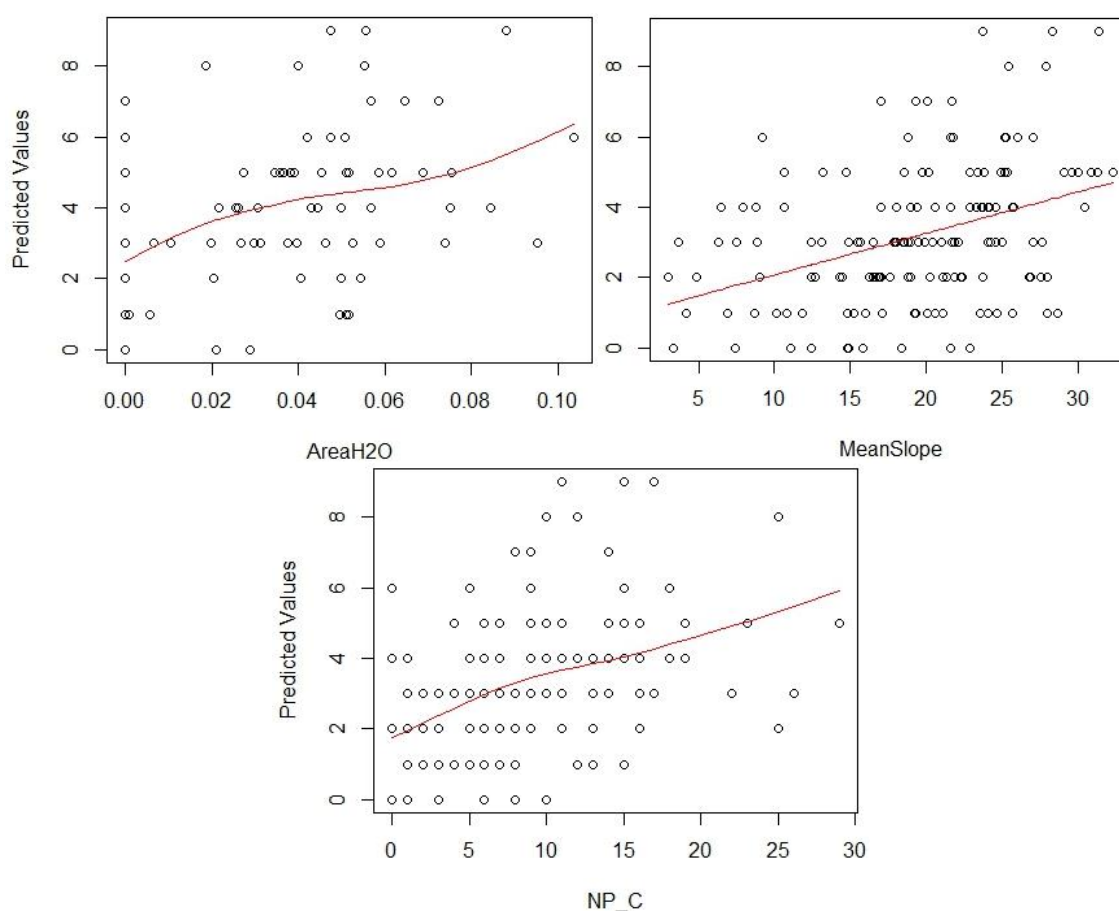


Figure 5 - Response curves of the informative variables from the drivers of bat species diversity model (AreaH2O, MeanSlope, NP_C) vs. Predicted Values

3.4 | Effect of habitat composition on bat species diversity

To understand how habitat shaped bat species richness in the study area a series of GLMM were calculated considering only the habitat variables. Univariate GLMM on habitat composition showed that the only habitat that does not provide information on bat species diversity is the area of shrubland (Table 10). The most informative variable was again the area of water bodies (AreaH2O), solidifying its importance to bats.

Although the remaining variables are informative (with the exception of AreaShrub already referred), only AreaUA, AreaNF and AreaAL were selected to be included in the candidate model, along with AreaH2O (Table 11). The area of urban areas was one of the least represented types of habitats in the transects total area, however, this variable had the second lowest AIC score, showing that this variable might be important for some bat species and supporting other studies with similar results (Russo and Jones 2003, Avila-Flores and Fenton 2005, Rainho 2007). Also, native forests are often referred in literature as important features for maintenance of bat species diversity (Russo and Jones 2003, Davy, Russo et al. 2007, Rainho 2007). On the other

hand, arable lands were one of the most represented types of habitats in the study area, and thus, it is important to understand its influence on bat species richness.

Table 10 - Univariate GLMM models on effect of habitat composition on bat species diversity, ranked by Aikake information criteria (AIC) and Aikake differences (Δ AIC). Each model is named by the variable tested (see Table 3 for variable abbreviations; in bold, the four models selected for the candidate model)

Model	AIC	Δ AIC
AreaH2O	624.0961	0.0000
AreaUA	657.6503	33.5542
AreaNF	657.9549	33.8588
AreaAL	660.4431	36.347
AreaRG	662.2363	38.1402
AreaEuc	662.4228	38.3267
AreaConif	666.0261	41.9300
AreaFF	666.1327	42.0366
<u>Null</u>	666.4325	42.3364
AreaShrub	667.2042	43.1081

Table 11 - Multivariate GLMM for drivers of bat species richness – candidate models. Reference for model identification (Ref.), Log-likelihood, Aikake information criteria (AIC), Aikake differences (Δ AIC), Akaike weights (AICw) and accumulated AIC weights; in bold, the two models with Δ AIC $\leq$ 5)

Ref.	Model	Log-likelihood	AIC	Δ AIC	AICw	Accumulated AICw
	<u>Null</u>	-329.2163	666.4325	52.9295	0.0000	
H1	AreaH2O + AreaUA + AreaNF + AreaAL	-298.7515	613.5030	0.0000	0.4711	0.4711
H2	AreaH2O + AreaUA + AreaNF	-300.2537	614.5073	1.0043	0.2851	0.7562
H3	AreaH2O + AreaUA + AreaAL	-300.9000	615.8000	2.2970	0.1494	0.9056
H4	AreaH2O + AreaUA	-302.7112	617.4223	3.9193	0.0664	0.9720
H5	AreaH2O + AreaNF + AreaAL	-303.3626	620.7252	7.2222	0.0127	0.9847
H6	AreaH2O + AreaNF	-304.8331	621.6662	8.1632	0.0080	0.9926
H7	AreaH2O + AreaAL	-305.2983	622.5966	9.0936	0.0050	0.9976
H8	AreaH2O	-307.0480	624.0961	10.5931	0.0024	1.0000
H9	AreaUA + AreaNF + AreaAL	-315.2718	644.5436	31.0406	0.0000	1.0000
H10	AreaUA + AreaNF	-318.0988	648.1975	34.6945	0.0000	1.0000
H11	AreaUA + AreaAL	-319.7514	651.5028	37.9998	0.0000	1.0000
H12	AreaNF + AreaAL	-321.1548	654.3097	40.8067	0.0000	1.0000
H13	AreaUA	-323.8251	657.6503	44.1473	0.0000	1.0000
H14	AreaNF	-323.9775	657.9549	44.4519	0.0000	1.0000
H15	AreaAL	-325.2216	660.4431	46.9401	0.0000	1.0000

Despite all the candidate models being more informative than the null model, a total of four models have a ΔAIC lower than 5: H1, H2, H3 and H4 (see Table 11). These four models together explain 97% of the data acquired.

The area of water bodies and urban areas were present in all the four best models and have positive relationship with the species diversity (Table 12). From the four variables selected for the candidate models, only the area of arable lands was a detractor of bat species diversity (Table 12); the remaining variable, area of native forests, seems to promote the presence of bat species though with a lower impact than the water bodies' area.

Table 12 - Candidate models' variables importance significance. Summary showing the variable name (see Table 3 for abbreviations), its Importance, n° of containing models, variable slope estimate (Estimate), variable slope estimate standard error (St. Error), Z-test score (Z value) and the probability of Z-test fit ($P(>|z|)$) (*) - 0.001; ** - 0.01; * - 0.05; . - 0.1; - 1)**

Variable	Importance	N containing models	Estimate	St. Error	Z value	Pr (> z)
AreaH2O	1.00	4	33.15230	5.91050	5.57	0.00000 ***
AreaUA	1.00	4	21.21330	16.56000	1.27	0.20390
AreaNF	0.78	2	1.54410	0.91220	1.68	0.09320 .
AreaAL	0.64	2	-1.15550	1.07980	1.06	0.28850

The predicted values for the water bodies' area confirmed that bat species richness is positively influenced by the presence of water bodies (Fig. 6). Despite the response curve of the AreaUA showing a positive relation with the diversity of bat species, the number of transects where this type of habitat was present was too low (Fig. 6). Furthermore, while native forests also are positively related with bat species richness, arable lands tend to decrease bat species diversity with the increase of proportion of area.

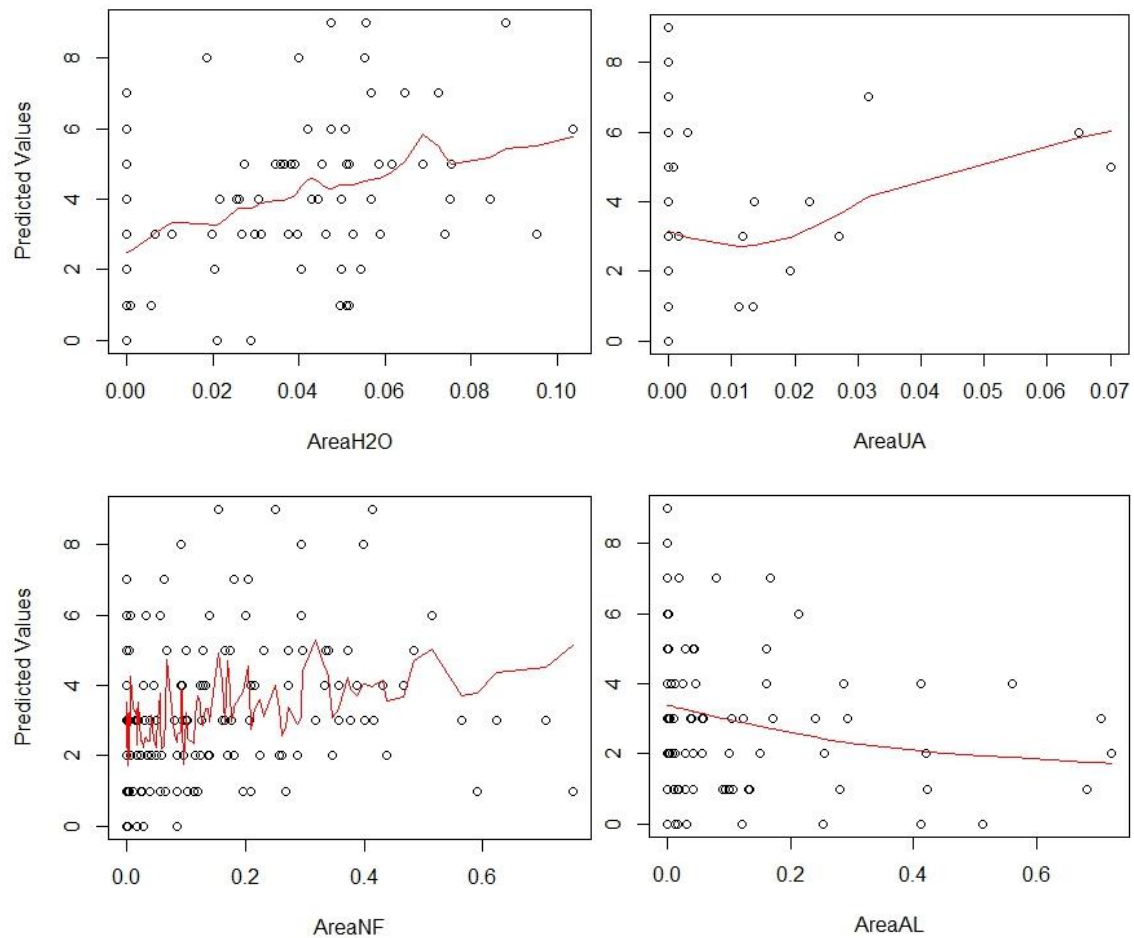


Figure 6 - Response curves of the four more informative variables from the effect of habitats on bat species model (AreaH2O, AreaUA, AreaNF and AreaAL) vs. Predicted Values.

3.5 | Effect of landscape structure on bat species diversity

To study the effect of landscape structure on bat species richness, eight variables were modelled in univariate GLMM. The results show that the number of patches of closed areas was the most informative variable (Table 13); patch richness of both closed and open areas also provide information on species richness of bats and, along with the number of patches of closed areas, were selected for the candidate model.

Using the selected variables for the candidate models, a total of seven multivariate models were made (Table 14). Only four of the candidate models showed a $\Delta AIC \leq 5$ (LS1, LS2, LS3 and LS4). Regarding the probability of the species richness being represented by these models, LS1 and LS2 are the most likely models, having AICw values of ≈ 0.43 and ≈ 0.38 , respectively.

Table 13 - Univariate GLMM models on the effect of landscape structure on bat species diversity, ranked by Aikaike information criteria (AIC) and Aikaike differences (Δ AIC). Each model is named by the variable tested (see table 4 for variable abbreviations; in bold, the three models with AIC value lower than the null model)

Model	AIC	Δ AIC
NP_C	645.7837	0.0000
PR_C	657.1647	11.3810
PR_O	665.4076	19.6239
<u>Null</u>	666.4325	20.6488
AREA_MN_C	668.7958	23.0121
ED_C	670.3002	24.5165
AREA_MN_O	672.9076	27.1239
NP_O	672.9150	27.1313
ED_O	673.5959	27.8122

Table 14 - Multivariate GLMM for drivers of bat species richness – candidate models. Reference for model identification (Ref.), Log-likelihood, Aikaike information criteria (AIC), Aikaike differences (Δ AIC), Akaike weights (AICw) and accumulated AIC weights; in bold, the two models with Δ AIC $\leq$ 5.

Ref.	Model	Log-likelihood	AIC	Δ AIC	AICw	Accumulated AICw
	<u>Null</u>	-329.2163	666.4325	20.6489	0.0000	
LS1	NP_C	-317.8918	645.7837	0.0000	0.4301	0.4301
LS2	NP_C + PR_O	-317.0213	646.0425	0.2589	0.3779	0.8081
LS3	NP_C + PR_C + PR_O	-317.3936	648.7872	3.0035	0.0958	0.9039
LS4	NP_C + PR_C	-318.4683	648.9366	3.1530	0.0889	0.9928
LS5	PR_C + PR_O	-321.2109	654.4217	8.6381	0.0057	0.9985
LS6	PR_C	-323.5823	657.1647	11.3810	0.0015	1.0000
LS7	PR_O	-327.7038	665.4076	19.6240	0.0000	1.0000

The variable that revealed the greatest importance for the effect of landscape structure on bat species diversity was the number of patches of closed areas which was present in the four candidate models (Table 15). Patch richness of both closed and open areas, were only present in two models though with a positive relationship with species richness.

All response curves from the effect of landscape on bat species diversity show a positive influence on bat species richness (Fig. 7).

Table 15 - Candidate models' variables importance significance. Summary showing the variable name (see Table 4 for abbreviations), its Importance, n° of containing models, variable slope estimate (Estimate), variable slope estimate standard error (St. Error), Z-test score (Z value) and the probability of Z-test fit ($P(>|z|)$) (- 0.001; * - 0.01; . - 0.05; - - 0.1; - 1)**

Variable	Importance	N containing models	Estimate	St. Error	Z value	Pr ($> z $)
NP_C	1.00	4	0.13626	0.02758	4.90	0.00000***
PR_C	0.48	2	0.39385	0.23011	1.70	0.08960.
PR_O	0.19	2	0.12329	0.20853	0.59	0.55750

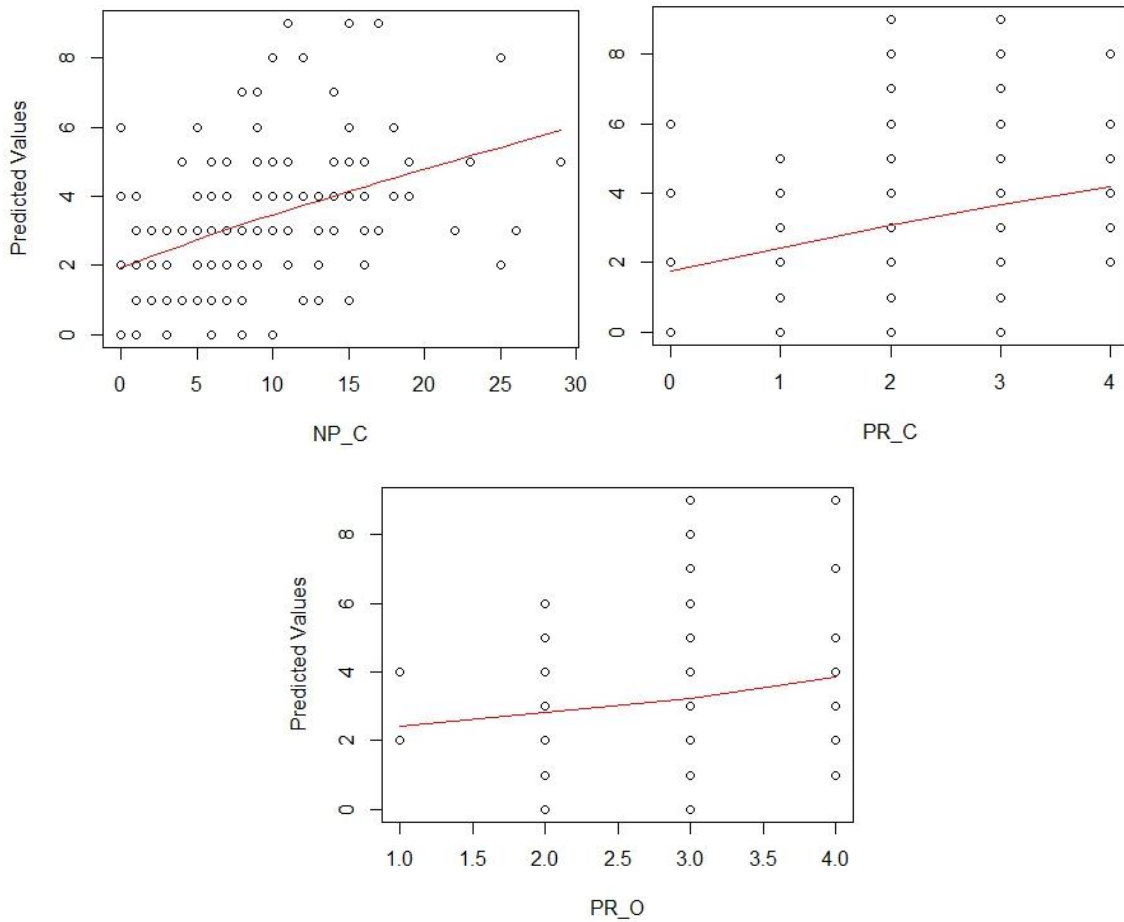


Figure 7 - Response curves of the informative variables from the effect of landscape structures on bat species model (NP_C, PR_C, and PR_O) vs. Predicted Values.

4 | Discussion

Results of this study suggest that bat species diversity is strongly associated with the existence of water bodies. The number of patches of closed areas and mean slope are features to take into account as well, regarding the conservation of bat species in the Sabor valley. Furthermore, an intermediate level of fragmentation and diverse landscape offers bats different roost and feeding opportunities (Neuweiler 1989, Fenton 1997).

The Sabor valley fragmented landscape offers a high level of habitat diversity and landscape structural features. The strong influence of water bodies showed in the results might be related with the aridity of the region, since most of the smaller streams and ponds dry out during the hot summers. On the other hand, patches are drivers of bat species diversity since they are used as commuting corridors between roosting and foraging habitats in a landscape dominated by wide agricultural open areas (Estrada and Coates-Estrada 2001).

The presence of species classified by the Portuguese Red Book of Vertebrates (Livro Vermelho dos Vertebrados de Portugal (2005)) as vulnerable (VU) or critically endangered (CR), such as *R.ferrumequinum* (VU), *R.hipposideros* (VU), *M.blythii* (CR) and *M.myotis* (VU), underpin the need to better understand what drives species diversity in the region.

4.1 | Drivers of bat species diversity

The importance of water bodies to bat species has consistently been acknowledged by several studies on bat habitat use in the Mediterranean (Carmel and Safriel 1998, Russo and Jones 2003, Russo, Almenar *et al.* 2005, Rainho 2007, Di Salvo, Russo *et al.* 2010, Lisón and Calvo 2011). This habitat provides not only fresh water for bats to drink, but also is one of its main foraging habitats due to insects availability.

As for the number of patches of closed areas, the results suggest that fragmentation favours bat species richness possibly because some species prefer more open areas, others closed, and mosaic favours both. Medina, Harvey *et al.* (2007) concluded that the presence of forest patches in agricultural landscapes is of high importance, once it provides food, shelter from predators and possible roost sites. Also, patches of closed

areas are important navigation references, since they increase edge effect, the interface between a closed and an open area, where bats have specialized in commuting over echo-clutter (Neuweiler 1989); in other words, the increase in the number of patches promotes the extent of available edges. These patches are also possible roosts for tree-dwelling bat species (Kunz 1982).

Finally, slopes are also important drivers of bat species diversity since they represent roost availability for crevice-dwelling species (Kunz 1982) and can be used as linear features for navigation as well (Lentini, Gibbons *et al.* 2012).

4.2 | Habitat importance

The results of the habitat model showed, once again, that water bodies are of main importance for bat species. Moreover, these habitats tend to register not only the greatest species richness, but also the highest bat activity (Lisón and Calvo 2011).

Other studies on bat habitat use (Jaberg and Guisan 2001, Rainho 2007) confirm that, urban areas and native forests are important habitats for bat species diversity. Urban areas are possible roosts for some species, like *Pipistrellus* species, that are also known to forage in these areas near street lamps (Rydell and Racey 1995), while for other species, such as *Myotis* spp., native forests are the type of habitat that provides those resources (Jaberg and Guisan 2001).

Unlike the previous variables, the results showed that arable lands have a detrimental effect on bat species richness. Wickramasinghe, Harris *et al.* (2003) concluded that bats are affected by agricultural intensification through the use of agrochemicals or monocultures, as insects are also less abundant in these habitats (Wickramasinghe, Harris *et al.* 2004), and consequently bats do not search arable lands for foraging. Moreover, being an open area, these habitats do not provide roosting opportunities and increase the exposition to predators (Downs and Racey 2006).

4.3 | Landscape structure

The effect of landscape structure on bat species diversity was shown to be complex on the Sabor valley. The number of patches of closed areas and the patch richness of either closed or open areas were the most important variables. These results are supported by other studies that concluded that bats select highly fragmented

landscapes composed by a mosaic of diverse habitats (Russ and Montgomery 2002, Bernard and Fenton 2007).

A higher patch richness of closed and open areas offers more foraging opportunities, since different species have different preferences when it comes to foraging areas (Neuweiler 1989).

Some level of landscape fragmentation might benefit bat species richness depending on the constitution of the surrounding matrix, and if the connectivity between foraging and roosting habitats is not lost (Russ and Montgomery 2002). An increased number of patches of closed areas can be used by bats as stepping stones, allowing the movement of bats in the landscape, decreasing isolation and providing shelter from predators (Estrada and Coates-Estrada 2001, Downs and Racey 2006). Also, habitats densely cluttered might create difficulties to bat flight due to the presence of a great number of obstacles (Bernard and Fenton 2007).

4.4 | Caveats, limitations and futures studies

Species identification from bat echolocation calls was one of the main limitations. Species have different detectability and whispering species, such as those from the genus *Plecotus*, can be difficult to record while other can be misidentified due to the similarity between their echolocation calls. The correct identification of a higher proportion of the total recorded calls would allow the analysis of which environmental variables promote the presence of each species individually. However, many species were identified consistently and are indicative of other species presence, allowing the analysis of bat species richness on the Sabor valley.

Future studies should focus on the contribution of landscape history to the formation of the landscape mosaic, and how bat species diversity and populations will evolve as a response to landscape alteration.

4.5 | Conservation implications

According to the models' results and the response curves, there are some environmental features to take into consideration when planning conservation measures for the maintenance of bat species diversity. Also, as bats are bioindicators,

conservation measures designed in order to maintain their diversity will promote diversity of other taxa as well.

The flooding of the river valley will transform a fragmented and diversified landscape in a wide single homogenized feature. The presence of small water bodies is acknowledged as a bat species diversity driver, suggesting that small streams and ponds should be maintained. Furthermore, there is limited information on wide areas of water (a maximum of $\approx 10\%$ of a buffer was occupied by water bodies), but the results show that bat species richness increases with habitat heterogeneity and densely vegetated areas.

As for the forest fragmentation, it is important to understand that an intermediate level of fragmentation will increase species richness. This way, a net of patches and/or corridors of native trees should be created in order to minimize the effect of increase of agriculture in the area.

At last, a monitoring plan on bat populations and roosts along cliffs and other previously identified roosts in the Sabor valley should be set, in order to follow how bat species richness will be affected by the construction of the two dams and respective reservoirs.

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