Universidade de Évora - Instituto de Investigação e Formação Avançada

Programa de Doutoramento em Biologia

Tese de Doutoramento

Landscape functional connectivity and animal movement: application of remote sensing for increasing efficiency of road mitigation measures.

Francesco Valerio

Orientador(es) | António Mira
Sara Maria Lopes Santos
Sérgio Rui Godinho

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To my father,
and the last legacy of his mortal life:

Resilienza, stoicismo ed aspettando Godot,
chiunque esso sia.

Resilience, stoicism and waiting for Godot,
whoever he may be.

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Landscape functional connectivity and animal movement: application of remote sensing for increasing efficiency of road mitigation measures

Abstract

Roads are a major threat to wildlife due to induced mortality and restrictions to animal movement. A central issue in conservation biology is the accurate site identification for the implementation of multispecies mitigation measures, on roads. Those measures entail high costs and methodological challenges and their efficiency highly depend on the right location. The aim of this PhD is to inform, through remote sensing and connectivity modelling, how to increase the efficiency of planning mitigation measures to reduce roadkill and promote connectivity; and demonstrate the usefulness of remote sensing in defining suitable areas for the conservation of an endangered species that often occurs in the vicinity of roads. To do so, we first assessed whether occurrence-based strategies were able to infer functional connectivity, compared to those more complex and financially demanding based on telemetry, with respect to daily and dispersal movements. Secondly, we assessed whether remote sensing data were sufficiently informative to identify key habitats for a threatened species around road verges. Thirdly, we assessed the predictive and prioritisation ability of road mitigation units intercepting multispecies corridors to prevent vulnerability to roadkill. Findings revealed that simple models are suitable as complex ones for both daily and dispersal movements, allowing for costly-effective connectivity assessments. Results demonstrated the ability of free remote sensing data to identify microhabitat conditions in verges and surrounding landscape, for a threatened rodent, allowing for the delimitation of refugee areas and definition of monitoring strategies for the species. Undemanding data (occurrence and remote sensing) were able to describe species-specific ecological requirements for birds, bats and non-flying mammals as well as roadkill patterns, possibly due to similar overlapping corridors and habitats, despite some mismatches that occurred for highly mobile species. This framework ensured high efficiency in prioritisation of multispecies roadkill mitigation planning, resilient to long-term landscape dynamics.

Keywords

Landscape connectivity; Landscape ecology; Multispecies; *montado* agro-forest-ecosystem; Remote sensing; Road ecology; Road verges; Road mitigation; Wildlife roadkill.

Conectividade funcional da paisagem e movimento animal: aplicação da detecção remota para aumentar a eficiência de medidas de mitigação em estradas.

Resumo

As estradas constituem uma enorme ameaça para a vida selvagem devido à mortalidade. Uma questão central é a identificação dos locais para implementar medidas de mitigação multi-espécies, em estradas. Essas medidas envolvem custos elevados e desafios metodológicos e sua eficiência depende muito da localização correcta. O objetivo deste doutoramento é informar, através de detecção remota e conectividade, como aumentar a eficiência do planeamento de medidas de mitigação para reduzir atropelamentos e promover a conectividade; e demonstrar a utilidade da detecção remota na definição de áreas adequadas para a conservação de espécies ameaçadas que podem ocorrer nas proximidades de estradas. Portanto, primeiro avaliamos se os dados resultantes de amostragens simples eram capazes de inferir conectividade funcional, em comparação com estratégias complexas, respeito aos movimentos diários e de dispersão. Segundo, avaliamos se os dados de detecção remota eram suficientemente informativos para identificar habitats-chave para uma espécie ameaçada em torno das margens das estradas. Terceiro, avaliamos a capacidade preditiva e de prioritização das unidades de mitigação de estradas que cruzam corredores multi-espécies para reduzir o risco de atropelamentos. Os resultados revelaram que os modelos simples são adequados quanto os complexos para os movimentos diários e de dispersão. Os resultados demonstraram a capacidade dos dados de detecção remota gratuitos em identificar condições de microhabitats nos habitats de berma e na paisagem circundante, para um roedor ameaçado, permitindo a delimitação de áreas de refúgio. Dados pouco exigentes (ocorrência e detecção remota) foram capazes de descrever os requisitos ecológicos específicos de aves, morcegos e mamíferos não voadores, bem como padrões de atropelamentos, possivelmente devido a corredores e habitats semelhantes, apesar de haver algumas incompatibilidades para espécies de maior mobilidade. Essa estrutura foi capaz de garantir uma elevada eficiência na prioritização de planeamento de mitigação de atropelamentos para multi-espécies, resiliente à dinâmica da paisagem de longo prazo.

Palavras-chave

Ecologia das estradas; Mortalidade por atropelamentode animais selvagens; Margens das estradas; Ecologia da paisagem; Detecção remota; Conectividade da paisagem; mitigação; multi-espécies; Sistema agro-florestal *montado*.

Chapter 1

General Introduction

Ecosystems and wild species contribute to the economic and social well-being of billions of people through the multiple benefits they provide: from food to cultural services, along with biotic regulation of climate and biogeochemical processes (Díaz et al., 2018; Malhi et al., 2020). Currently, the footprint of human activities and landscape changes are acting as key drivers of ecosystems and species decline, with extinction rates accelerating 1,000 to 10,000 times faster than normal rates in Earth' past life history (Dirzo et al. 2014; Primm & Joppa, 2015). Such dramatic loss supports the evidence that Earth is entering the sixth mass extinction, presenting a critical challenge to the Convention on Biological Diversity (CBD), where nations are currently negotiating the Post-2020 Global Biodiversity Framework to halt biodiversity loss (Bongaarts 2019). Biodiversity, referred as the variety of organisms, is being wiped out as anthropogenic pressures increase, likewise reflecting the ongoing development of human societies (Dirzo et al. 2014; Young et al. 2005). Infrastructure expansion is closely linked to economic growth worldwide, and is considered a major deterioration factor for ecosystems, habitats and biodiversity (Coffin 2007; Forman & Alexander, 1998). Thus, in light of the multifaceted negative ecological impacts from expanding linear infrastructures (van der Ree et al., 2015a), cost-effective solutions are increasingly needed to optimise wildlife conservation measures, particularly along roads (Clevenger & Huijser, 2011; Polak et al., 2019; van der Grift et al., 2017).

Background

1.1 ***Road effects on wildlife populations***

Earth terrestrial biodiversity is declining in tandem with human growth and the subsequent expansion of roads, increasing global conservation concerns (IPBES, 2019). Earth surface is approximately covered by 36 million kilometres of major roads, considered responsible for disrupting native patches into smaller fragments, nowadays estimated to be around 600000 units mostly with an area lesser than 1km^2 (Ibisch et al., 2016). Roads are widespread worldwide (Figure 1.1.1), and are expected to expand considerably in the next decades, which will likely place additional negative impacts on biodiversity (Laurance et al., 2014).

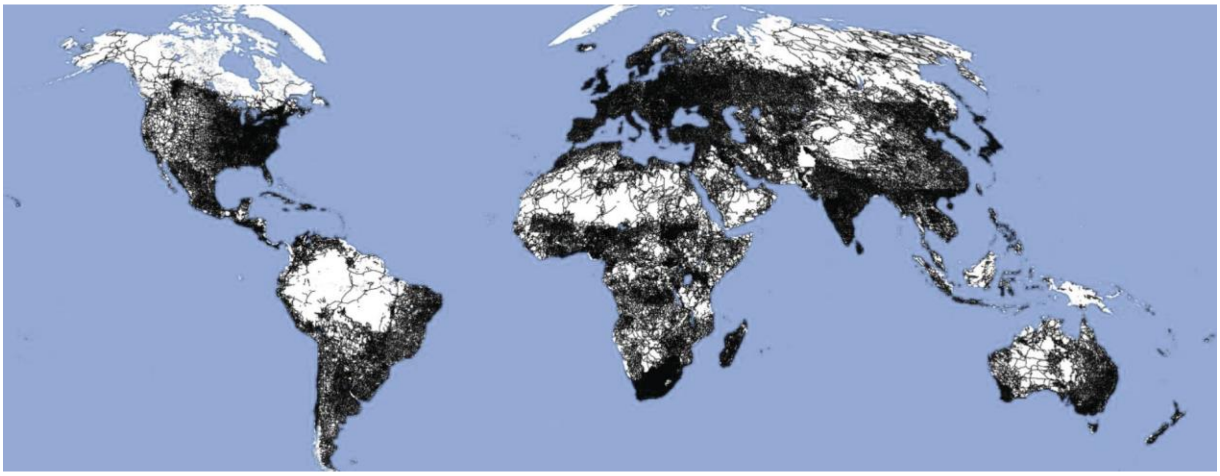

Figure 1.1.1. Road extension (in black) in actual world. Adapted from Laurance et al. (2014)

Mounting evidence suggests that roads are affecting natural areas far more adversely than other types of human development; and as a result, attention is turning towards environmental impact assessments (EIAs) as a legal protocol to diminish their negative impacts (Geneletti, 2003; Thompson et al., 1997). Since 1990, the ecological impacts of roads have received interest at an international level (Andrews, 1990; Pagany, 2020), culminating into the conceptualisation of "road ecology", an emerging discipline aiming the study of "the ecological effects (both positive and negative) of roads on environments and wildlife populations" (Coffin, 2007; Forman et al., 2003; Forman & Alexander, 1998). Over the past decades, research in this area has progressively uncovered the direct and indirect negative impacts derived from roads, that had long been jeopardising both habitats and ecosystems functionality (Bennett, 2017). The first and most obvious impact from roads begin with its construction, resulting into the physical disruption of landscapes and habitat patches, the latter being completely destroyed in the worst cases, or drastically altered in their compositional and configurational properties (Geneletti, 2003; Young et al., 2005). Habitats, at the interface with roads, underwent subdivision into smaller patches with a more pronounced edge, giving way to further habitat fragmentation and reduced landscape connectivity (Bennett, 2017; Wilson et al., 2016). Habitat degradation and loss are among the biggest threats to biodiversity (Dirzo et al. 2014), and in this regard, roads are considered the most prevalent landscape alteration trigger since the early 20th century (Geneletti, 2003; Trombulak & Frissell, 2001), with an expansion up to 60% (25 million of kilometres) foreseen until 2050 (Laurance et al., 2014). Such a considerable expansion is a matter of concern also due to the roads associated "Pandora' box" of many additional ecological problems: facilitated access to natural resources, accelerated deforestation, wildlife mortality, as well as encouraged speculative activities (Benítez-López et al, 2010; Gross, 2016; Laurance et al., 2014). The road expansion and construction phase represent a direct serious threat to wildlife (e.g., for species with low mobility; Trombulak & Frissell, 2001), even though mortality may also persist over longer term in the form of wildlife collisions with vehicles (Coffin, 2007; Forman et al., 2003; Forman & Alexander, 1998).

Mortality from vehicle collisions, henceforth referred to as roadkill, constitutes the second most important source of anthropogenic mortality (Forman & Alexander, 1998; Schwartz et al., 2020), and because of that, it is receiving a central attention in conservation biology worldwide. This interest is not a sole consequence of the infrastructure expansion and mortality effects over pristine natural areas (Laurance et al., 2014), but also of the high mortality rates on roads, where traffic volume is intensifying. On this issue, for most roads, a substantial growth in vehicle volume traffic is expected for the next decade, corresponding to a doubling of motor vehicles on roads, roughly from one billion in 2010 to two billion by 2030 (Gross, 2016). This will reasonably lead to higher vulnerability for some wildlife populations, challenging transport agencies and conservation scientists to reduce mortality risks, as well as to restore landscape connectivity. Connectivity of wildlife populations can be indeed compromised by a prolonged exposure to mortality, along with a behavioural avoidance of individuals from roads (Jacobson et al., 2016). Nevertheless, progresses with the aim of mitigating road-related mortality seem insufficient, and while growing awareness is triggering efforts from citizen initiatives (Bíl et al., 2020), jointly with conservation scientists and governments through policy directives (Pagany, 2020), alarming rates are yet routinely reported and rising. In the United States, the frequency of roadkill has doubled since the 1990s, reaching approximately one million vertebrate deaths along roads per day (Forman & Alexander, 1998; Schwartz et al., 2020), then in Brazil with more than 8 million birds and 2 million mammals per year in last decades (González-Suárez et al., 2018), whilst 194 million birds and 29 million mammals correspond to the annual estimated mortality on European roads (Grilo et al., 2020). Such losses have the potential to undermine wildlife population demography and viability, leading to local extinctions and deteriorating ecosystem functioning (Oddone Aquino, 2021; van der Grift et al., 2017). Roadkill mitigation is relevant from both functionality of ecosystems and ethical standpoints, though is also intertwined with motorist safety and economic damages, since wildlife on roads represents a serious wildlife-human conflict and a global socio-economic danger (Oddone Aquino, 2021; van der Grift et al., 2017; van der Ree et al., 2011). In particular, ungulate-vehicle collisions are problematic for transport agencies, since may reach up to two million of roadkilled animals in Europe, hundreds of human deaths per year in the US, whilst costing tens of millions of Euros in human fatalities (Europe), and over a billion in property damage (US) (Bissonette et al., 2008; Putman et al., 2011). Importantly, human fatalities can be attributed to species with large body size such as ungulates, but also to smaller fauna, as drivers can fatally crash on other vehicles or objects after hitting or avoiding individuals on roads (Williams & Wells, 2005).

Apart from above listed negative effects, linear infrastructures can have also positive effects when promoting the presence and maintenance of semi-natural habitats, such as verges (Coffin, 2007). The presence of verges and hedges along roads is recognised to exert beneficial effects on biodiversity (Coffin, 2007), and studies show that such elements have the potential to provide suitable habitat for various taxa, including plants (Tikka et al., 2001), insects (Phillips et al., 2019), birds (Hinsley & Bellamy, 2000) and mammals (Bellamy, 2000). This also includes habitat specialist fauna of conservation concern (e.g., rare or threatened), where verges are often considered a refuge, typically when the surrounding landscape is subject to severe alterations (Ascensão et al., 2012; Pita et al., 2007; Santos et al., 2007; Smart et al., 2006). Still, the multiple beneficial effects of roadside verges as habitat and corridors can be context dependent, while varying according to geographical location (Smart et al., 2006), and can have side-effects (e.g. for attracting predators; Silva et al., 2019). Further, concerns have also been raised whenever road verges may lead to a serious compromise in road safety, as they may provide shelter for certain species approaching the roads (e.g. ungulates) whilst restricting visibility for drivers (Keken et al., 2019). Thus, roads can exert both negative and positive effects (Figure 1.1.2), which require different goals and targets to increase their sustainability and soften wildlife-human conflicts.

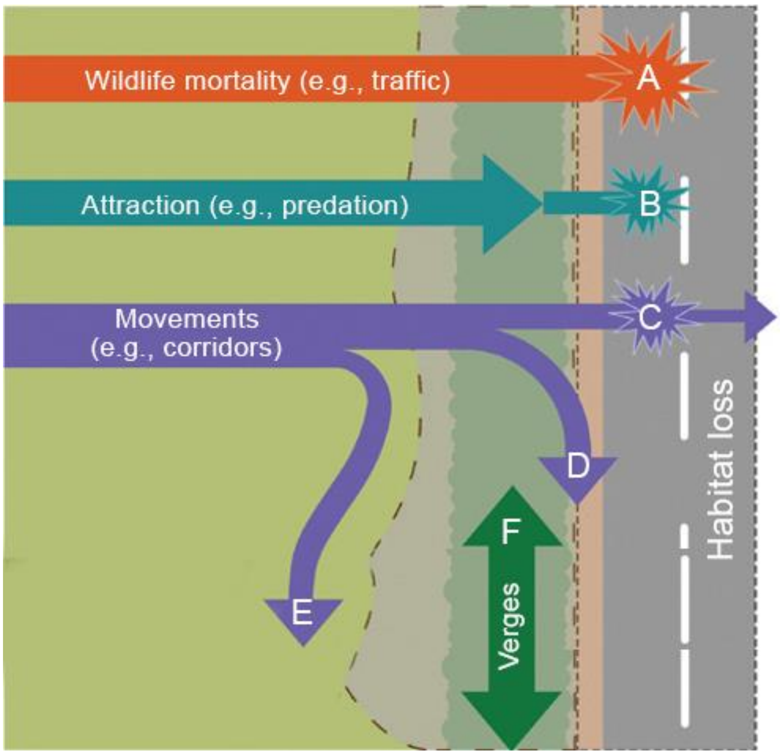

Figure 1.1.2. Major effects associated with roads on wildlife. The most obvious negative effect for wildlife is a directly induced mortality through vehicle collision (A). This mortality effect may be triggered from attraction of wildlife on road verges (B), or may be associated to landscape corridors, at the interface with roads (C), whether roads act as semi-permeable barrier. However, roads may be also not permeable, a condition that may act as a deterrent for wildlife crossing (D), or in worst cases, as avoidance (E). Conversely, the most obvious positive effect for wildlife is related with the presence of road verges (F), as those may constitute potential habitat and corridors for some wildlife species, even though with collateral effects in mortality risk (B). Adapted from van der Ree et al. (2015a).

1.2 *Mitigation measures and their implementation*

Biodiversity protection is considered in mitigation planning and decision-making owing to the negative influences from human activities worldwide, where legislation procedures are becoming explicitly dedicated to biological conservation and increasingly mandatory in numerous countries. Nations around the world are committed to reducing rates of biodiversity loss following the adoption of the Strategic Plan for Biodiversity, set out in the Convention on Biological Diversity (CBD), and representing an ambitious goal on different fronts (Chandra & Idrisova, 2011). In particular, the stipulated paragraph 1 of Article 14 of the CBD identifies EIAs as a legal protocol and decisive tool for conservation and mitigation (Secretariat of the Convention on Biological Diversity, 2006). An EIA is carried out whenever is expected a detrimental effect on special environments or protected area from the development of a new infrastructure, such as roads (Jaeger, 2015). On the other side, linear infrastructures actually exist in particular areas of biodiversity interest and/or ecological significance, meaning that were conceived and developed at the dawn of the road ecology discipline, or when attention to EIAs protocols was minimal (Forman & Alexander, 1998). This contributed to inadequacy in conservation planning, which coupled with lack of conservation actions, has led to insufficient efforts with respect to species protection, as revealed in the Post-2020 Global Biodiversity Framework report on the lack of compliance of the CBD 2010 targets (Xu et al., 2021), namely to mitigate many causes affecting biodiversity, including those from roads. Furthermore, the presence of roads, as previously mentioned, may impair the ecosystems' functionality (Coffin, 2007; van der Ree et al., 2011), which is considered at the foundation of human well-being (Díaz et al., 2018; Dirzo et al., 2014; Malhi et al., 2020). Identically, transport infrastructure is also relevant for the human well-being, which can trigger conflicts of interest between economic growth and biodiversity preservation, such as highlighted by Ibisch et al., (2016), that have pointed such conflict with respect to the United Nations' agenda (e.g. Sustainable Development Goals). In light of all this, if on one hand conservation scientists are now challenged to develop global conservation policies capable of addressing the negative issues associated with roads (Ibisch et al., 2016; Pagany, 2020), on the other, focus is likewise required on roads already built, with EIAs-based practical and local solutions limiting their negative impacts (Jaeger, 2015; Karlson et al., 2014; Rytwinski et al., 2015; van der Grift et al., 2015). In Europe, the EIA procedure has become mandatory according to the EU Directive 85/337/EEC, 2011/92/UE and 2014/52/UE with regard to projects, while the EU Directive 2001/42/EC with regard to public plans and programmes (e.g., land use, agriculture, transport, etc.). In this direction, the EU also outlined a Biodiversity Strategic Plan for 2030 (European Commission, 2020) to restore biodiversity in Europe, which includes a series of guidelines, including EIAs (see Karlson et al.

2014), for the compensation of depleted ecosystem services, the fragmentation mitigation and functional connectivity restoration.

Mitigation efforts, along roads, are mostly conducted aiming human safety, animal welfare and wildlife conservation issues (van der Grift et al., 2015). Particularly for the latter, several mitigation measures are considered to restore connectivity and readdress population viability, considered as the most important objectives (Sijtsma et al., 2020; Smith et al., 2015). This includes the construction of road crossings structures which aim to reduce roadkill rates and promote animal movement (Huijser et al., 2009; Lesbarreres & Fahrig, 2012). These structures can have several designs and dimensions such as overpasses, underpasses and/or culverts (Smith et al., 2015; Figure 1.2.1a-b) and must be complemented with fences (van der Ree et al., 2015b; Figure 1.2.1c) to facilitate and redirect safer movements (Clevenger & Huijser, 2011; Crooks & Sanjayan, 2006; Sijtsma et al., 2020).

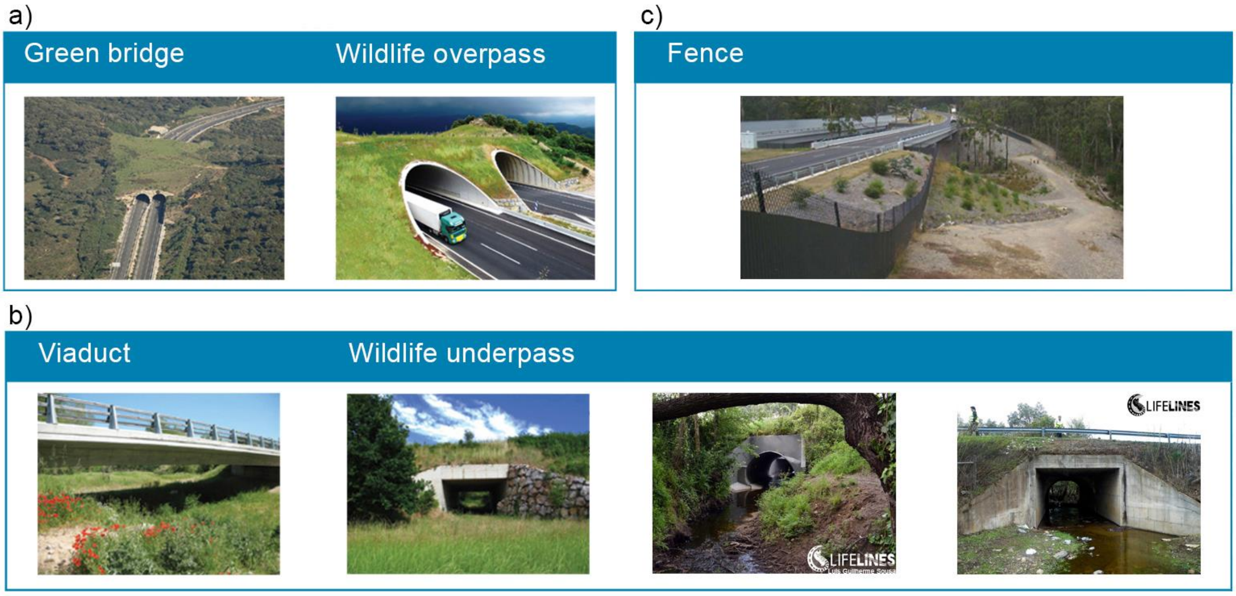

Figure 1.2.1. Examples from main typologies of mitigation structures. In the upper part are depicted typical wildlife crossing structures, respectively a) overpasses and b) underpasses. In the lower part are depicted c) fences, with examples of management strategies combining fencing for multiple species. The figure combined and adapted illustrations from: Smith et al., (2015) and van der Ree et al., (2015b) and LIFE-LINES project (LIFE14 NAT/PT/001081; https://lifelines.uevora.pt/).

The proper implementation of such structures has the potential to increase population connectivity, allowing easier access to resources, reproductive success, and transfer of genetic material, all of which can increase long-term population viability (Clevenger & Huijser, 2011; Rytwinski & Fahrig, 2015a; van der Grift et al., 2015). Still, it is worth noting that those supposed benefits are currently a topic of discussion, as if it is recognised that wildlife crossings may improve connectivity (Clevenger & Huijser, 2011; Crooks & Sanjayan, 2006; Mimet et al., 2016), on the contrary, their increased use by wildlife and the facilitated movements might not necessarily lead to improved population viability. This is mainly because an explicit measure on viability is difficult to yield, primarily due to the lack of field data for long periods and poor research designs (Rytwinski et al., 2016). This means that roadkill frequencies, movement rates between populations and genetic variability, are often the only parameters for assessing the effectiveness of mitigation structures, even if they are not directly related to population viability (Rytwinski et al., 2016; van der Grift et al., 2015). For the effectiveness of mitigation structures, attention is given with regards to size, material and design (Brunen et al., 2020; Craveiro et al., 2019; Smith et al., 2015), but prior the construction phase, a careful consideration of the planning phase is critical for the effectiveness of mitigation measures (Glista et al., 2009; van der Grift et al., 2013; van der Grift et al., 2015; Rytwinski et al., 2015b). A better understanding of risks undermining biodiversity gradually emerged along recent decades, with improvements from road-based EIAs assessments, though uncertainties and inefficiencies still remain, especially during decision-making and planning processes. In a comprehensive review, Karlson et al. (2014) pointed out that most EIAs are based on a mere description of biodiversity, carried out through expert-based decision-making consisting of subjective arguments regarding putative sites for applying measures, or descriptive rather than quantitative evaluations, with the issue of connectivity often omitted. Such decisions can have the consequence of biasing planning in a counter-productive manner, with significant expenditure of resources. In spite of this, research is progressively moving for measuring impacts quantitatively, and EIA-based modelling tools are increasingly being more valued (Gontier et al., 2006), especially when integrating different complementary disciplines (e.g., ecology, connectivity, remote sensing; Zetterberg et al., 2010; De Leeuw et al., 2010). Such an integration may lower limitations and gaps when estimating road ecological impacts on biodiversity in human-animal conflictual areas, resulting as more adequate for distributing mitigation measures able to restore population viability and connectivity (Gontier et al., 2006; Karlson et al. 2014; Smith et al., 2015).

1.3 Assessing functional connectivity as a surrogate of wildlife movements

Historically, connectivity is defined as "the degree to which the landscape facilitates or impedes movement" (Taylor et al., 1993), where permeability and resistance indicate the ability of natural and semi-natural landscape elements to, respectively, facilitate or impede wildlife movement (Ament et al., 2014; Crooks & Sanjayan, 2006). Promoting connectivity plays a determinant role to halt biodiversity decline, conserve and adapt wildlife populations and ecosystem services, which is of special interest in view of landscape and climate changes (Malhi et al., 2020). A variety of goods and services for human well-being are provided by wildlife during their movements/activities (e.g., food, pollination, pest control, etc.), which also depend on a preserved landscape connectivity (Ament et al., 2014; Crooks & Sanjayan, 2006; Malhi et al., 2020). In this direction, the EU has outlined, throughout the Biodiversity Strategy 2030, many targets within and outside protected areas to halt the degradation of biodiversity and ecosystems, where target 2 in particular aims to improve connectivity by creating green infrastructure and restoring at least 15% of degraded ecosystems. These initiatives highlight the importance of mitigating road-related mortality and undertaking defragmentation programs towards degraded ecosystems by road impacts (e.g., Towards green infrastructure for Europe, 2007). To improve connectivity, defragmentation measures can focus on creating conservation areas with ecological components of natural and semi-natural areas to act as corridors and stepping stones (Ament et al., 2014; Hilty et al., 2020). Alternatively, abovementioned site-specific measures such as crossing structures and fences along roads can directly address some corridor-blocking effects, such as fragmentation and roadkill (Andrews, 1990; Crooks & Sanjayan, 2006; Hilty et al., 2020). The geographical identification of locations to implement mitigation measures along roads can be based, besides expert-opinion, throughout a plethora of modeling approaches, divided into two main categories: those that spatially prioritise sites along roads on the basis of the relation between roadkill and environmental drivers (e.g., D'Amico et al., 2015; Pagany, 2020), and those that spatially prioritise sites by assessing connectivity on the basis of the relation between observed movements and environmental drivers (e.g., Zeller et al., 2020a). These two approaches can be further combined by relating roadkill patterns and movement patterns from connectivity assessments (e.g., Koen et al., 2014).

Connectivity can include both structural and functional components, with structural connectivity referring to a physical component that describes the geographical arrangement of patches in a landscape, such as habitats, whereas functional connectivity refers to a behavioural component that describes a species' response to the landscape (Crooks & Sanjayan, 2006; Zeller et al., 2012). Structural connectivity models were the first to be formulated, and are defined as '*patch-matrix*' (Brudvig et al., 2017). In fact, these models are binary and rooted in the theory of island biogeography (MacArthur and Wilson, 1967), in which habitat patch areas are conceived as islands surrounded by an inhospitable matrix of non-habitat areas (Forman, 1995). Structural connectivity is often analysed as a graph network, geographically represented in the form of nodes (habitats) and edges (links) (Ament et al., 2014; Urban & Keitt, 2001). In contrast, functional connectivity models are post-hoc formulations and are defined as '*continuous*' models (Brudvig et al., 2017), reflecting the willingness of wildlife movements across the landscape, represented for instance through resistance surfaces models (Ament et al., 2014; Zeller et al., 2012). The integration of functional within structural connectivity may have practical outcomes as it may help identifying unique paths (corridors), for instance with the least-cost path theory (Etherington, 2016), or multiple alternative paths, for instance with the circuit theory (McRae et al., 2008) (Figure 1.3.1).

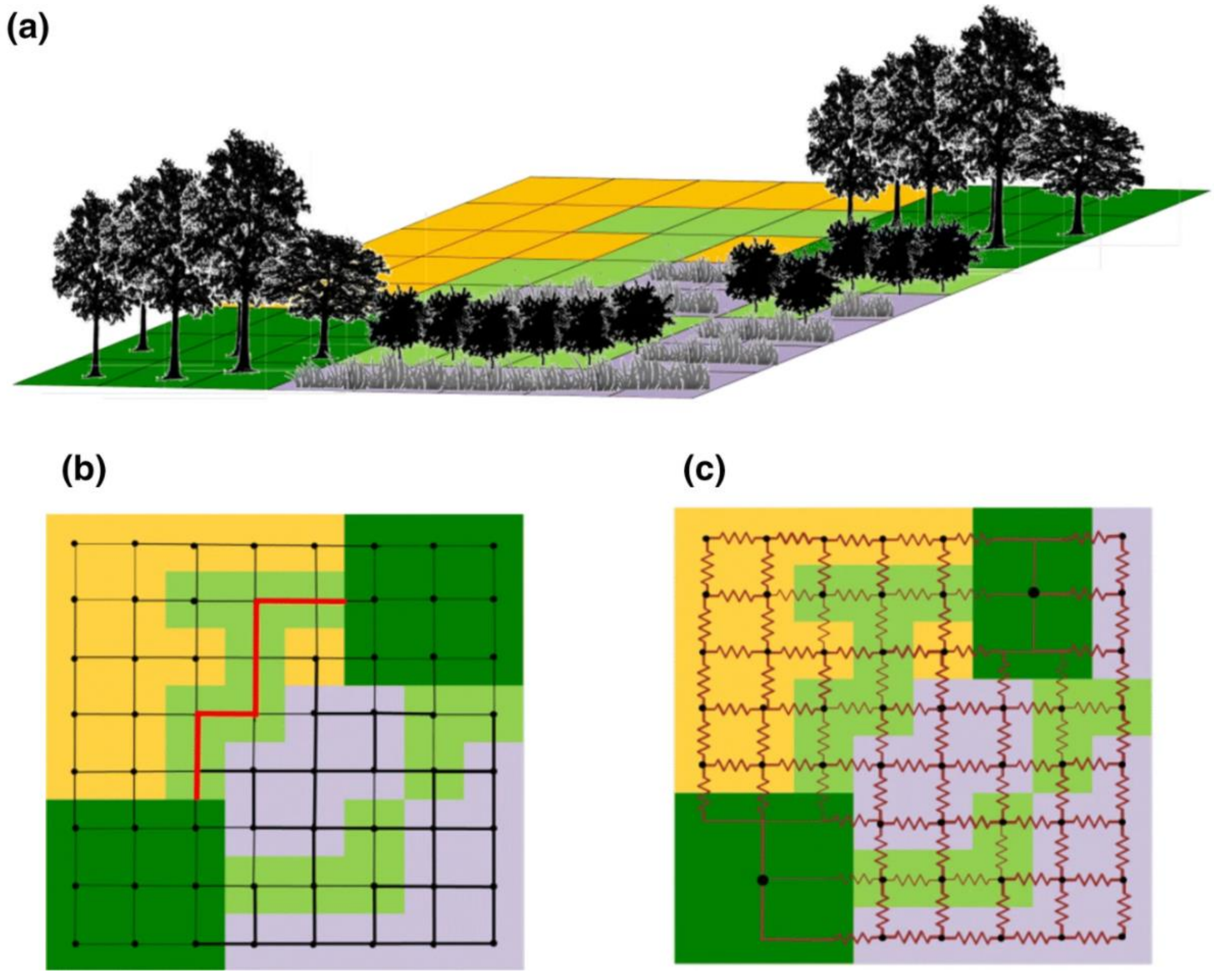

Figure1.3.1. A typical exemplification of a landscape connectivity framework, where the resistance surface reflects the willingness of an organism to move across habitat types (a), and combined with a graph network of nodes connected by weighted edges, may help identify source/sink paths (corridors) with least-cost models (b), or by substituting edges with resistors, to identify multiple alternative source/sink paths (corridors) with circuit theory (c). Adapted from Diniz et al. (2020)

Yet, the way how these models complement each other (structural connectivity within functional, or viceversa) may depend on the ultimate goal, as one study may find more appropriate to emphasise structural connectivity (e.g., identifying the best connected patches), or functional connectivity (e.g., identifying most important wildlife corridors), notwithstanding both approaches have the potential for solving planning prioritisation questions [e.g., see Mimet et al., (2016) for emphasising structural connectivity, whilst Koen et al., (2014) for functional]. Priorities are set during the mitigation planning phase to identify suitable sites (or units) along roads, meaning that: (1) wildlife crossing sites can be predicted (Ament et al., 2014; Zeller et al., 2020a), likewise (2) roadkill have non-random distributions but occur in a spatially aggregated way along roads (Santos et al., 2015), frequently at locations positively related with traffic, habitat availability and functional connectivity (Gunson et al. 2011; Pagany et al., 2020; Santos et al., 2013).

Quantifying functional connectivity can aid in identifying priority areas that facilitate movement, dispersal, and gene flow between populations or sub-populations (Taylor, 2006; Taylor et al., 1993), though it remains an utmost critical step (Diniz et al., 2020; Fletcher et al., 2016). For this purpose, two traditional spatially explicit methodologies can be adopted, namely a '*coarse filter*' and a '*fine filter*' (Ament et al., 2014). A '*coarse-filter*' approach is also called a '*species-agnostic*' or '*species-neutral*', which is relatively quick to characterise and with a low level of complexity, representing a typology of connected natural and semi-natural habitat areas as a possible corridor (Ament et al., 2014), thereby representing functional connectivity as a generalisable surrogate across species (e.g., Koen et al., 2014). Yet, functional connectivity is species-specific (Zeller et al., 2012), implying that when determining the willingness of species to move across a landscape, a '*coarse filter*' approach should be less informative and reliable than a '*fine filter*', by definition dedicated to a target species (Ament et al., 2014). On the other side, a '*fine filter*' approach requires movement data (e.g., path movements) typically based on telemetry devices (Diniz et al., 2020; Elliot et al., 2014), to assess the so-called actual connectivity (Fletcher et al., 2016). Actual connectivity is so termed because is assessed by relating realistic movement with landscape or anthropogenic constraints, and it is finalised through specific analysis such as path selection functions (PathSFs; e.g., Carvalho et al., 2016). However, the acquisition of movement data requires a large amount of time and money, representing a persistent and long-standing problem. This is because it involves the acquisition of telemetry devices sufficiently advanced to record wildlife movements, in large quantities to cope with malfunctioning technology and to simultaneously track several individuals (Carvalho et al., 2016; Diniz et al., 2020). Secondly, is often required an appreciable manpower, namely skilled technicians, to ensure the success of some sensible steps (e.g., individuals capturing and radiocollaring), as well to adequately capture individual responses to landscape features, especially roads. The latter is achieved with long sampling campaigns spent in the field (Carvalho et al., 2016), though more advanced technologies increasingly affordable have facilitated this issue (e.g., satellite telemetry; Hofman et al., 2019). Third, the sparsity of movement data represents the greatest drawback in multispecies research initiatives, owing to limited funding that routinely hinders conservation (Dalerum, 2014), also considering the difficult realisation of radiotracking some small species (e.g., passerines; Salgueiro et al., 2021). So, a possible compromise in this regard for maintaining a '*fine filter*' approach without relying on movement data, may consist in inferring movements (e.g., occurrences; Zeller et al., 2012) through potential connectivity (Fletcher et al., 2016). Potential connectivity assessments are particularly worthy of attention, because empirical models that are based on theoretical assumptions may face substantial uncertainties, and consequently, independent data for evaluations is demanded (Fletcher et al., 2016). Because potential connectivity is seldom validated, studies involving road areas may validate their robustness through independent roadkill data to increase effectiveness of mitigation planning decisions (Laliberté et al., 2020).

Beyond theoretical assumptions, a further complication for EIAs is that functional connectivity, and consequently movement decisions, are a reflection of animal behaviour, which may vary across life history stages, namely between movements within home range (e.g., foraging), and dispersal (Cosgrove et al., 2018; Elliot et al., 2014; Pe'er et al., 2011). In detail, environmental constraints (e.g., resistance surfaces) on a species pertaining daily movements within home ranges, may be more relaxed during dispersal movements (Ims, 1995). This has progressively changed the previously "inhospitable matrix", taken as an absolute concept in the past, in favour of alternative matrix types (Brudvig et al., 2017). In fact, is known that roadkill events may be associated to different life history stages, namely to movements within habitats (home-range; e.g., D'Amico et al., 2015; Gunson et al. 2011; Pagany et al., 2020), but also to dispersal (Grilo et al., 2011). Consequently, to better relate dispersal events (e.g., roadkill) with functional connectivity, the formulation of different movement costs should be required (Pe'er et al., 2011). On this issue, implications for correct assessments also rely on the distance potentially travelled by a species during dispersal, as it can influence the accessibility of habitat areas, which combined with patch size, in turn can influence the likelihood of colonisation/extinction mechanisms (Heinrichs et al., 2016). All of these factors are largely ignored when determining wildlife movements, which might significantly compromise the effectiveness of management decisions (Elliot et al., 2014). Concisely, the representation of multiple corridor routes intersecting roads, hence connectivity, should ideally be based on three principal aspects, synthesised according to Vasudev et al., (2015), that are capable of influencing mortality risk and likely to differ from species to species: a) spatial constraints (structural connectivity), depending on the arrangement of habitat features, b) environmental constraints (behavioural-based movement costs; functional connectivity), and c) intrinsic constraints (species limits), depending on travelled distance by a species. By accounting this, the research goal moreover requires to be contextualised according to the organism hierarchical level of interest, namely individual (home range), population (distribution) and community (biodiversity) (Fletcher et al., 2016). Indeed, while it is important to consider the road effects on individual species within their home-range (e.g., Carvalho et al., 2016), on the other side, is also pivotal to examine the negative effects from roads at the community level, intended for multiple species (Fletcher et al., 2016), as the locations selected for the placement of the mitigation structures should cover as many species as possible (Clevenger & Huijser, 2011; Polak et al., 2019; van der Grift et al., 2017).

Finally, for conservation efforts and management decisions, apart from understanding the relationships between functional connectivity and roadkill, it must be considered the "scale of effect", as ecological processes operate at multiple spatial scales (Diniz et al., 2020; Wiens, 1989). A multiscale study design is always recommended when there is no prior knowledge of the best scale of a particular ecological mechanism/impact (Fletcher et al., 2016), and more specifically, it is necessary to understand at what scale environmental/anthropogenic features are appropriately correlated with road-related mortality events (Gunson et al., 2011;). Depending on the connectivity context and addressed issue, it is essential to define spatial extent (e.g., patch, landscape, region, etc.), and temporal scale (daily life, lifetime, multiple generations) on which the effects that are negatively affecting connectivity occur, as this can lead to different mitigation outcomes (e.g. focused on resource accessibility, demographic exchange, gene flow) (see more details in Cosgrove et al., (2018) (Figure 1.3.2).

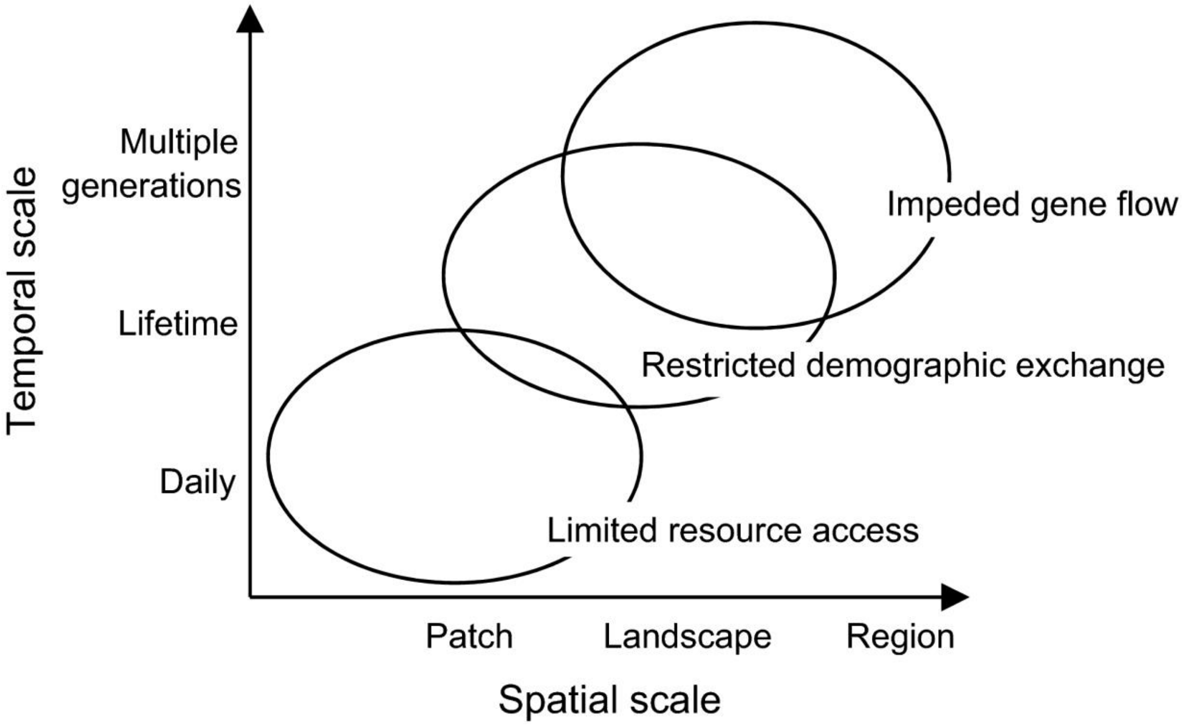

Figure 1.3.2. A conceptualisation depicting the mechanisms by which negative impacts can take place, when wildlife movements are hampered across spatial and temporal scales. Adapted from Cosgrove et al. (2018).

On this issue, while the vast majority of EIAs have focused from fine to large spatial scales when analysing roadkill, they lacked to incorporate large temporal scales, relying on static "snapshots" of landscape representations (e.g., categorical land use classes; Gunson et al. 2011; Pagany et al., 2020). Conservation planners in road ecology need to monitor environments at larger temporal scale and put forward advanced EIA-based modeling tools able to track the dynamism of landscapes in space and time, being this considered a pivotal step for the effectiveness of long-term mitigation strategies (Clevenger & Huijser, 2011).

1.4 *The advances of Earth Observation Satellites for conservation biology*

Conservation biology is a discipline that aims to monitor and protect the Earth' habitats and ecosystems, along with the biological organisms inhabiting them. Being historically grounded in ecology, field data have often constituted the only accessible information on the state of habitats/ecosystems and their spatiotemporal variations, as well as the negative impacts affecting them, though sampling strategies predominantly suffer from a lack in large spatiotemporal coverage. This has progressively led to the obsolescence of conventional environmental monitoring programs, especially considering that, over large spatial and temporal scales, anthropogenic pressure is increasingly alarming (Dirzo et al. 2014), while prolonged planned-survey programs are extremely difficult to fund (Malhi et al., 2020). Conservation biologists are searching for alternative monitoring techniques and data sources that can provide sound and effective responses in relation to global environmental changes and anthropogenic threats. In this direction, scientists and practitioners are turning their attention to remote sensing science as a possible solution, given its exceptional growth in recent decades and the possibility to detect multiple aspects of Earth biotic and abiotic elements (Kerr et al., 2003; Pettorelli et al., 2014a; Pettorelli et al., 2014b). Remote sensing is "the science and art of obtaining information about an object, area, or phenomenon through the analysis of data acquired by a device that is not in contact with the object, area, or phenomenon under investigation" (Lillesand et al., 2015). Through remote sensing, data can be cyclically collected for ecosystems monitoring and ecological studies, which can be accomplished by one or more automatic sensor devices, minimising human errors. In addition to ground-based (*in situ*) sensor systems (e.g., cameras), which may have limited extension capabilities, information is formally available from airborne sensors via unmanned aerial vehicles (UAVs; e.g., drones), aircrafts with human pilots (on board) and Earth Observation Satellites (EOS) (Emilien et al., 2021; Lillesand et al., 2015).

The first difference in remote sensors information acquisition lies in the dichotomy between passive and active sensors. The passive ones are optical sensors, also called spectrometers (Zhu et al., 2018) to passively receive natural information, namely energy signals emitted by the Earth in the form of radiation, within a particular portion of the electromagnetic spectrum (Ose et al., 2016; Lillesand et al., 2015). The electromagnetic spectrum (Figure 1.4.1) is composed of different frequency oscillations and wavelengths, and those detectable by optical sensors commonly range in the solar wavelength region of the human visible spectrum VIS (0.40-0.65 µm), part of the infrared IR, namely the near-infrared NIR (0.65-1.0 µm) and the short-wave infrared SWIR (1.0-3.0 µm) (Lillesand et al., 2015; Xiong et al., 2018). Information from optical sensors may reveal the Earth biotic components such as biophysical and biochemical characteristics, useful for ecological applications and insights (Kerr et al., 2003; Ose et al., 2016; Zhu et al., 2018).

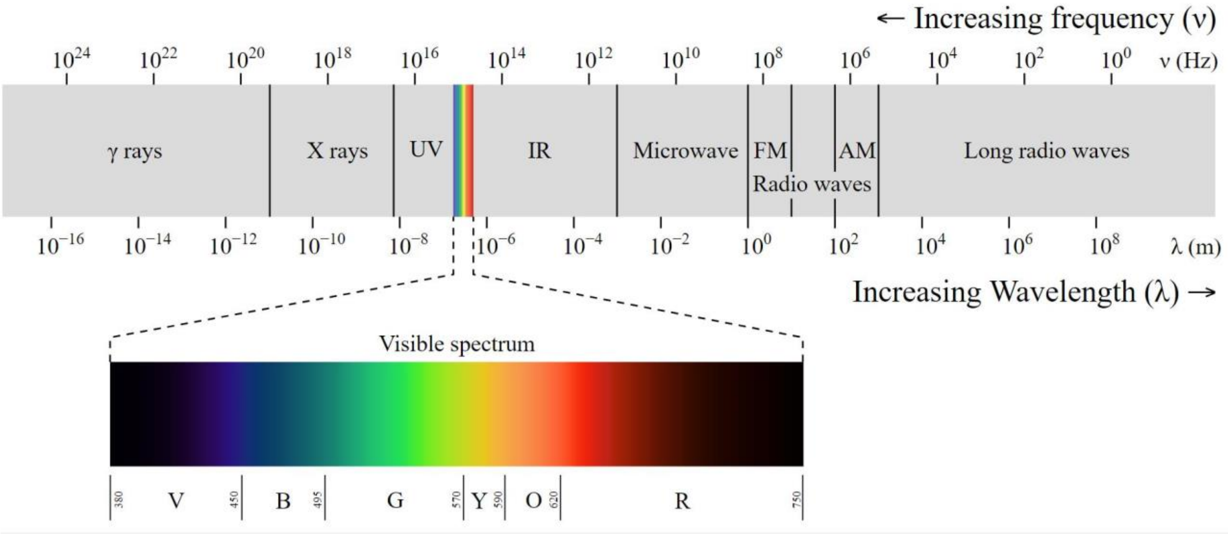

Figure 1.4.1. The electromagnetic wave spectrum, composed of frequencies of electromagnetic radiation. Source: https://commons.wikimedia.org/wiki/File:EM_spectrum.svg

Distinctly, active sensors are radar (Radio Detection And Ranging) such as the notorious satellite-based SAR (Synthetic Aperture Radars), also called radiometers (Zhu et al., 2018) for actively emitting (and subsequently receiving) energy pulses in the form of microwave radiations (a form of electromagnetic radiations; Figure 1.4.1), hence representing a different region of the electromagnetic spectrum than optical sensors (wavelengths approximately 0.9–0.001 m) (Leslie, 2018), and primarily used to unveil' abiotic components such as temperature, humidity and physical characteristics (Emery & Camps, 2017; Leslie, 2018; Zhu et al., 2018). Another important active remote sensing method is the lidar (Light Detection and Ranging). It is based on optical and light technology, with a wavelength region encompassing the IR infrared (10 µm) and the UV ultraviolet (250 nm) (Figure 1.4.1), and has great potentiality for structural vegetation measurements, biomass estimations and topographic mapping (Pettorelli et al., 2014a; Zhu et al., 2018). For both active and passive EOS sensors, the acquired wavelength information is organised into bands (or channels), and the way this is realised defines the sensor technology and its quality (Lillesand et al., 2015; Zhu et al., 2018). EOS optical sensors can store wavelength information within: (1) a single band, which is a typical feature of panchromatic PAN imaging systems (e.g., Ikonos [PAN]; SPOT; GeoEye-1; Zhu et al., 2018), (2) multiple bands, each containing a specific range of wavelengths from a portion the electromagnetic spectrum - multispectral MS imaging systems (e.g., Landsat; Sentinel; Ikonos [MS]; Zhu et al., 2018), (3) dozens of bands, each containing a narrower wavelength range, while covering a wider portion the electromagnetic spectrum belonging to superspectral imaging systems (e.g., MODIS; MERIS; Zhu et al., 2018), then (4) hundreds of bands from hyperspectral imaging systems (e.g., Hyperion;

PRISMA; Kpalma et al., 2014), each containing an even narrower wavelength range, while representing a complete, or nearly-complete, portion of the VIS/NIR/SWIR electromagnetic waves spectrum. Wavelength information pertaining each band (or channel) is digitally prepared through a conversion into a georeferenced and rasterised layer, where multiple bands compose a multilayer image (Lillesand et al., 2015). It is important to underline that the radiometric resolution of an image identifies the range storing capacity of the electromagnetic spectrum, while the spectral resolution reflects the number of bands upon which the information of the represented electromagnetic spectrum is sub-divided. Both features have substantial implications for a higher accuracy of the targets or objects to be identified, such as landscape habitat features (Kpalma et al., 2014; Ose et al., 2016; Zhu et al., 2018). Obviously, an accurate landscape classification also depends on the spatial and temporal resolution of an image, and likewise spectral and radiometric resolution, those features likely differ between EOS sensors, even when belonging to the same imaging system (Kpalma et al., 2014; Lillesand et al., 2015; Zhu et al., 2018). Spatial resolution refers to the grain (or pixel size) size at which wavelength information is acquired by a sensor, and can be classified from coarse (greater than 250m; e.g., NOAA), moderate (around 250m; e.g., MODIS), high (around 30m; e.g., Landsat and Sentinel), to very high resolution (around 0.5cm; e.g., GeoEye-1) (Alleaume et al., 2018; Emilien et al., 2021; Ose et al., 2016). Temporal resolution instead refers to the time required, by a sensor, to revisit and image the same geographic area, and it that can range from high temporal resolution (1-5 days; e.g., NOAA, MODIS; Sentinel), moderate (about 15 days; e.g., Landsat), to coarse temporal resolution (1-5 years; e.g., NAPP) (Lillesand et al., 2015; Ose et al., 2016; Zhu et al., 2018). An appropriate revisit time is crucial for determining the status and trends of terrestrial ecosystems, hence their dynamics (e.g., phenology of vegetation from seasonality, or landscape changes; Alleaume et al., 2018; Pettorelli et al., 2014a). Importantly, the description of sensor features also regards spatial and temporal range coverage, where spatial range coverage indicates the so-called "swath", namely the spatial extent capability of a sensor when imaging an area, whereas temporal range coverage indicates the duration over which the sensor has been operational in collecting information (Zhu et al., 2018; Xiong et al., 2018).

Therefore, in addition to the basic theory knowledge beyond remote sensing, it is required a solid background with the types of sensor technologies and missions, and the specific purposes they are designed for. This means that trade-offs are necessary towards the strategic selection of candidate sensors and associated quality and quantity data, which may vary on the basis of considered conservation goal (Alleaume et al., 2018). For instance, sensors that are mounted on devices such as UAVs and airborne may have great potential in spectral, radiometric and spatio-temporal resolution (e.g., hyperspectral cameras), but may lack spatio-temporal coverage compared to EOS, resulting more indicated for local studies (Emilien et al., 2021). UAV and airborne-based technology are also difficult to manage owing to devices with low battery capacity and depending on large storage capacity, along with the necessity of skills in piloting devices and legal permissions for flies. EOS, on the other hand, are autonomous in the long run, do not require pilots, transmit information to online repositories, but do not have the spatio-temporal resolution of UAVs (Kerr et al., 2003; Lillesand et al., 2015). Also, as previously anticipated, even within EOS sensors there are substantial differences, as some may have high temporal resolution but very poor spatial resolution, such as MODIS and NOAA, compared to others such as Hyperion, which in contrast has low temporal resolution (Kpalma et al., 2014; Xiong et al., 2018). Other EOS sensors may have impressive spatial and temporal resolution, such as the panchromatic GeoEye-1 sensor, but are commercial, meaning they are often financially prohibitive for long-term studies (Lillesand et al., 2015; Zhu et al., 2018). A vast constellation of EOSs exist and many are actually operative, with those public conventionally developed by the NASA and ESA, or under their partnership (Figure 1.4.2).

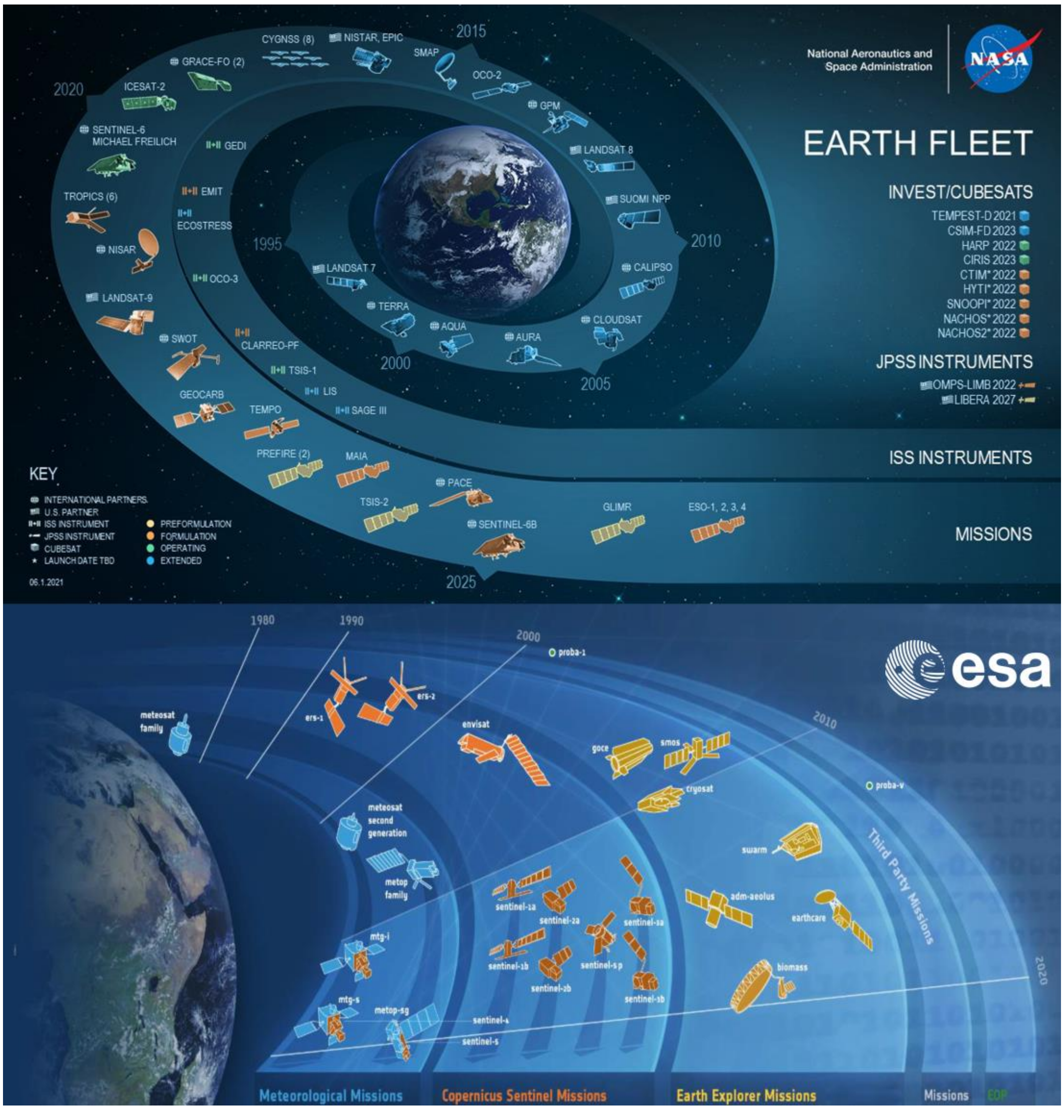

Figure 1.4.2. The constellations of EOS developed under the Nasa and ESA programs. Image have been merged from two different sources: NASA (https://science.nasa.gov/) and ESA (https://www.esa.int/).

A common issue in characterising terrestrial ecosystems and habitat dynamics in time and space is that abiotic and biotic measurements should be sensitive to spatial and temporal changes, as well as covering large extents (Pettorelli et al., 2014). In this respect, potential biotic information may come from a family of EOS with optical sensors designed for environmental monitoring, namely from Landsat and Sentinel missions, given the compromise between spectral and radiometric resolution, but high spatiotemporal resolution and range coverage, hence particularly suitable for fine-scale and wide range ecological applications (Emilien et al., 2021; Xiong et al., 2018; Zhu et al., 2018). More specifically, Landsat is a joint NASA/U.S. Geological Survey (USGS) programme, and since 1967, its sensors (Thematic Mapper, TM [Landsats 1-5]; Enhanced Thematic Mapper Plus, +ETM [Landsats 7]; Operational Land Imager OLI and Thermal Infrared Sensor TIRS, OLI/TIRS [Landsats 8-9]) have been monitoring the Earth' surface, representing today one of the most comprehensive EOS data (Xiong et al., 2018). Instead, Sentinel satellites and in specific Sentinel-2 have been developed by the European Space Agency (ESA) as part of the Copernicus initiative, consisting into an Earth observation mission of two satellites operative since 2015, namely Sentinel-2A and Sentinel-2B, consequently with a shorter time coverage than Landsat. For both missions, data is provided at zero cost and rapidly disposable from the sensor acquisition. By combining several bands through specific formula, indicators (or metrics) are calculated to monitor the environmental conditions of habitats and ecosystems (Pettorelli et al., 2005; Pettorelli et al., 2014a), which are preferred at a detailed resolution to better guide conservation planning (Alleaume et al., 2018). The number and typology of indicators describing biophysical features is quite vast, with distinct potentialities depending on the biome studied. Some examples (e.g., bands, habitat metrics, texture variables) can be found in Godinho et al. (2017) regarding Sentinel-based metrics, and in Oeser et al. (2020) for Landsat. Nevertheless, little concordance exists on the typologies of metrics from wildlife ecology studies, and more importantly, few studies integrated physical measurements. A convenient choice for this purpose concerns SAR sensors, in particular L-band PALSAR developed by JAXA' ALOS-PALSAR and ALOS-PALSAR2 missions (Emery & Camps, 2017; Zhu et al., 2018). Across all SAR-based bands, L-band is particularly suitable for describing the structural properties of landscape features, notably of vegetation, given its ability to penetrate canopy cover and transmit information pertaining the understory (for more details on radar bands, see Zhu et al., 2018).

Remotely sensed information from passive and active sensors can help mapping and monitoring habitat and ecosystems (Alleaume et al., 2018; Pettorelli et al., 2014a; Pettorelli et al., 2014b), and for their characterisation, relevant remotely sensed descriptors are integrated together following a procedure called "data fusion" (e.g., Alleaume et al., 2018). According to Schulte to

Bühne & Pettorelli (2018), a particular variant data integration relies on species distribution models (SDMs) (also known as habitat suitability or niche models) (Elith & Leathwick, 2009; Franklin, 2010). SDMs represent an empirical approach to identify species distribution patterns by relating their occurrence to environmental characteristics (Elith & Leathwick, 2009; Franklin, 2010), and their reliability is proven by thousands of studies that throughout SDMs have addressed numerous ecological issues (Basile et al., 2016; Franklin, 2010; Zimmermann et al., 2010), biodiversity conservation and planning (Elith & Leathwick, 2009), as well as interesting applications targeting endangered and rare species (Mestre et al., 2017). SDMs are divided into mapping species distribution directly or indirectly, involving the inclusion of traditional abiotic and biotic descriptors (e.g., bioclimatic variables, land cover products, etc.), though new opportunities are coming from remote sensing science (He et al., 2015). The information of environmental descriptors from remote sensing data may give higher advantages in shaping species distribution, as may allow the description of new biophysical properties like habitat spectral signature, production, senescence, biomass, and structural properties (Alleaume et al., 2018; Jones & Vaughan, 2010) at a fine spatiotemporal scale. However, remote sensing has been poorly applied with SDMs regarding wildlife communities and through continuous descriptors, consisting into a topic deserving further attention (Coops & Wulder, 2019). Continuous remote sensing data may be useful for the identification of landscape elements difficult to find, as previously explained, in particular for sparsely distributed and localised habitats, and for small species (Kerr & Ostrovsky 2003). EOS data have become one of the most widely used sources for Earth monitoring, comprising decades of recorded information that can be used to learn about the state of ecosystems/habitats (Jones & Vaughan, 2010; Zhu et al., 2018), and may have a great contribute for monitoring wildlife populations status (both relative and absolute; He et al., 2015). Finally, remote sensing has been taken into account to support environmental policies (e.g., EIA; De Leeuw et al., 2010) and by EU Member States to comply with the Habitats Directive (Alleaume et al., 2018), which includes an improved protocol for monitoring landscape changes, for the surveillance of corridors and areas of conservation interest, as well the delineation of new areas and corridors at high precision (Schmidt et al., 2021).

1.5 *Thesis focus and main goals*

The large amounts of resources to invest on ground in species protection and to restore connectivity means that planning strategies have to reduce uncertainties. Managers from transportation agencies need further to consider that road impacts often cover large extents (Clevenger & Huijser, 2011; van der Ree et al., 2011), meaning that prioritisation procedures are indispensable. A foremost gap in systematic conservation planning remains on how to optimise the implementation of costly structures such as fences and crossing structures for multiple species (Jaeger, 2015; Polak et al., 2019; Rytwinski et al., 2016). Conservation practitioners are increasingly shifting from expert-based decisions, to the integration of connectivity in mitigation planning relying on a better understanding of animal movements, and on the identification of where and when roads intersect and block/compromise wildlife corridors (e.g., Carvalho et al., 2016; Zeller et al., 2020a). Still, due to limited feasibility, very few studies have quantified functional connectivity for each species separately (Brennan et al., 2020). Multispecies assessments may be facilitated by the possibility to infer wildlife movements, through functional connectivity relying on occurrence data, which however remains an unclear subject (Zeller et al., 2012), with sparse research currently existing on the topic (Jennings et al., 2020). Therefore, it makes sense that the robustness and performance of occurrence data models need to be compared with those based on movements, with regards to roadkill predictability and thus mitigation sites identification (Laliberté et al., 2020). Nor have attempts been made to relate roadkill with different resistance costs (resistance surfaces), which reflect the strength of environmental constraints on a species during movements, with a lower influence during dispersal (Elliot et al., 2014; Ims, 1995). A further step is the multispecies integration into spatial mitigation planning along roads, as no studies targeted species with different characteristics from multiple taxonomic groups (Polak et al., 2019). To address the shortcomings and enhance the effectiveness of mitigation measures, EIAs should unravel the species-specific effect of remotely sensed habitat, functional connectivity and road-related characteristics in driving mortality for each species, and verify the risk agreement across taxa on road planning sites. Finally, taking into account all of the above, it is important also to consider landscape dynamics in conservation planning (Jennings et al., 2020), which is critically overlooked in road ecology (Oddone Aquino, 2021).

On the more specific issue of landscape dynamism, the inter-annual variability in composition and configuration of habitat patches and matrix quality derived from natural or human-derived factors may affect the location of movement corridors. This aspect is not only relevant to the effectiveness of placing mitigation structures (Clevenger & Huijser, 2011), but also to effective planning of conservation areas (Margules & Pressey, 2000). The inter-annual landscape variability, it is easier to take into account nowadays, given the greater accessibility of remote sensing by compiling data over long periods, such as Landsat satellites (Schulte to Bühne & Pettorelli 2018; Zeller et al., 2020b). This data may offer benefits in strategical mitigation planning, by designating structures able to offer resilience to landscape dynamism over long-term. Besides, the higher spatiotemporal resolution of the recently launched Sentinel satellites allows the identification of unique habitat elements, while examining landscape dynamics within a year (Coops & Wulder, 2019; Kerr & Ostrovsky 2003). More specifically, considering highly detailed spatiotemporal information may be particularly useful for the identification of suitable habitat conditions for species along linear landscape elements of difficult detection, notably road verges, which, for plants and small animals often represent a last refuge in human-dominated landscapes (Bellamy et al., 2000; Pita et ta al., 2006; Santos et al., 2006; Tikka et al., 2001). Those arguments are especially valid when conservation and management measures are needed within landscapes characterised by a pronounced intra- and inter-annual variability, such as the Mediterranean region (Mazzoleni et al., 2004), including the area selected to conduct the research questions (Figure 1.5.1).

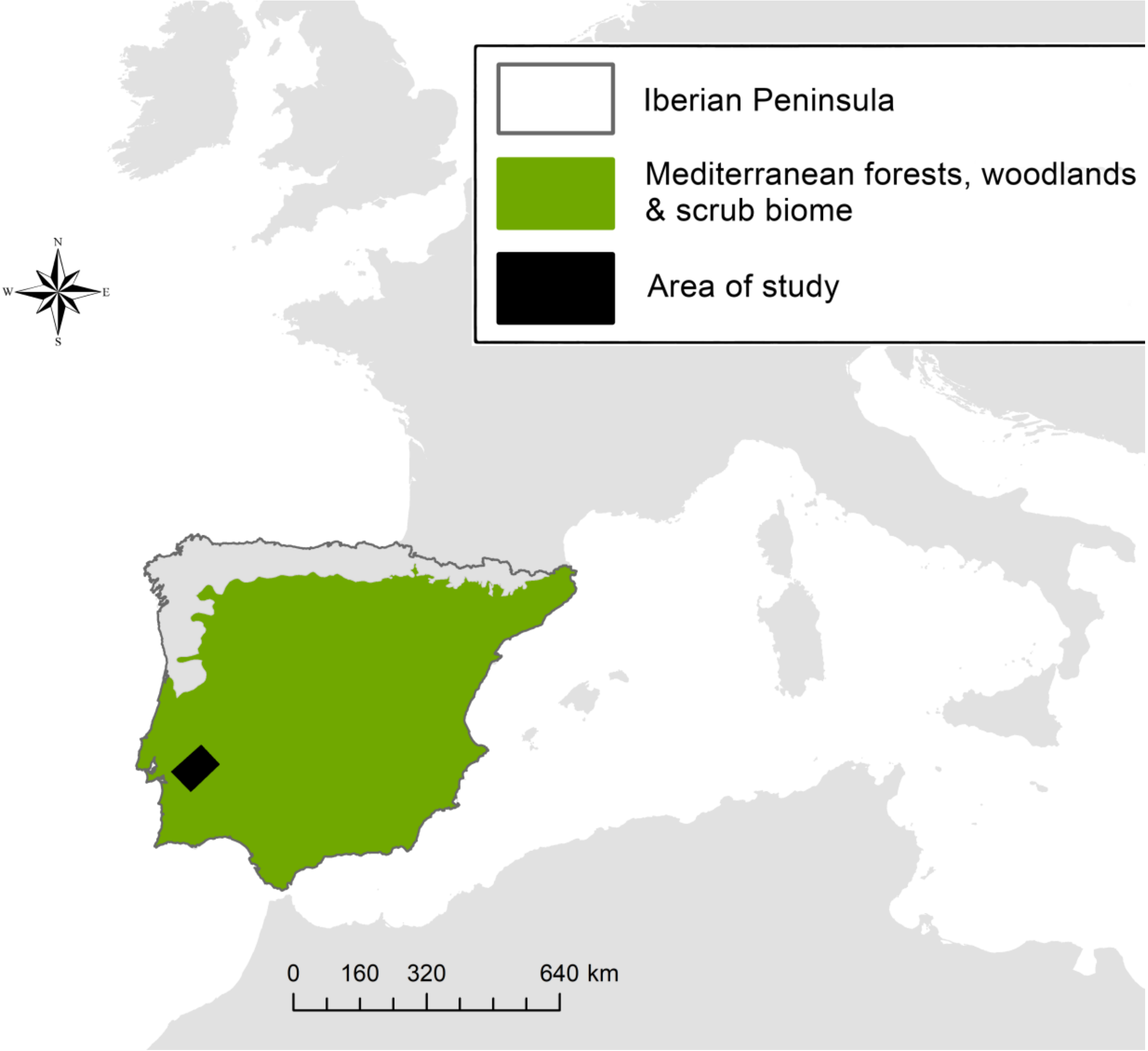

Figure 1.5.1. Study area, where the thesis research has been conducted. The area is located in the Iberian Peninsula, and comprised within the Mediterranean biome.

The area is located in the Alentejo region, southern Portugal, comprised within the ecoregion of Southwest Iberian Mediterranean sclerophyllous and mixed forests (Joffre et al., 1999; Mazzoleni et al., 2004). As such, the area is dominated by a Mediterranean climate with a pronounced intra-annual variability, namely by rainy winters (5-12 °C) and dry summers (16-30 °C), with average annual precipitations exceeding 600 mm, falling mostly from October to March

(IPMA, 2021). In addition, the area is characterised by a mild and gentle relief (<500 a.s.l.), ranging from flat to hilly terrain, with low slopes. The landscape is dominated by *Quercus suber* and *Quercus rotundifolia*, stands, the semi-natural *montado* agroforestry system, which is considered a "High Nature Value farming system", and is intrinsically associated with high socio-economic values, together with high biodiversity (Pinto-Correia & Godinho, 2013). The *montado* also typically comprises a complex understory of scrub, annual crops and grassland with variable distribution of perennial and annual grasses (Joffre et al., 1999). The *montado* in the study area is intermixed with open agricultural areas (pastures, vegetable crops). Other less represented land uses are vineyards, olive trees, pine and eucalyptus forests. With regard to inter-annual variability, in recent decades the Alentejo region and the study area have been subject to a process of land use intensification (e.g. overgrazing and overexploitation of trees), which have contributing for the degradation trend of the *montado* agro-forestry system (Pinto-Correia & Godinho, 2013; Machado et al., 2020). This negative trend, concomitantly with land abandonment, is increasingly becoming a matter of concern as it is worsening across the entire Alentejo region (Pinto-Correia & Godinho, 2013), as well in other Mediterranean regions, such as Spain and Italy (Cillis et al., 2021). Focusing on roads, the study area includes the most an important terrestrial transport corridor linking Lisbon to Madrid (Figure 1.5.2), which along with some other national and regional roads, cause high roadkill rates, habitat fragmentation and degradation of functional connectivity (Carvalho & Mira, 2011).

As such, it is crucial to develop effective mitigation taking advantage from EOS, namely by integrating *montado* spatiotemporal conditions, and concomitantly considering the surrounding landscape and its alterations, to better allocate strategical resources on roads over long-term, as well as to improve the delineation of conservation areas along verges. Thus, for our research goals, multiple species were selected, which inhabit the *montado* system, being one of the most representative and widely diffused land uses in the region. By monitoring the entire landscapes at large extent, EOS information ensures the possibility of extrapolation inferences in a more cost-effective way, and the use of advanced remote sensing techniques, jointly with functional connectivity, represent prime components of this thesis, given the benefits in their application for optimising mitigation and conservation approaches.

The first part of the study consists in demonstrating the reliability of developing useful functional connectivity models relying on data easily available. In the second part, we demonstrate that EOS with high spatiotemporal detail can be used to identify suitable areas for endangered species often occurring on road verges, and that this information can be used for better planning conservation measures. Finally, we integrate useful EOS into functional connectivity modelling and show the potential of this approach to increase, in the long term, the effectiveness of planning mitigation measures targeting multiple taxa.

Considering the abovementioned, the thesis will address three main research goals:

I. Comparing the performance of two data types to build functional connectivity models for road mitigation plans.
II. Exploring the capability of EOS for identifying suitable microhabitats for small species of conservation concern in a Mediterranean ecosystem.
III. Developing an innovative methodological framework by combining remote sensing data, SDMs, and functional connectivity through circuit theory to optimise multitaxa roadkill mitigation planning.

To achieve these main goals, six specific objectives were defined, namely:

- Research Goal I:
    1) compare the performance of resistance surfaces derived from telemetry data with surfaces defined with occurrence data in identifying road-crossing locations of a forest carnivore;
    2) assess the influence of movement type (daily vs. dispersal) on the performance of models identifying those road-crossing locations.
- Research Goal II:
    3) test the usefulness of Sentinel-2 EOS derived predictors for identifying suitable microhabitats for small and elusive species of conservation concern.
- Research Goal III:
    4) evaluate the utility of remotely sensed habitat metrics (Landsat EOS) in describing the occurrence of forest-dwelling species of taxa with different life-histories (body size, home-range and dispersal).
    5) quantify the relative contribution of species-specific habitat, functional connectivity and road metrics in explaining spatial patterns of road mortality for multiple species across different taxa.
    6) identify road locations with the highest agreement in long-term mortality predictions, for the greatest number of species in each group and across groups.

1.6 *Outline of the thesis*

In this thesis some challenges and opportunities regarding road ecology were investigated, along with shortcomings that still remain unsolved in systematic conservation planning, with the overall aim to enhance the effectiveness of wildlife conservation measures. The thesis was structured into five main chapter: the introduction, three scientific manuscripts already published (chapters 2 and 3), or submitted for publication (chapter 4) in peer reviewed international journals, and a final chapter with overall conclusions and new insights (chapter 5). The chapters are structured in the following way:

- Chapter 1 frames the general introduction with focus on the road ecology discipline, as well as gaps and limitations during EIAs and the planning phase, rising possible solutions across the interdisciplinary areas of landscape connectivity and remote sensing to enhance application of conservation measures along roads. On the foundation of this chapter are formulated and structured the objectives of the thesis.
- Chapter 2, introduces the possibility of developing functional connectivity models for predicting road-crossing sites from real movements, underlining that their collection often requires the disposal of high budgets. Therefore, by using as case of study a forest dwelling species inhabiting the *montado* the common genet (*Genetta genetta*), it is examined whether roadkill predictions from more affordable connectivity-based data type models, namely species distribution models (SDMs), are comparable to those much more costly and data demanding, such as path selection functions (PathSFs) derived from telemetry data. This chapter addresses objectives 1 and 2.
- Chapter 3 focuses on the usefulness of high resolution spatiotemporal remotely sensed information (Sentinel-2) in estimating the distribution of an endangered species, the Cabrera vole (*Microtus cabrerae*), often occupying habitats along road verges, as well as areas nearby. Here it was explored the intra-annual variation, in *montado* systems with focus on more open habitats (understorey) to foster a more accurate identification of microhabitat, hence heightening the detail and effectiveness of conservation measures along road verges. The objective 3 is here realised, which paved the way for using EOS habitat metrics and accounting for inter-annual landscapes dynamics in chapter 4.
- Chapter 4 focuses on the effectiveness of EOS data in supporting multitaxa long-term mitigation plans while covering long temporal scales and integrating species-based functional connectivity modelling. The chapter is divided in three-stage statistical analysis to unveil road sites for mitigation in dynamic landscapes: 1) several habitat metrics are calculated and habitat suitability, based on species distribution models are developed, for each species of the three animal groups considered (non-flying mammals, birds, and bats); 2) the relative effects of habitat, functional connectivity and road metrics, on roadkill are disentangled for each species to assess the roadkill

vulnerability; 3) the variation in roadkill predictability is estimated to check prediction consistency, in order to allow flexibility for mitigation plans for biodiversity conservation, resilient in the long-term to inter-annual landscape dynamics. The objectives 4, 5, and 6 are addressed in this chapter.

- Chapter 5 summarises the core findings of chapters 2, 3 and 4, embedding them in the state-of-the-art of road ecology. It also points out to new insights linking the objectives, and towards future prospective and challenges from the interplay of remote sensing and connectivity disciplines, bridging road ecology and systematic conservation planning.

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Chapter 2

Accounting for Connectivity Uncertainties in Predicting Roadkills: a Comparative Approach between Path Selection Functions and Habitat Suitability Models

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Abstract

Functional connectivity modeling is increasingly used to predict the best spatial location for over- or underpasses, to mitigate road barrier effects and wildlife roadkills. This tool requires estimation of resistance surfaces, ideally modeled with movement data, which are costly to obtain. An alternative is to use occurrence data within species distribution models to infer movement resistance, although this remains a controversial issue. This study aimed both to compare the performance of resistance surfaces derived from path versus occurrence data in identifying road-crossing locations of a forest carnivore and assess the influence of movement type (daily vs. dispersal) on this performance. Resistance surfaces were built for genet (Genetta genetta) in southern Portugal using path selection functions with telemetry data, and species distribution models with occurrence data. An independent roadkill dataset was used to evaluate the performance of each connectivity model in predicting roadkill locations. The results show that resistance surfaces derived from occurrence data are as suitable in predicting roadkills as path data for daily movements. When dispersal was simulated, the performance of both resistance surfaces was equally good at predicting roadkills. Moreover, contrary to our expectations, we found no significant differences in locations of roadkill predictions between models based on daily movements and models based on dispersal. Our results suggest that species distribution models are a cost-effective tool to build functional connectivity models for road mitigation plans when movement data are not available.

Keywords

movement data, occurrence data, telemetry; species distribution models, mitigation; dispersal period

2.1 Introduction

Linear infrastructures such as highways, roads, railroads, and electric power lines are globally recognized as a major cause of habitat and biodiversity loss (van Der Ree et al. 2011), whose impact is expected to rise considerably in future decades (van der Ree et al. 2015). Roads are responsible for landscape fragmentation, changing animal routes, for direct mortality due to vehicle impact (Coffin 2007; Benítez-López et al. 2010), and for jeopardizing the gene-flow exchange in some species (Riley et al. 2006; Bischof et al. 2017). To address such issues, mitigation measures such as over- or underpasses are usually built at specific locations along roads (van Der Ree et al. 2011; Santos et al. 2018). The decision about where to locate such measures is often critical, given the uncertainties related to the ecological process to preserve, the scale of analysis, the intrinsic biases (e.g., expert experience, model uncertainties) and the existing budgetary limitations (Tulloch et al. 2016). Thus, besides the commonly used roadkill hotspots (Bissonette and Cramer 2008; Santos et al. 2011), which may not always be accurate (Santos et al. 2015) or available, road ecologists have further considered the benefits of understanding wildlife movements to prioritize resource allocation for mitigation (Allen and Singh 2016). Although the coincidence between crossing sites and roadkill sites is not always verified (Neumann et al. 2012), functional connectivity modeling is increasingly used as a tool to predict the best spatial location for animal road-crossing structures by relating functional connectivity with roadkills (Roger et al. 2012) as well as road crossing (Cushman et al. 2014).

Functional connectivity is a "species-specific trait that measures the degree by which landscape impedes or facilitates wildlife movements, and depends on characteristics of the landscape and on species ecology and behavior" (Taylor et al. 1993). Functional connectivity modeling is usually addressed through landscape resistance surface analysis (Zeller et al. 2012; Carvalho et al. 2016; Correa Ayram et al. 2016). This quantifies "the reluctance of an animal when moving through the landscape and ultimately depicts its physiological cost or mortality risk" (Zeller et al. 2012). Resistance surfaces are thus a crucial step in functional connectivity analysis and can be modeled through numerous approaches (Zeller et al. 2012). The simplest is the "expert-based" approach, which consists in one or more experts classifying the resistance of different land use categories (or other landscape elements) based on their experience or on a literature review (Grilo et al. 2011; Wade et al. 2015). However, the above approach has been widely criticized (Wade et al. 2015; Correa Ayram et al. 2016; McClure et al. 2016), and resistance surfaces are now frequently obtained from model estimations (Pullinger and Johnson 2010; Carvalho et al. 2016; Ziółkowska et al. 2016).

There are multiple criteria to parametrize models for estimating resistance surfaces. Some authors use sequential points in time, i.e., steps (two consecutive points) or paths (e.g., consecutive steps followed during one foraging event) obtained from telemetry data. This allows the development of functions that can be used to predict the probability of animal movement across the study area (Zeller et al. 2012), such as path selection functions (PathSFs; e.g., Elliot et al. 2014; Carvalho et al. 2016). Another possible approach is to develop species distribution models (SDMs) that use species occurrence locations (Guisan and Zimmermann 2000; Correa Ayram et al. 2016) which can result from direct sightings of animals or other detection methods (tracks, scats, vocalizations, and nests). This approach implies that "the same environmental predictors influence both animal movements and habitat selection" and thus it is assumed that "high habitat suitability values correspond to low landscape resistance values" (Ziółkowska et al. 2016).

Given the types of empirical data required for each modeling approach, occurrence data are usually easier to obtain. However, occurrence data are limited to a specific time and space, meaning that "movement cannot be really measured, but only inferred" (Zeller et al. 2012). Sequential points obtained through telemetry are usually preferred over independent occurrence data for deriving resistance surfaces because when they are collected at short time intervals, they reflect the exact pathway taken by an animal (Zeller et al. 2012; Ziółkowska et al. 2016). However, path data gathered through telemetry requires a large quantity of information that is more difficult and expensive to obtain, and thus requires more time to convert into a resistance surface (Zeller et al. 2012).

Another potential difficulty of path data is that they may include distinct types of movement that can be made during an animal's lifespan (Coffin 2007; van der Grift and van der Ree 2015; Blazquez-Cabrera et al. 2016). There are two main behavioral states: daily movements, made within the home range to fulfill the animals' primary needs (e.g., foraging, shelter, mating), and dispersal movements to colonize new territories (Pe'er et al. 2011). Some young dispersing animals are known to venture into suboptimal habitats (e.g., extensive open farmland areas), putting their lives at risk, unlike established (resident) animals that use more suitable habitats within their home ranges, such as the Iberian lynx (Blazquez-Cabrera et al. 2016) and lions (Elliot et al. 2014). Conflating these movement types may produce inaccurate resistance surfaces, and it is thus generally recommended to separate the movement-related behaviors before using them in the paths for connectivity analyses (Zeller et al. 2012; Blazquez-Cabrera et al. 2016). In this context, the use of SDMs may not be sensible, given the uncertainty around which independent occurrence points represent movement within the home range and which represent dispersal movement.

The hypothesis that "animals move in the same way as they select habitat" still remains a matter for discussion (Zeller et al. 2012), focusing on which contexts SDMs can reflect movement models. SDMs can be used at diverse spatial scales, ranging from fine (daily movements or within home range) to broad scales (dispersal) (e.g., Vergara et al. 2015). Yet this methodology has been partly contested when applied to assessing functional connectivity. For example, Ziółkowska et al. (2016) proved that SDMs and step selection function models were equally good at predicting brown bear movements only for small scales, while for road mitigation measures, Blazquez-Cabrera et al. (2016) advocate that SDMs can provide suitable estimates of movement resistance. Road-crossing mitigation structures aim to restore habitat connectivity and to reduce the number of roadkills (Bissonette and Cramer 2008; van der Ree et al. 2015). Occurrence of roadkills is commonly driven by species-specific habitat preferences (Gunson et al. 2011). If we assume a direct relationship between roadkills and habitat features (Gunson et al. 2011), this should imply efficient performances in predicting roadkills from habitat suitability models (Roger et al. 2012). It is also commonly assumed in SDMs that opportunistically collected fine-scale occurrences (used to fit the models) represent mostly daily movement, as dispersal is an infrequent event in the lifetime of one animal (Cosgrove et al. 2018). Although dispersal is rare, occurring usually once a year, high kill rates have been detected during this biological period (Červinka et al. 2015; Carvalho et al. 2018). Crossing roads is inherently risky and roads may impair daily movement routes through avoidance or barrier effects (Coffin 2007; Bissonette and Cramer 2008; Cosgrove et al. 2018). However, species may be “forced” to cross roads during natal dispersal to reach new territories (Fey et al. 2016; Carvalho et al. 2018). Consequently, SDM predictions could be significantly biased when validated through high rates of roadkills containing dispersal movements. Daily movements, in fact, should be better related to landscape resistance surfaces than dispersal movements (Hanski 1995; McClure et al. 2016). Thus a transformation function was recently developed for application on habitat suitability outcomes (Trainor et al. 2013; Keeley et al. 2016) so that dispersal resistance surfaces could be better simulated.

To our best knowledge, little is known about the comparative performance of landscape resistance estimates in predicting animal movements, and there still persists a lack of consensus on which biological data type to use (paths vs. occurrences) (Zeller et al. 2012; Ziółkowska et al. 2016). Quantifying the degree of uncertainty in ecological risk assessment is necessary to provide guidance to decision makers about the efficiency of spatial ecological models (Jaeger 2015; Wade et al. 2015; Tulloch et al. 2016). The understanding of how resistance surfaces can influence the accuracy of road-crossing predictions is a critical issue, given the strong conservation implications and road agency investments in mitigation measures potentially derived from such analyses. Identification of locations for appropriate application of mitigation structures that enhance local animal routes is thus of crucial importance for maintaining abundance (Benítez-López et al. 2010) and dispersal movements, thereby ensuring gene flow (Riley et al. 2006; Cosgrove et al. 2018).

Our primary objective was to compare the performance of resistance surfaces derived from (a) path (PathSFs) and (b) occurrence (SDMs) data in predicting road-crossing locations of a forest carnivore. Secondly, we assessed the influence of movement type (daily vs. dispersal) on the above performance, using measures of roadkill prediction accuracy. We hypothesized that (H1) daily resistance surfaces derived from occurrence data (SDMs) are as suitable in predicting movement (roadkills) as are path data (PathSFs), since suitable habitat characteristics are a key driver of roadkill patterns (Grilo et al. 2011; Gunson et al. 2011). Consequently, (H2) resistance surfaces derived from simulated dispersal scenarios, using either PathSFs or SDMs, are expected to have lower roadkill predictive performance than daily resistance surfaces. However, (H3) predictive performance of resistance surfaces derived from dispersal scenarios can be improved when roadkill data are restricted to the species' dispersal period.

Medium-sized forest carnivores are highly vulnerable to losses in landscape connectivity and to fragmentation by roads (Grilo et al. 2015). Within this group, we selected the genet (*Genetta genetta*) as a target species because it is a relatively abundant carnivore, specialized in forest habitats and negatively affected by roads (Galantinho and Mira 2009; Carvalho et al. 2014).

We built PathSFs and SDMs that combined environmental predictors with either path or occurrence data through a multimodel ensemble of generalized linear mixed models (Duchesne et al. 2010) and generalized linear models (GLMs; Guisan and Zimmermann 2000), respectively. The performance of data type (path vs. occurrence) and movement type (daily vs. dispersal) in predicting roadkills was evaluated through the fit of GLMs using discrimination ability and explained variance (Guisan and Zimmermann 2000).

2.2 ***Materials and Methods***

Study Area

The study area is located in southern Portugal (Fig. 1a; 38°21′48″–39°00′35″N, and 8°23′45″–7°35′08″W) and covers about 4408 km2. The climate is Mediterranean, dominated by mild winters (5.8–12.8 °C) and hot dry summers (16.3–30.2 °C), with a mean annual rainfall of 609.4 mm, falling chiefly from October to March (IPMA 2016). The area is covered mostly by Mediterranean cork/holm oak woodland, with varying tree cover and (open) agricultural areas in approximately equal proportions, lying in a smooth undulating (<420 m a.s.l.) landscape. The area is crossed by several roads, including one highway, and some national and regional roads. For the present study, 102 km of national roads (EN114, EN18, and EN4) were monitored for roadkills of vertebrates (Fig. 1). A further description of the study area is provided in Carvalho et al. (2016).

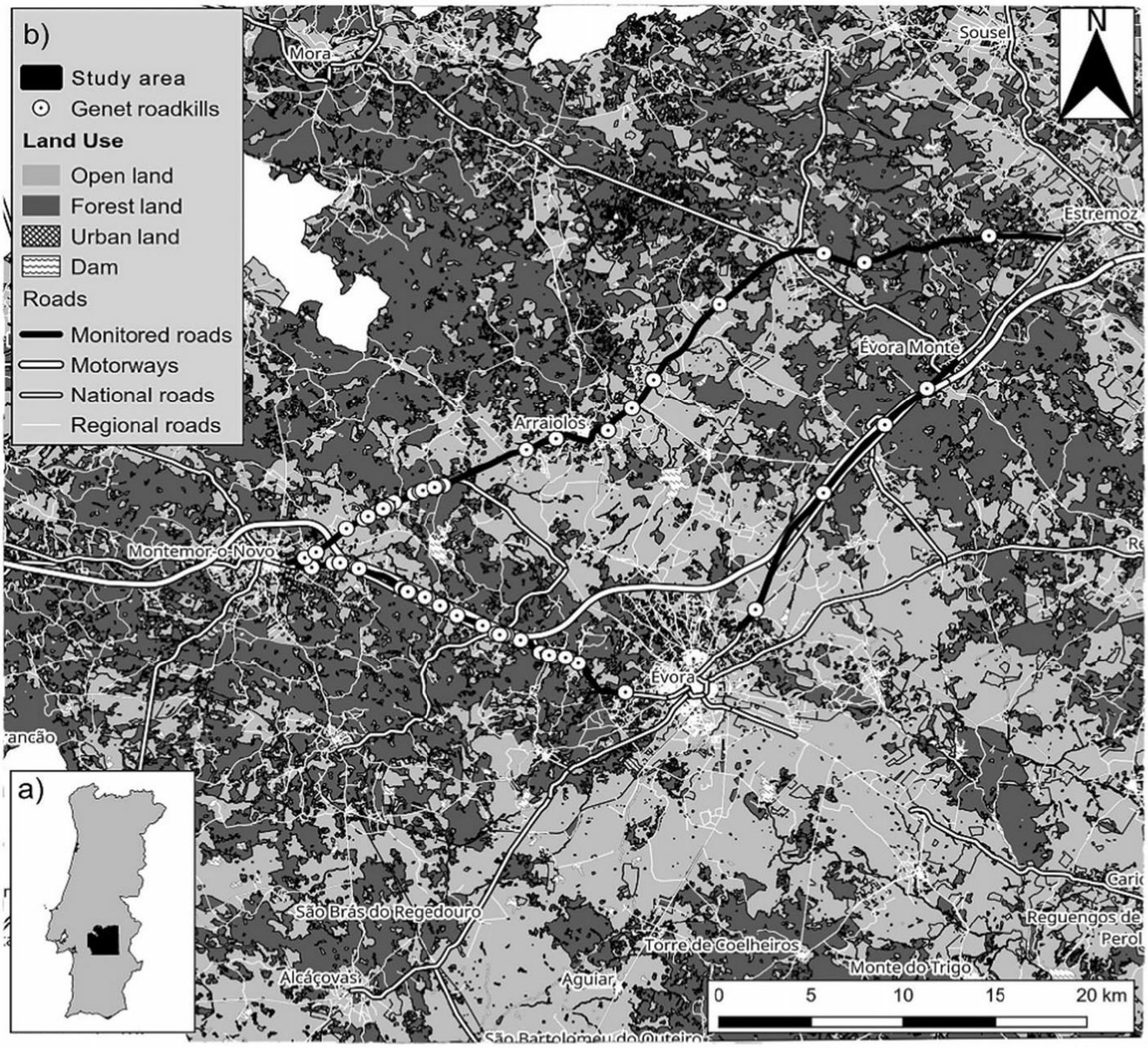


Fig. 1. **a** Location of the study area in Portugal. **b** Spatial distribution of genet roadkills and monitored roads in the study area, overlaid on a layer with the main land uses.

Modeling Procedure

We summarize all modeling steps in a flow chart, from predictor extraction and model settings to movement types used for roadkill predictions (Fig. 2), and we detail below the analytical procedures.

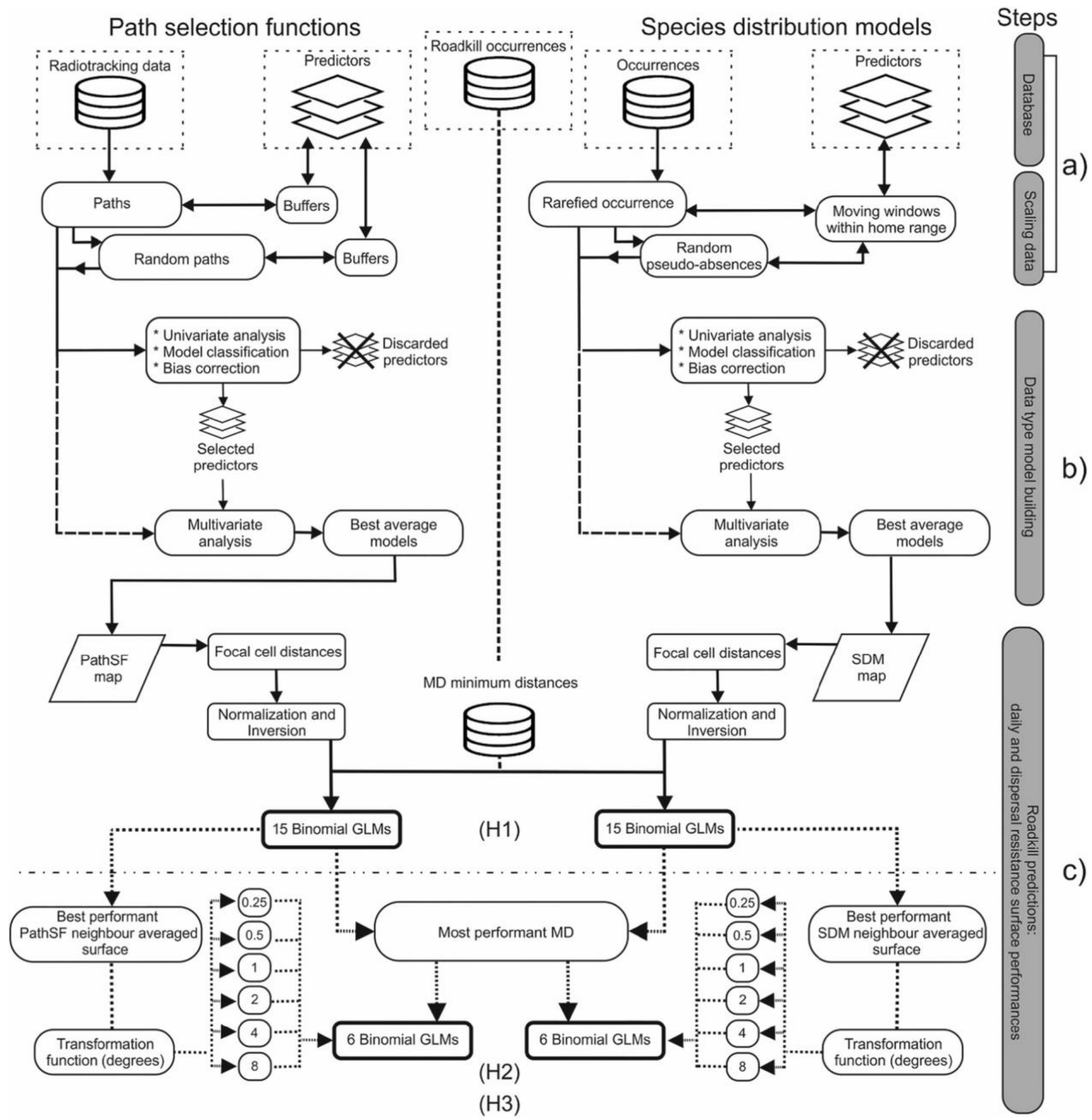


Fig. 2. Flow chart representing model building and comparisons of roadkill predictions between path selection functions (PathSFs) and species distribution models (SDMs), accounting for both movement types (daily and dispersal). See the main text for details.

Step (a) Data Acquisition

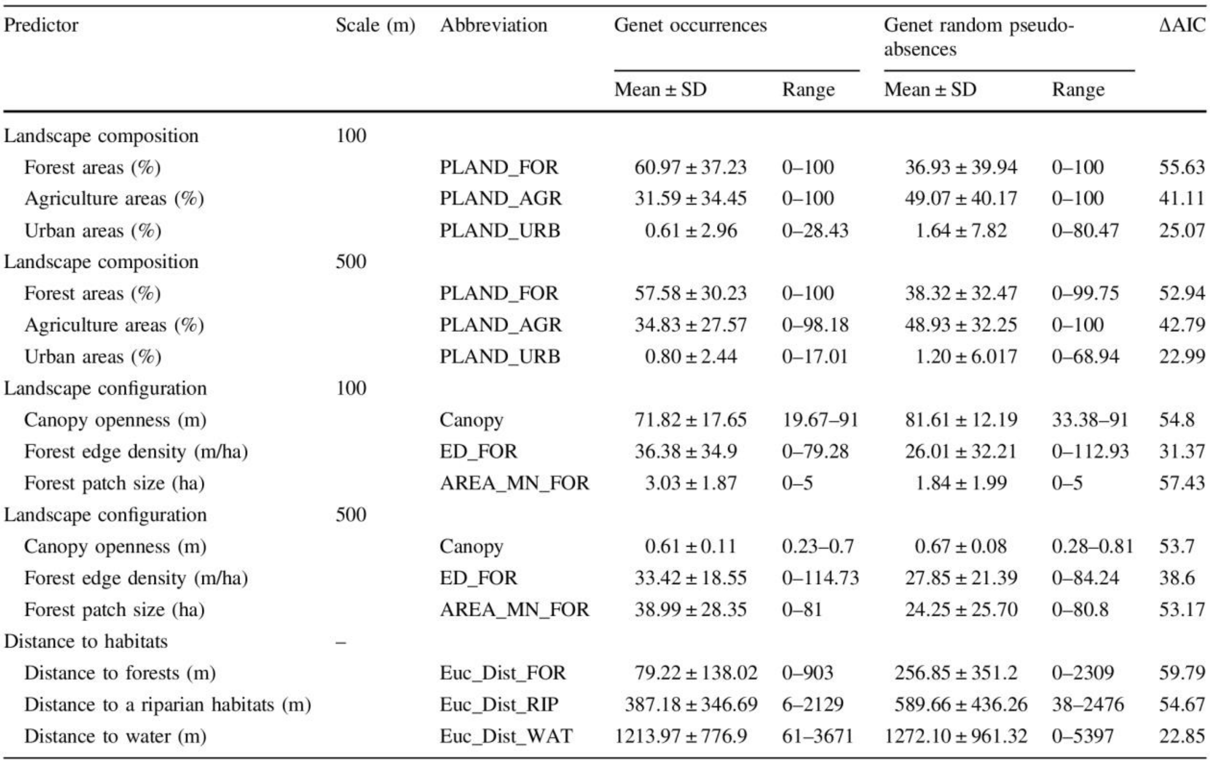
Genet data

The movement data regarding the study area were obtained within a previous study conducted between 2010 and 2012 (Carvalho et al. 2016). The study included telemetry records of 22 resident animals within their home ranges, corresponding to a total of 2850 consecutive location records taken at about half-hour intervals, which produced 198 time-independent paths (separated by more than 60 min). For each observed path, nine random paths were generated (Carvalho et al. 2016). We also gathered data on species distribution from the UBC database (Conservation Biology Unit, University of Évora), which included both occurrences of the genet in the study area, and roadkill locations from 2005 to 2016 (Fig. 2a). These data included 1203 occurrence records with potential to be used in the SDMs. To avoid spatially clustered occurrence patterns and minimize spatial auto-correlation, we applied a rarefaction procedure (Kramer-Schadt et al. 2013). In this process, only occurrences that are at least 1000 m away from each other (ca 300 ha buffer, an approximation to the mean home range size of this species, Santos-Reis et al. 2005; Carvalho et al. 2014) were kept for further analyses (Fig. 2a). Thus, 175 records of genet occurrence were finally included in SDMs. We then defined an equal proportion of pseudo-absences (n = 175; Fig. 2a) assigned randomly from the extent of the study area (Barbet-Massin et al. 2012), excluding the nonrarefied occurrence cells.

Genet roadkills consisted of 77 locations of genet carcasses identified on monitored paved roads (Fig. 1a). Surveys were done previously by one experienced observer driving at 20–40 km/h. All genet carcasses, once detected, were registered with a GPS and removed from the road to avoid double counting (e.g., Santos et al. 2013).

Environmental predictors

SDMs predictors and the analysis protocol were as similar as possible to those used in previous PathSFs models (Carvalho et al. 2016) to allow comparability of the resistance surface performance (Table 1). We prepared a set of landscape predictors at 100-m resolution using CORINE Land Cover data (Guiomar et al. 2009) to characterize paths (observed and random) and occurrence points (Fig. 2a). These include landscape composition (percent cover of oak forests, open agricultural land, and urban areas), landscape configuration (mean forest patch size, forest edge density, and tree canopy openness), and distance to important landscape resources (forests, riparian habitats, and water bodies; Table 1; Carvalho et al. 2016). The predictors concerning landscape composition and configuration were measured for two spatial scales (McGarigal et al. 2016): within a 100 m buffer (immediate surroundings perceived by the animal when following a path) and within a 500 m buffer (the larger area available to the animal when making movement decisions). The distance predictors were scale independent (see Carvalho et al. 2016 for details). We used round moving windows (McGarigal et al. 2012) for the landscape class-level metrics, and focal foci to assess optimal neighborhood scale (Holland and Yang 2016) for the canopy openness predictor.



Predictor	Scale (m)	Abbreviation	Genet occurrences		Genet random pseudo-absences		ΔAIC
			Mean ± SD	Range	Mean ± SD	Range	
Landscape composition	100						
Forest areas (%)		PLAND_FOR	60.97 ± 37.23	0–100	36.93 ± 39.94	0–100	55.63
Agriculture areas (%)		PLAND_AGR	31.59 ± 34.45	0–100	49.07 ± 40.17	0–100	41.11
Urban areas (%)		PLAND_URB	0.61 ± 2.96	0–28.43	1.64 ± 7.82	0–80.47	25.07
Landscape composition	500						
Forest areas (%)		PLAND_FOR	57.58 ± 30.23	0–100	38.32 ± 32.47	0–99.75	52.94
Agriculture areas (%)		PLAND_AGR	34.83 ± 27.57	0–98.18	48.93 ± 32.25	0–100	42.79
Urban areas (%)		PLAND_URB	0.80 ± 2.44	0–17.01	1.20 ± 6.017	0–68.94	22.99
Landscape configuration	100						
Canopy openness (m)		Canopy	71.82 ± 17.65	19.67–91	81.61 ± 12.19	33.38–91	54.8
Forest edge density (m/ha)		ED_FOR	36.38 ± 34.9	0–79.28	26.01 ± 32.21	0–112.93	31.37
Forest patch size (ha)		AREA_MN_FOR	3.03 ± 1.87	0–5	1.84 ± 1.99	0–5	57.43
Landscape configuration	500						
Canopy openness (m)		Canopy	0.61 ± 0.11	0.23–0.7	0.67 ± 0.08	0.28–0.81	53.7
Forest edge density (m/ha)		ED_FOR	33.42 ± 18.55	0–114.73	27.85 ± 21.39	0–84.24	38.6
Forest patch size (ha)		AREA_MN_FOR	38.99 ± 28.35	0–81	24.25 ± 25.70	0–80.8	53.17
Distance to habitats	–						
Distance to forests (m)		Euc_Dist_FOR	79.22 ± 138.02	0–903	256.85 ± 351.2	0–2309	59.79
Distance to a riparian habitats (m)		Euc_Dist_RIP	387.18 ± 346.69	6–2129	589.66 ± 436.26	38–2476	54.67
Distance to water (m)		Euc_Dist_WAT	1213.97 ± 776.9	61–3671	1272.10 ± 961.32	0–5397	22.85

Table 1. Summary univariate statistics of predictors used for SDMs models, with indication of scale used, abbreviation, mean, and SD values for sites with genet occurrence and pseudo-absence. Predictors are grouped into three categories (landscape composition, configuration, and distance to habitats). The same three landscape class-level metrics used on PathSFs analysis were also calculated for SDMs and regarded the same land cover types. Land cover types considered were as follows: *FOR* forest areas, *AGR* agriculture areas, and *URB* urban areas

Step (b) Model Building

Prior to model building, predictors were evaluated for normality and the effect of extreme values was softened through angular or logarithmic transformations, and standardized to zero mean and unit variance to allow comparability of their effects (see Carvalho et al. 2016). The degree of collinearity among candidate predictors was evaluated through a pairwise Pearson correlation test. For pairs with $|r| > 0.7$, only the variable with the highest biological meaning, based on previous studies of the species, was retained (see Carvalho et al. 2016).

Because path and occurrence data have different spatial properties, the modeling approaches were also slightly different (Fig. 2b). PathSFs were built with mixed effects conditional logistic regression, to account for stratification of random paths and inter-individual heterogeneity in path selection (Duchesne et al. 2010; Carvalho et al. 2016). For SDMs, GLMs with binomial error distribution and a logistic link function were applied (Guisan and Zimmermann 2000). Both approaches compared landscape characteristics of observed paths (or occurrences) with random paths (or pseudo-absences).

Predictor screening in both approaches was conducted with univariate analysis based on mixed models or GLMs, respectively. We compared each single-predictor model with a null model using the Akaike information criterion (AIC; Burnham and Anderson 2002). Only predictors producing models with strong support (ΔAIC > 10) were selected for the subsequent multivariate analyses (Table 1).

We built PathSFs and SDMs multivariate models with all possible combinations of the selected predictors (including the null and the full models), and used a multimodel inference procedure (Burnham and Anderson 2002) to rank the models based on their Akaike weights (w_i). The average parameters and unconditional standard errors (SEs) were estimated for the set of models that differed by less than four in their AIC from the most supported model (Burnham and Anderson 2002). The model coefficient of a predictor was considered statistically significant if estimates of its 95% confidence interval did not include zero. The contribution of each predictor was based on the sum of Akaike weights for the models in which that predictor was contained (w_+) and on the magnitude of the average model coefficient. We evaluated model performance through the amount of explained deviance (D-squared; Guisan and Zimmermann 2000) and discrimination ability was assessed by the area under the receiver operating characteristic (ROC) curve (AUC) (Fielding and Bell 1997; Swets 1988). The averaged PathSFs and SDMs model predictions were applied to estimate movement probability on a grid of 100-m scale (Carvalho et al. 2016).

Step (c) Roadkill Predictions: Habitat and Dispersal Resistance Surface Performances

We derived additional movement probability maps (for PathSFs and SDMs) in order to evaluate the optimal neighborhood scale (Cushman et al. 2014; Holland and Yang 2016). For each pixel, we extracted movement probability values from five neighbor distances (0, 50, 100, 200, and 300 m; Fig. 2c). We applied a normalization procedure to guarantee that pixel values of each movement probability map remained within the range of 0–1. Each of these movement probability maps was transformed into a resistance map by linearly inverting the probability values (Wade et al. 2015).

To test H1 (PathSFs and SDMs produce equally good habitat-based resistance surfaces for predicting roadkills), we compared the performance of each type of resistance surface in explaining the occurrence pattern of roadkills by using binomial GLMs. In order to apply these models, roadkill pseudo-absences were randomly generated (with a 50% prevalence, i.e., the same number of observed presences and pseudo-absences) along the monitored roads. Accordingly, we defined three datasets (presence/absence of roadkills) that vary in the minimum distance (MD) between an absence and a roadkill location: 100, 500, and 1000 m. This procedure intends to minimize false-negative bias (Barbet-Massin et al. 2012; Fielding and Bell 1997). Each MD roadkill dataset (MD100, MD500, and MD1000) was used as the response variable in univariate GLMs, and each of the five defined resistance surfaces were used as predictors (resulting in combinations of 15 GLMs per data type model; Fig. 2c). Model fit was verified through the AUC (Fielding and Bell 1997), the amount of explained deviance (Guisan and Zimmermann 2000), AIC and a ΔAIC comparison between the best-performing model of PathSFs or SDMs vs the of PathSFs or SDMs (for each MD). We used a ΔAIC threshold of four to evaluate the magnitude of support between models (Burnham and Anderson 2002; Carvalho et al. 2016; Salgueiro et al. 2018). Results were averaged across ten replicates run on the training (75%) and randomly chosen test (25%) datasets. The performance differences of PathSFs and SDMs in predicting roadkills (H1) was assessed through a DeLong test that compares ROC curves between different models (1000 permutations). Statistical divergence between curves was set to $p < 0.05$.

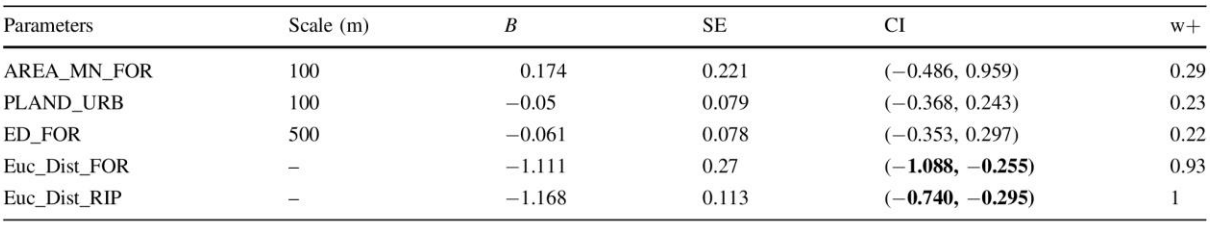
In order to test the working hypotheses concerning dispersal movements (H2 and H3), we obtained simulated dispersal resistance maps by applying a negative exponential transformation function (Keeley et al. 2016) on the previously evaluated PathSFs and SDMs best resistance surfaces models in H1 (Fig. 2c). When landscape resistance is estimated from SDMs, it is usually assumed to be a negative linear function of suitability; in other words, the resistance decreases at a constant rate as suitability increases (Keeley et al. 2016). Using an exponential transformation means that larger portions of the landscape will be coded as low resistance to movement (Keeley et al. 2016), thus approaching more generalist habitat choices during dispersal events. We used as an exponential transformation function the following equation: $R = 100 - 99 \times ((1 - exp(-c \times h))/(1 - exp(-c)))$, in which R is resistance, h is probability of movement taken from PathSFs or SDMs models, and c is the degree of the function corresponding to 0.25, 0.5, 1, 2, 4, or 8 (Fig. 2c). For each of the seven transformations, resistance is minimum when probability of movement is maximum. At $c = 0.25$, the relationship is almost linear, being equivalent to the previous daily resistance surface. As c increases, resistance values change from a linear towards an exponential decay function, with resistance to movement decreasing more rapidly (and reaching the asymptote sooner) with increasing habitat suitability (Keeley et al. 2016). Thus, we calculated six dispersal resistance surfaces for each data type model (PathSFs and SDMs). Simulated dispersal resistance maps were validated against roadkills using GLMs, as in previous analyses. However, for hypotheses H2 and H3, we used only one MD dataset (that with the best performance in previous analysis; Fig. 2c). To determine whether roadkill identification performance significantly decreased (H2) or improved (H3), we used the ΔAIC by comparing the performance of each model vs the best model (for PathSFs and SDMs separately). Similar analyses were used to test H2 and H3. However, for H2, all-year-round roadkill records were included, while for H3 roadkill data were restricted to the dispersal period of the genets (September–March; Larivière and Calzada 2001). Because such movements are more common among juveniles or subadults (Fey et al. 2016; Carvalho et al. 2018), we further excluded all identified road-killed adults (animals with a lower probability of being in dispersal) from H3 testing.

Analyses were conducted with R v.3.3.1 (R Development Core Team. 2016), QGIS v2.6 (Quantum GIS Development Team 2015), and Fragstats v4.2 (McGarigal et al. 2012). The following R packages were applied: “spThin” (Aiello-Lammens et al. 2015), “raster” (Hijmans 2016), “MuMIn” (Barton 2016), “ModEvA” (Barbosa et al. 2016), and “pROC” (Robin et al. 2011).

2.3 *Results*

General Results on SDMs

The SDMs results were in accordance with those of previously built PathSFs models. The average SDMs model included five predictors. Genet presence is more likely in areas close to riparian and forest habitats, as these predictors have high support in the average model ($w_+ > 0.90$; Table 2). In addition, areas with large forest patches, reduced urban presence, and reduced forest edge density also contribute to the increase in probability of species presence (Table 2). This model showed a D-squared value of 0.124 and resulted in a good discrimination ability (AUC = 0.7), which is close to PathSFs results ($\text{R-squared}_{\text{PathSFs}} = 0.33$, $\text{AUC}_{\text{PathSFs}} = 0.8$; Carvalho et al. 2016).



Parameters	Scale (m)	*B*	SE	CI	w+
AREA_MN_FOR	100	0.174	0.221	(−0.486, 0.959)	0.29
PLAND_URB	100	−0.05	0.079	(−0.368, 0.243)	0.23
ED_FOR	500	−0.061	0.078	(−0.353, 0.297)	0.22
Euc_Dist_FOR	–	−1.111	0.27	**(−1.088, −0.255)**	0.93
Euc_Dist_RIP	–	−1.168	0.113	**(−0.740, −0.295)**	1

Table 2. Summarized results for the averaged SDMs model: standardized regression coefficients (B), unconditional standard errors (SEs), 95% confidence intervals of coefficient estimates (CI), and selection probability (w_+) for each predictor in SDMs. Coefficient estimates whose 95%CI exclude zero are in bold. The PathSFs average model results showed similar predictors having high support (forest predictors and distance to riparian habitats) and are presented in Carvalho et al. (2016). Original names and associated calculated metrics of the abbreviation parameters are referred in Table 1.

Resistance Surfaces for Prediction of Daily Movements (H1)

For both types of models (PathSFs and SDMs) we found that model performance in explaining roadkill sites (AUC, D-squared, and AIC) increased with increasing MD datasets, with the best models corresponding to MD1000 dataset (Fig. 3a–c). These models had the highest AUC values and explained deviance (AUC > 0.80; D-squared > 0.15) and presented the lowest AIC scores (Fig. 3a–c).

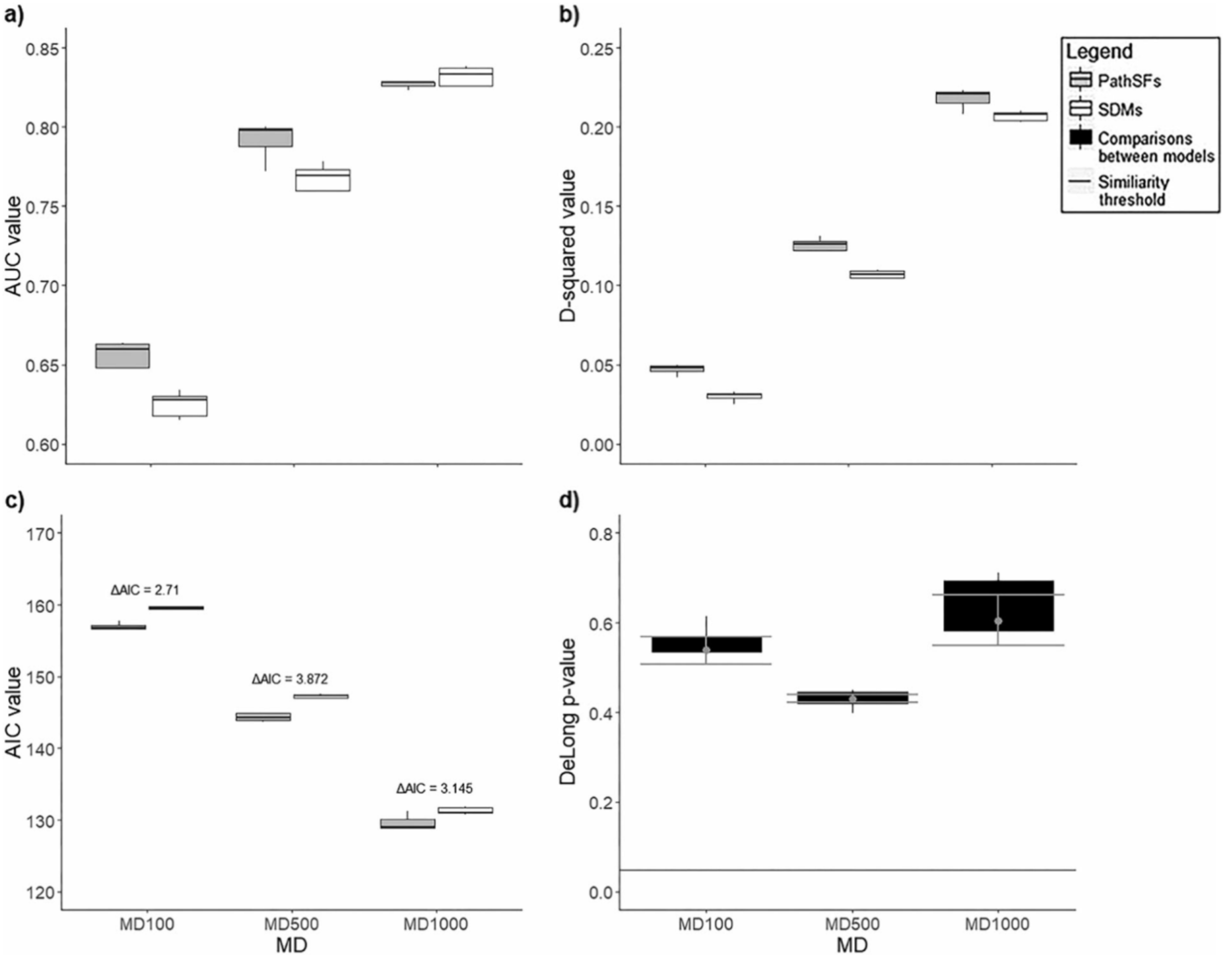


Fig. 3. Performance comparison results of path data (PathSFs) and occurrence data (SDMs) in predicting genet roadkills using all calculated neighbor distances (0, 50, 100, 200, and 300 m) and roadkill dataset (MD100–MD1000): **a** Area under the ROC curve (AUC); **b** amount of explained deviance (D-squared); **c** Akaike's information criterion (AIC); and **d** comparison of ROC curves (DeLong test) and p-value similarity threshold (horizontal black line).

When comparing the performance of each type of resistance surface in explaining roadkill patterns, we found that SDMs performed similarly (AUC and D-squared) in explaining roadkill data when compared with PathSFs, a result consistent through all roadkill datasets (MD100–MD1000; Fig. 3a, b). Moreover, the differences in AIC and in ROC curves were not statistically significant for neither datasets ($\Delta AIC < 4$; *p* values of DeLong tests > 0.05; Fig. 3c, d; Table 3). The similarities between SDMs and PathSFs resistance maps are shown in Figs 4a–b and 5. We thus conclude that the resistance surfaces derived from occurrence data (SDMs) are as suitable in predicting roadkills as are path data (PathSFs), thereby supporting hypothesis H1.

PathSFs					SDMs					Performance comparison	
MD	Neighbor distances	AUC	AIC	D-squared	MD	Neighbor distances	AUC	AIC	D-squared	ΔAIC	DeLong test (*p*-value)
1000	0	0.823	131.231	0.208	1000	0	0.838	131.875	0.203	0.644	0.642
1000	50	0.832	128.961	0.222	1000	50	0.837	130.979	0.209	2.018	0.711
1000	100	0.828	128.727	0.223	1000	100	0.833	130.790	0.21	2.063	0.583
1000	200	0.828	128.938	0.221	1000	200	0.737	132.084	0.208	3.146	0.398
1000	300	0.826	130.082	0.215	1000	300	0.826	131.795	0.204	1.713	0.693

Table 3. Data type models (PathSFs and SDMs) specific results regarding all the focal foci associated to

the most performant MD (MD1000). Accuracy scores were based on AUC, D-squared, AIC, ΔAIC, and p-values of the DeLong test. For further details regarding all the MD results see Table S1.

Resistance Surfaces for Prediction of Dispersal Movements (H2 and H3)

We also compared the performance of models including dispersal resistance surfaces in explaining a complete dataset (all-year-round roadkills; H2) and a dispersal-only dataset (H3). The dispersal resistance maps (e.g., $c = 4$ and $c = 8$) are presented in Fig. 5 for both models and their similarities are apparent. When using the complete roadkill dataset (H2), we found that ΔAIC scores showed decreasing performance from daily ($c = 0.25$) to dispersal movements ($0.5 < c < 8$; Fig. 4a). However, this AIC variation is <4, meaning that models do not statistically differ and thus rejecting H2. The scenario is similar for the dataset including only roadkills within the dispersal period (ΔAIC < 4), and H3 is also not supported (Fig. 4b). For further details on H2 and H3 results see Table S2.

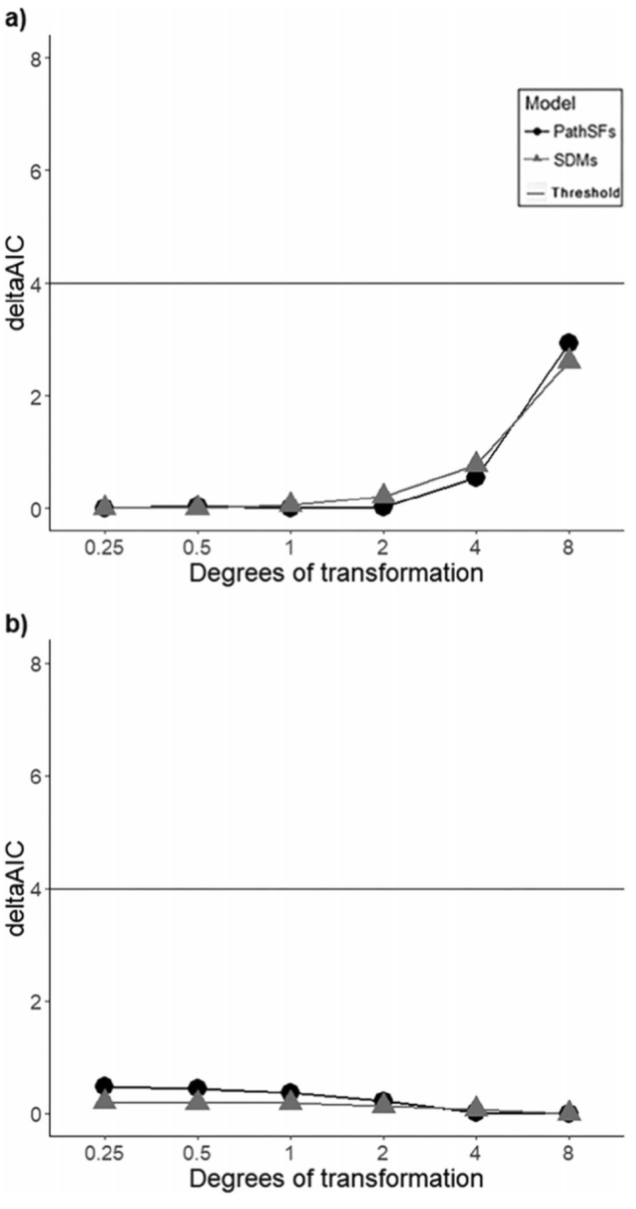


Fig. 4. Comparison of performance results (ΔAIC) between path data (PathSFs) and occurrence data (SDMs) in predicting genet roadkills for daily movement ($c = 0.25$) and for simulated dispersal

movement ($0.5 \leq c \leq 8$); **a** considering all roadkills and **b** considering only roadkills within the dispersal period. Horizontal black lines indicate the significance threshold ($\Delta AIC = 4$), meaning that differences under the line are interpreted as nonsignificant.

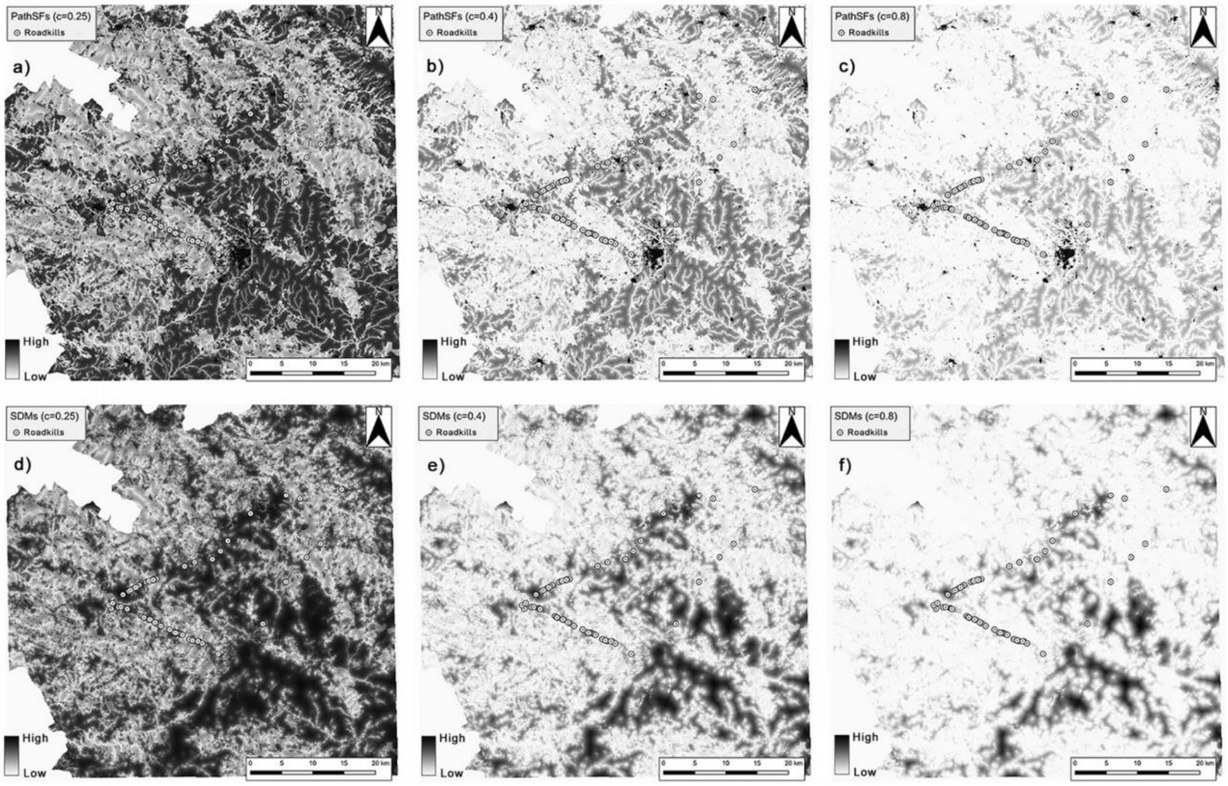


Fig. 5. Comparison of resistance maps between PathSFs and SDMs for the movement of genets (higher resistance values in black, lower resistance values in white) overlaid with roadkills sites; **a** PathSFs daily movement scenario ($c = 0.25$); **b** PathSFs intermediate dispersal movement scenario ($c = 4$); **c** PathSFs high-level dispersal movement scenario ($c = 8$); **d** SDMs daily movement scenario ($c = 0.25$); **e** SDMs intermediate movement scenario ($c = 4$); **f** SDMs high-level dispersal movement scenario ($c = 8$).

2.4 *Discussion*

Connectivity models often suffer from lack of validation with independent data and are therefore subject to uncertainty (Zeller et al. 2012; Wade et al. 2015; Correa Ayram et al. 2016). We used genet roadkill locations to validate landscape connectivity models built with “real movement” and “occurrence” data. Moreover, we compared the performance of models using daily movements with models using inferred dispersal, as they correspond to different life-events associated with individual survival and long-term population persistence. Juvenile as well as subadult are the most road-killed age classes for the genet and causalities are higher during the dispersal period (Carvalho et al. 2018). Both these facts suggest that dispersal is a critical event explaining temporal patterns of genet roadkills.

Our spatial analysis confirmed that landscape characteristics can be a valuable proxy of functional connectivity, and wherever roads approach or bisect suitable habitats, they are more prone to be crossed by animals, as maintained by others (Grilo et al. 2011; D’Amico et al. 2015; Kari and

Zimmermann 2015). We found a relationship of roadkills with connectivity maps derived from SDMs, which is consistent with previous studies (Roger et al. 2012) and also with maps derived from PathSFs, for which we have no knowledge in the literature. In particular, for both model types, the substantially improved performance with increasing MD distances seems to be related to a decrease in false absence error rates, resulting in a smaller negative effect of false absences (Fielding and Bell 1997). There is a growing body of scientific literature that suggests caution be taken when accounting for false absence bias (Barbet-Massin et al. 2012) and we highlighted its importance. Indeed, this is an underestimated issue in road ecology that can compromise the reliability of prediction models (Santos et al. 2018).

Comparison of Model Performance

The similarity in performance of roadkill predictions between SDMs and PathSFs suggests that connectivity models of both types are determined by similar predicted patterns and supports the hypothesis that movement is most facilitated in suitable habitats. For the genet, a higher probability of movement (and roadkills) is expected within or in the proximity of forest habitats, which often contain more stable and abundant resources (Carvalho et al. 2016) and thereby can be translated into lower variability of inter movement decisions (Duchesne et al. 2010). Thus, simple models based on occurrence data may be as able to determine how environmental parameters affect movements as more complex models based on path data. Moreover, considering the link between scales and the ecological process under investigation (Holland and Yang 2016; McGarigal et al. 2016), PathSFs and SDMs results may have been converged given the scale-dependent relationship of roadkills with movement type and land-use patterns. Specifically, scales within species’ home range are adequate to capture landscape characteristics that should be taken into account when applying mitigation measures (Gunson et al. 2011; Červinka et al. 2015), thereby representing a feasible “scale of management” (Allen and Singh 2016; Cosgrove et al. 2018).

Besides, when we compared roadkill prediction performance regarding the impact of behavior, whether daily movements or dispersal, the latter responded similarly, meaning that models based on daily movements can be as good as those based on dispersal, when choosing the locations for roadkill mitigation measures. One possible explanation for the lack of support for H2 might stem from a potential behavioral contamination, due to inclusion of the nondispersal period in the initial analysis. However, in a further analysis, dispersal-based prediction of roadkills did not improve when using only data for the dispersal period, contrary to expectations, thus also leading to the rejection of H3. Interpretation of such results may not be straightforward and needs prudence because terrestrial carnivore dispersing strategies may be influenced by individual variability in movement decisions (Oriol-Cotterill et al. 2015), which may be triggered as a mechanism of antipredator response toward a human-caused mortality risk (Jacobson et al. 2016). Such dispersing plasticity may include juveniles that adopt movements associated normally to adults (e.g., throughout favorable habitats). For example, this often happens when juvenile offspring, with no prior knowledge of the landscape, are accompanied by their more sensitive-to-risk mothers in their early exploratory movements (Beier 1995; Oriol-Cotterill et al. 2015). Moreover, riparian habitats, which had the most pronounced effects for both data type models (Carvalho et al. 2016), are often used as corridors during dispersal movements and simultaneously provide optimal residence habitat for multiple mesocarnivores species including genet (Carvalho et al. 2016; Grilo et al. 2016). On the other hand, adult genets may undertake extraterritorial habitat movements across supposedly avoided roads (Carvalho et al. 2018) which might contribute to reducing potential bias effect due to the small number of identified dispersing juveniles in the datasets. Although road avoidance is likely to exclude carnivore road-crossing events, increases in mortality rates have been documented following decreases in traffic volume (Alexander et al. 2005) or after rainy nights when road culverts become flooded (Craveiro et al. 2019). Thus, roads may occasionally act as a filter rather than being an absolute barrier (Jacobson et al. 2016; Ceia-Hasse et al. 2018).

Wide-ranging animals, such as carnivores, may encounter roads during different types of movements and behaviors (Ceia-Hasse et al. 2018), as outlined above. Once again, this issue is unlikely to be tackled in most road ecology studies, and when we accounted for it, the interpretation of our results is that the biasing behavior toward our employed roadkill datasets (global and dispersal) was not capable of significantly undermining the habitat suitability-roadkill relationship. Globally, our results reinforce the importance of roadkill mitigation measures that allow for the promotion of movements associated with different ecological processes, as explained below.

Management Implications

Functional connectivity models are generally more efficient when they include movement data. However, despite the advantages of pathway data, this information is costly to obtain in terms of time, manpower and money, and conservation actions are constantly faced with limited funding, which plays a paramount role when choosing the final solution (e.g., Santos et al. 2015). Placement of mitigation structures can be optimized by using models that draw on more easily available data, such as SDMs, because such models may adequately reflect the interactions between species and environment across spatial scales (Guisan et al. 2013). The SDMs cost-benefit tradeoff is enhanced when we take into account the growing demand for multiple species assessments (Khosravi et al. 2018), as the collection of a comprehensive multispecies pathway dataset is hampered by limited budgets. We therefore support such an application, in the context addressed herein, for other species and with different behaviors or life stages, as long as the SDMs modeling framework is explicit and justified within the context of conservation purposes (Guisan et al. 2013; Tulloch et al. 2016).

Conservation can operate at different scales, and previous studies required the selection of a proper planning scale that would integrate a pattern-process linkage associated with roadkills (Ims 1995; Kari and Zimmermann 2015; Tulloch et al. 2016; Cosgrove et al. 2018). Indeed, the type of mitigation should depend on both the target species and movement to promote (Smith et al. 2015; van der Grift and van der Ree 2015; Allen and Singh 2016). The analysis showed that daily and dispersal movements are simultaneously accounted for by SDMs. This fact, together with their simplicity (compared with PathSFs), makes them useful tools to guide the installation of road mitigation measures targeting both dispersers and residents. SDMs may be useful to support conservation managers as they can identify suitable locations (e.g., wherever roads intersect high connectivity areas) for roadkill mitigation measures (e.g., fauna passages, culvert adaptations, etc.), ensuring the accomplishment of daily routines associated with feeding, protection from predators and reproduction, and simultaneously promoting the gene flow, associated with dispersal, which is critical for long-term population persistence (van Der Ree et al. 2011).

Nevertheless, distinguishing between resident experienced adults and subadult dispersers may be important as long corridors linking distant suitable habitat patches might be mostly used by few dispersing individuals. Consequently, it should be noted that although locally placed crossing structures might be successful in ensuring the gene flow between subpopulations (Sawaya et al. 2014; Carvalho et al. 2018), higher scales of planning like regional/international coordinated strategies are still needed for the promotion of large ecological networks and inter-population gene flow (van Der Ree et al. 2011; Jaeger 2015). Ecological corridors applied across wide study areas (e.g., regional scales or higher) normally better rely on dispersal resistance surfaces, since when constructed through habitat suitability or movements within the home range, such corridors may greatly overestimate resistance to movement (Blazquez-Cabrera et al. 2016; Ziółkowska et al. 2016). In such contexts, the use of a negative exponential transformation function (Trainor et al. 2013; Keeley et al. 2016) may be more relevant as the generally lower resistance to movements renders distant patches more reachable by a few dispersers, thereby increasing the confidence of corridor placement (e.g., Khosravi et al. 2018). Lastly, we argue that these corridors might be likely to benefit other dispersing forest mammal species, given the similar dimension of home range to the species we examined, which in turn can be translated into a capability of similar dispersal distances or suchlike (Bowman et al. 2002).

2.5 *Conclusions*

In this paper, our spatial analysis showed a consensus of two comprehensive frameworks to identify road-crossing sites by a forest carnivore. We specifically demonstrated that, regardless of the model complexity (occurrence or paths), landscape elements characterizing species habitat may be a helpful proxy of functional connectivity in explaining roadkills and therefore in allocating management resources. Our results presented herein afforded valuable insights to solve uncertainties in predicting roadkills regarding some of the most widely used data type models, providing support for the use of SDMs. Moreover, we demonstrated that our models simultaneously consider daily movements and dispersal associated to roadkills, emphasizing the reliability of both SDMs and PathSFs in targeting both dispersers and residents at the local scale. We suggest that SDMs are a viable option to build resistance surfaces for functional connectivity models either in single species or multispecies projects, and with similar results for different life stages and behaviors. This is especially important for large scale connectivity assessments, such as multispecies defragmentation plans of high road-density landscapes (Santini et al. 2016).

2.6 *Acknowledgements*

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2.8 *Supplementary material*

Table S1 **-** Data type models (PathSFs and SDMs) specific results regarding all the neighbor distances associated to the selected MD (MD100, MD500, MD1000). Accuracy scores were based on AUC, D-squared, AIC, ∆AIC and p-values of the DeLong test.

PathSFs					*SDMs*					*Performance comparison*	
MD	**Neighbor distances**	**AUC**	**AIC**	**D-squared**	**MD**	**Neighbor distances**	**AUC**	**AIC**	**D-squared**	**∆AIC**	**DeLong test (p-value)**
100	0	0.613	157.799	0.042	100	0	0.615	160.509	0.025	2.71	0.427
100	50	0.663	156.715	0.049	100	50	0.628	159.771	0.029	3.056	0.535
100	100	0.664	156.542	0.05	100	100	0.634	159.465	0.031	2.923	0.568
100	200	0.66	156.799	0.048	100	200	0.618	159.364	0.032	2.565	0.614
100	300	0.648	157.195	0.046	100	300	0.63	159.263	0.033	2.464	0.547
500	0	0.772	145.656	0.108	500	0	0.76	149.528	0.093	3.872	0.437

500	50	0.788	144.260	0.126	500	50	0.769	147.617	0.105	3.357	0.399
500	100	0.798	143.406	0.131	500	100	0.773	146.958	0.109	3.552	0.421
500	200	0.799	143.895	0.128	500	200	0.716	146.861	0.11	2.966	0.445
500	300	0.8	144.853	0.122	500	300	0.778	147.363	0.107	2.51	0.45
1000	0	0.823	131.231	0.208	1000	0	0.838	131.875	0.203	0.644	0.642
1000	50	0.832	128.961	0.222	1000	50	0.837	130.979	0.209	2.018	0.711
1000	100	0.828	128.727	0.223	1000	100	0.833	130.790	0.21	2.063	0.583
1000	200	0.828	128.938	0.221	1000	200	0.737	132.084	0.208	3.146	0.398
1000	300	0.826	130.082	0.215	1000	300	0.826	131.795	0.204	1.713	0.693

Table S2- Comparison of performance results (ΔAIC) of path data (PathSFs) and occurrence data (SDMs) in predicting genet roadkills for daily movement (c=0.25) and for simulated dispersal movement (0.5 ≤ c ≤ 8); considering all roadkills and considering only roadkills within the dispersal period. Best model are highlighted in bold.

		All roadkills		*Roadkills (dispersal period)*	
Data type model	**Degree value**	**AIC**	**∆AIC**	**AIC**	**∆AIC**
PathSFs	0.25	115.3251	0.007	**68.50356**	0
PathSFs	0.5	115.2892	0.04	68.92253	0.01
PathSFs	1	**115.2478**	0	69.27417	0.22
PathSFs	2	115.2859	0.03	69.46472	0.37
PathSFs	4	115.8156	0.56	69.55991	0.45
PathSFs	8	118.1737	2.92	69.60698	0.49
SDMs	0.25	111.9867	2.61	70.96025	0.21
SDMs	0.5	110.1393	0.77	70.94298	0.18
SDMs	1	109.5806	0.21	70.9087	0.18
SDMs	2	**109.4261**	0.056	70.84137	0.13
SDMs	4	109.3828	0.013	70.71298	0.07
SDMs	8	109.3698	0	**70.48763**	0

Chapter 3

Predicting Microhabitat Suitability for an Endangered Small Mammal Using Sentinel-2 Data

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Abstract

Accurate mapping is a main challenge for endangered small-sized terrestrial species. Freely available spatio-temporal data at high resolution from multispectral satellite offer excellent opportunities for improving predictive distribution models of such species based on fine-scale habitat features, thus making it easier to achieve comprehensive biodiversity conservation goals. However, there are still few examples showing the utility of remote-sensing-based products in mapping microhabitat suitability for small species of conservation concern. Here, we address this issue using Sentinel-2 sensor-derived habitat variables, used in combination with more commonly used explanatory variables (e.g., topography), to predict the distribution of the endangered Cabrera vole (*Microtus cabrerae*) in agrosilvopastorial systems. Based on vole surveys conducted in two different seasons over a ~176,000 ha landscape in Southern Portugal, we assessed the significance of each predictor in explaining Cabrera vole occurrence using the Boruta algorithm, a novel Random forest variant for dealing with high dimensionality of explanatory variables. Overall, results showed a strong contribution of Sentinel-2-derived variables for predicting microhabitat suitability of Cabrera voles. In particular, we found that photosynthetic activity (NDI45), specific spectral signal (SWIR1), and landscape heterogeneity (Rao's Q) were good proxies of Cabrera voles' microhabitat, mostly during temporally greener and wetter conditions. In addition to remote-sensing-based variables, the presence of road verges was also an important driver of voles' distribution, highlighting their potential role as refuges and/or corridors. Overall, our study supports the use of remote-sensing data to predict microhabitat suitability for endangered small-sized species in marginal areas that potentially hold most of the biodiversity found in human-dominated landscapes. We believe our approach can be widely applied to other species, for which detailed habitat mapping over large spatial extents is difficult to obtain using traditional descriptors. This would certainly contribute to improving conservation planning, thereby contributing to global conservation efforts in landscapes that are managed for multiple purposes.

Keywords

remote sensing; species distribution models; habitat metrics; wildlife conservation; rare species; Cabrera vole

3.1 ***Introduction***

Anthropogenic activities, concurrently with human population growth, are responsible for wiping out wildlife species at rates never experienced before [1]. In particular, agricultural intensification and infrastructure proliferation (roads, railways, etc.), which are considered among the main causes of habitat loss/fragmentation and populations declines, have been rapidly rising to an alarmingly level worldwide [2,3]. Traditionally, wildlife conservation priorities have been focused on megafauna, since species with a large body size have been associated with high extinction risks [4]. However, small body size can also be an important extinction driver [5], possibly exacerbated by species limiting ecological traits (e.g., short dispersal distances), restricted, and/or fragmented distribution and habitat specialization [6].

The Cabrera vole (*Microtus cabrerae*) is an Iberian-endemic small mammal, classified as "Vulnerable" in Portugal and Spain [7,8], and as "Near-threatened" by IUCN [9]. Within its restricted distribution range, the species presents a fragmented distribution [10], typically associated with marginal areas of agricultural systems, with local populations largely restricted [10,11,12] to sparse patches of tall and dense wet grasslands [11,13]. The major threats for this species include agriculture and grazing intensification [14], which destroy its preferred habitats, forcing individuals to disperse and occupy small habitat patches (often <500 m^2 [14]) like field margins or road verges [12,15,16]. The Cabrera vole often presents a metapopulation-like spatial structure, which together with the regular destruction and turnover of suitable habitat patches, makes the designation of special areas of conservation for this species a particularly challenging task. The designation of these conservation areas is however demanded by the European Union, as the species is listed in both Bern Convention (Appendix II; 82/72/CEE) and Habitats Directive (Annexes II and IV; Council Directive 92/43/EEC). The selection of those key areas should be supported by detailed and up-to-date species' distribution at multiple scales, and the use of efficient tools and frameworks able to appropriately identify them [17]. In this context, correlative species distribution models (SDMs), or habitat suitability/niche models [18], which provide probabilistic estimation of occurrence patterns over broad areas by relating species occurrences with environmental characteristics [18], have become a popular tool to develop potential species range maps.

Numerous studies have extensively reported the utility of SDMs for addressing a variety of ecological questions [19,20,21], related to biodiversity monitoring and conservation planning [17,22,23], including for the Cabrera vole [10,24]. Yet, SDMs applications on Cabrera voles, or other small and elusive species, at a local or regional scale are still challenging, likely due to their low detectability and/or narrow distribution, which may complicate data collection [25,26]. Moreover, the integration of fine grain habitat requirements for which suitability may change within short time periods makes SDM' building another challenging task, due to the lack of spatially explicit predictor variables able to capture habitat characteristics at small scales [27], as well as to account for species occupancy turnover and landscape dynamism [28], the latter being markedly pronounced in Mediterranean-type ecosystems [12,29,30]. Specifically, most available digital habitat proxy information (e.g., land cover/use maps) have low detail precision and have a static time nature (they are not expected to vary within the year) [28,31], and thus may fail to provide relevant ecological information for small species inhabiting dynamic habitat patch networks.

We used Cabrera vole as a model to create up-to-date spatially and temporally detailed habitat suitability maps for species with fine-scale habitat requirements occurring in dynamic landscapes. Opportunities to do this come from Earth Observation Satellites (EOS) due to their multispectral and systematic characteristics, which allows the identification of the vegetation composition and structure, as well as its physiological condition [32,33,34]. The usefulness of remote-sensing data for species habitat suitability mapping has been reported in numerous studies, as outlined in the review by He et al. [27]. In this review, the spatial-continuous nature and the reasonable time frequency of satellite-based data are highlighted as an added value to overcome SDMs limitations. By integrating this high-quality data into SDMs, their accuracy can be effectively increased as availability of resources may be better described [28,35,36,37]. Moreover, remote-sensing data can be used for modelling changes in species distribution across time and understand how vegetation changes might affect patch quality and influence demographic parameters, including reproduction and dispersal movements [28].

While it may be straightforward to map habitat suitability areas, for example, for large mammal species [35,38], having broad-scale home range sizes (e.g., >1000 m^2), modelling species responding to fine-scale landscape requirements (e.g., small mammals or insects) is challenging from the remote-sensing perspective due to limitations associated to conventional imageries when identifying local resource patches [38,39]. Indeed, until recently, the available information from remote sensing (e.g., land-cover) was too coarse or too expensive to be properly applied on fine-scale modelling [28]. The Copernicus Program from the European Commission (EC) in partnership with the European Space Agency (ESA) has been developing several satellite missions under the scope of the Sentinel program [40]. Within this program, a constellation of two multi-spectral satellites called Sentinel-2A (launched on 23 June 2015) and Sentinel-2B (launched on 7 March 2017) are together collecting information at high spatial (up to 10 m), spectral (13 bands), radiometric (12 bits), and temporal (each five days) resolution [41]. Due to its technical features and the open data policy, Sentinel-2 brings new opportunities and capabilities for evaluating wildlife spatio-temporal response to habitat features [27] and dynamic processes [36], which may be of particular importance for SDMs developed for small species inhabiting dynamic systems (e.g., grasslands [42]) such as the Cabrera vole. To the best of our knowledge, modelling fine-scale habitat suitability for wildlife conservation, specifically with open-access remote-sensing data and with Sentinel-2 imagery, is still scarce in the literature. Besides, as Sentinel-2 derived-products mostly reflect biotic environmental attributes, the integration of these variables with abiotic descriptors (e.g., topography) into SDMs likely provide more realistic results than using each type of variables alone [28,36,43].

Therefore, by taking advantage from spectral, temporal, and spatial characteristics of Sentinel-2 sensors, the main goal of this study is to assess the usefulness of Sentinel-2 derived predictors for identifying suitable microhabitats for small and elusive species of conservation concern, using the Cabrera vole in a Mediterranean ecosystem as a model. In particular, we aimed to:

i. Quantify the importance of Sentinel-2 derived predictors relative to more conventional predictors (e.g., topographical and distance to landscape elements) in predicting vole microhabitat suitability;

ii. Identify which Sentinel-2 derived predictors best explain vole distribution at fine spatial scales.

Overall, we predict that Sentinel-2-based variables should provide an important contribution for improving fine-scale habitat mapping of endangered small species, such as the Cabrera vole, thus supporting the view that remote-sensing products should greatly contribute for conserving biodiversity associated to small marginal areas in human-dominated landscapes. For this purpose, a methodological approach was devised for predicting suitable habitat areas for the Cabrera vole by using Boruta Random Forest algorithm [44] and different Sentinel-2-derived data (multispectral data, spectral indices, and textural and diversity indices), topographic variables, and distance to landscape key elements (roads, built-up areas, and water ponds).

3.2 ***Materials and Methods***

Study Area

The study was conducted in a ~176,000 ha area located in the Alentejo region, Southern Portugal (centroid: 586545 - 4281192; EPSG: 32629-WGS 84/UTM 29N; **Figure 1**a). The area is characterized by an altitude ranging from 80 to 500 m a.s.l. with a gently undulating relief [and included within a bioclimatic zone commonly associated to the Cabrera vole, namely the meso-Mediterranean,29]. Climate is typically Mediterranean, with hot and dry summers (August: 31 °C Tmax), mild and wet winters (January: 6 °C Tmin), and medium annual rainfall (>600 mm) (Évora 1981–2010 [45]). The landscape is largely dominated by an agrosilvopastoral system called montado (or dehesa), an open woodland of cork (*Quercus suber*) and/or holm oak (*Quercus rotundifolia*) trees [46]. The system is characterized by high spatial variability in tree density and an understorey mosaic of annual crops, grasslands (intermixed perennial and annual herb communities), and shrublands [47]. While the montado is considered as one of the highest biodiversity-rich ecosystems of the western Mediterranean Basin [48,49] having been classified as a High Nature Value farming system (HNV) [50], it is also referred as one the most threatened in terms of conservation, mainly due to land use intensification [51].

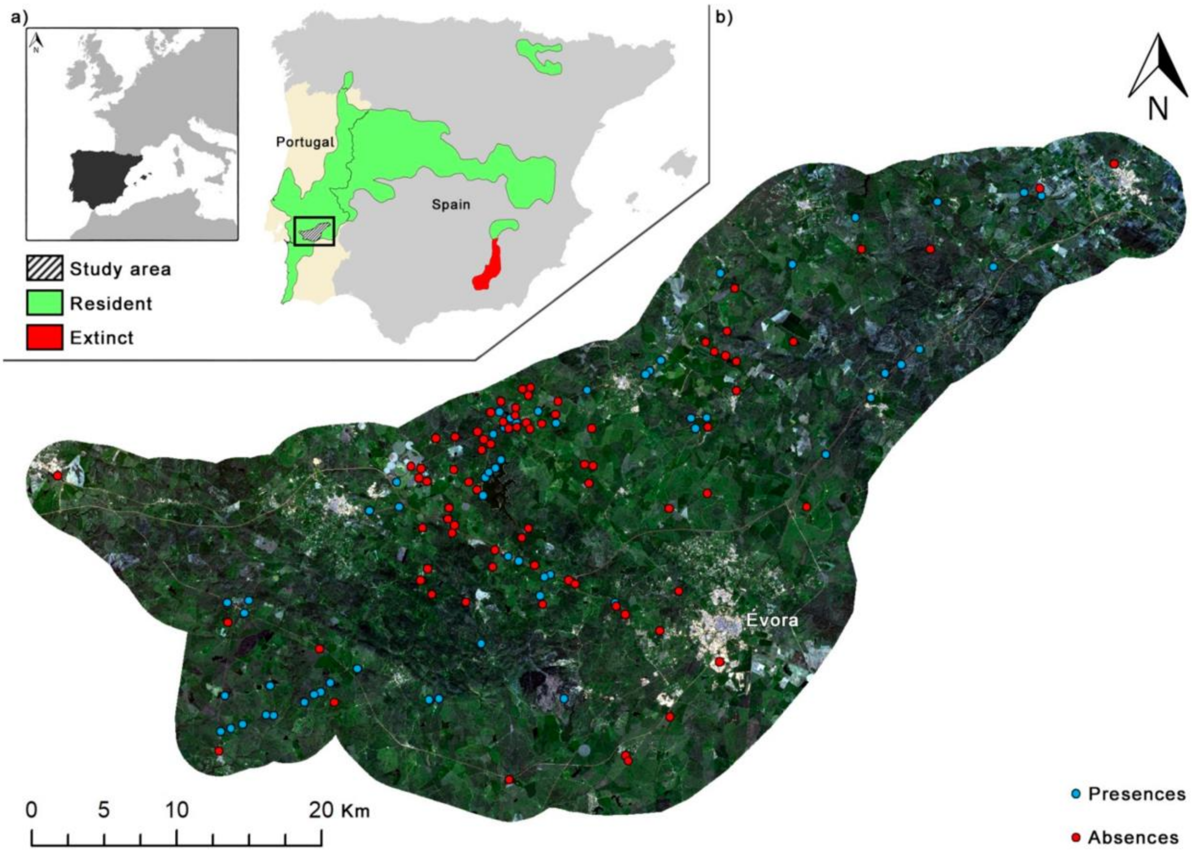


Figure 1. Location of the study area: (**a**) Iberian Peninsula and actual Cabrera vole distribution range are represented jointly with the study area, located within the Alentejo region (Southern Portugal); and (**b**) Cabrera vole sampling points layered with the T29SNC, T29SND, T29SPC, and T29SPD Sentinel-2A RGB composite imageries delimited by the study area.

Cabrera Vole Field Surveys

Cabrera vole surveys were conducted through stratified random selection by initially identifying in the field suitable and unsuitable grass patches. A total of 146 patches with dense and tall perennial grasses and/or sedge/rush communities growing in high soil moisture conditions [13,14] were defined as locations of potential occurrence and 79 patches were considered not suitable for the species, due to very dry soil conditions and/or lower cover and height. Each of the selected patches was carefully surveyed by two observers for presence signs typical of this species (surface runways, grass clippings, and typical small, dark green faeces associated with latrines) to assess its presence [14]. These signs are easily recognizable, and together provide a reliable sampling method, at least when other species producing similar signs (e.g., *M. agrestis*) are absent in the area [11,13], as it is the case of our study region. Each surveyed habitat patch was classified according to the presence/absence of the species, and georeferenced with an accurate GPS device (Garmin eTrex 30x; Projected coordinate system: EPSG: 32629-WGS 84 / UTM 29N; precision up to 3 m). The absences were further classified as absences with and without suitable habitat conditions (as the first ones may correspond to patches potentially used by voles, but that were not occupied at the time of the survey); [12]. Although each patch was surveyed once, samplings were conducted in two sessions to account for habitat variation, namely soil humidity, vegetation dryness, and structure. The first session ran in Spring (February–April 2017), which is when Cabrera vole populations are typically close to their peaks and breeding activity is presumably higher, due to increased soil humidity and vegetation growth (e.g., green grasses) [14]. The second session was conducted in autumn (October–early December 2018); when soil humidity was significantly lower due to the typical hot and dry summer conditions in the region, which were exceptionally hard and extended in 2018 (IPMA Évora 2018 [45]). This second session was also coincident with the period when more fallow areas can be found, being those of special interest for species' conservation [52]. A total of 97 and 128 herbaceous patches were surveyed in the first and second sessions, respectively. In order to lower model biases, all absences recorded in habitats identified as suitable were discarded from the dataset, as these may have resulted from possible low detectability [18,26]. We further applied a 500 m grid spatial filtering procedure, resulting in a roughly balanced dataset of 62 presences and 79 absences (Figure 1b).

Predictor Variables

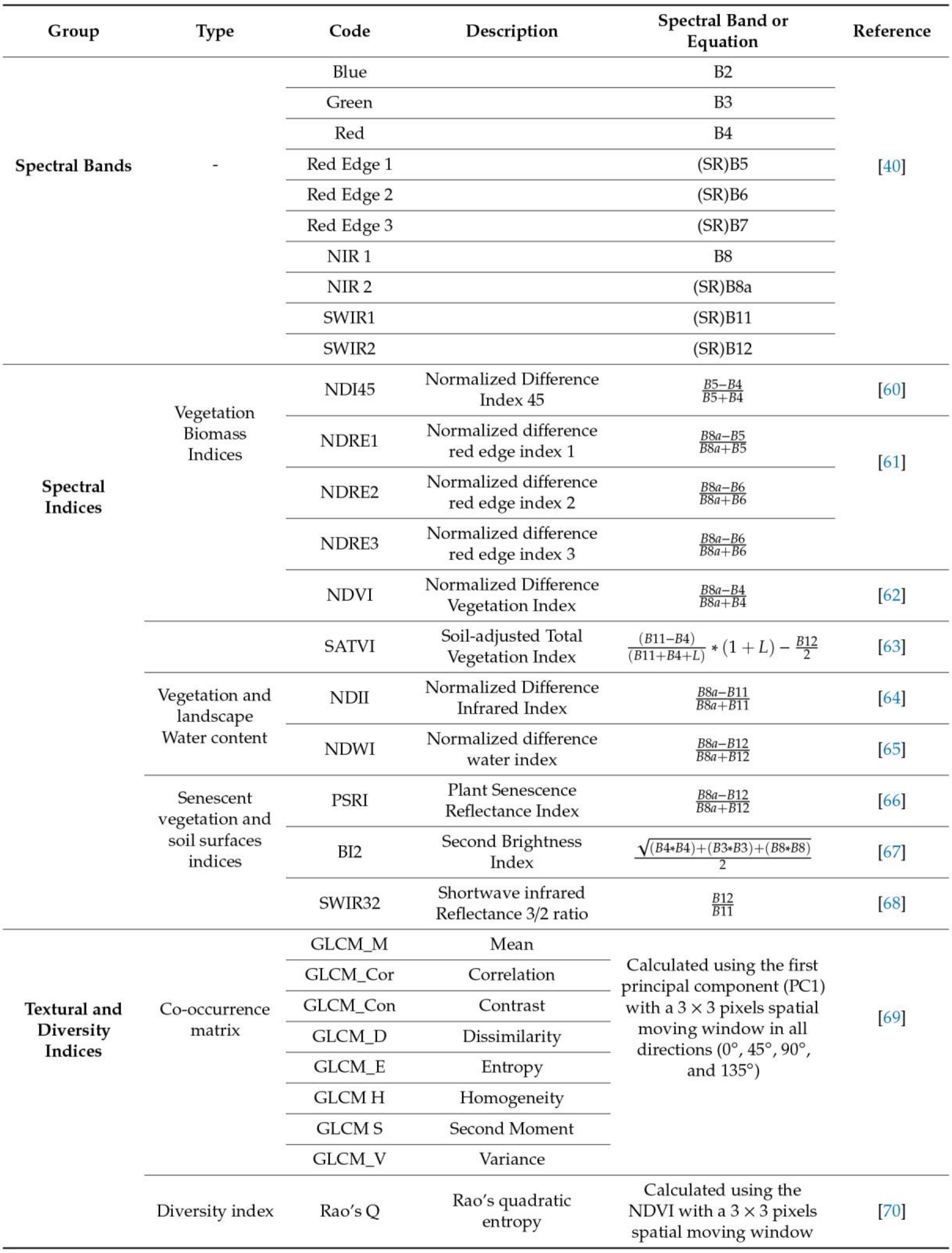
Three categories of predictors were defined: (1) Sentinel-2-derived predictors, (2) topographical, and (3) distance to key landscape elements.

Sentinel-2 Derived Predictor Variables

To better assess the capability of Sentinel-2 imagery in predicting Cabrera vole habitat suitability areas, three different types of Sentinel-2-derived variables were used: (1) Spectral bands, (2) spectral indices, and (3) textural and diversity indices.

Sentinel-2 multispectral images (Sentinel-2A MSI Level-1C) used in this study were downloaded from the Copernicus Science Data Hub portal (https://scihub.copernicus.eu/dhus/). For each of the study periods, the image with the lowest percentage of clouds was selected to represent environmental conditions at the time of vole surveys (5th April 2017 and 7th October 2018 in the case of the first and second period, respectively). The study area was entirely covered by the union of 4 multispectral images (0%–1% of clouds) for each selected period, which followed an atmospheric correction procedure using the Sen2Cor code implemented in the SNAP software [53].

Only the Sentinel-2 bands with 10 and 20 m spatial resolution were considered in this study, namely the B2 (blue), B3 (green), B4 (red), B5 (Red edge 1), B6 (Red edge 2), B7 (Red edge 3), B8 (NIR1), B8a (NIR2), B11 (SWIR1), and B12 (SWIR2) bands (Table 1).



Group	Type	Code	Description	Spectral Band or Equation	Reference
Spectral Bands	-	Blue		B2	[40]
		Green		B3	
		Red		B4	
		Red Edge 1		(SR)B5	
		Red Edge 2		(SR)B6	
		Red Edge 3		(SR)B7	
		NIR 1		B8	
		NIR 2		(SR)B8a	
		SWIR1		(SR)B11	
		SWIR2		(SR)B12	
Spectral Indices	Vegetation Biomass Indices	NDI45	Normalized Difference Index 45	$\frac{B5-B4}{B5+B4}$	[60]
		NDRE1	Normalized difference red edge index 1	$\frac{B8a-B5}{B8a+B5}$	[61]
		NDRE2	Normalized difference red edge index 2	$\frac{B8a-B6}{B8a+B6}$	
		NDRE3	Normalized difference red edge index 3	$\frac{B8a-B6}{B8a+B6}$	
		NDVI	Normalized Difference Vegetation Index	$\frac{B8a-B4}{B8a+B4}$	[62]
		SATVI	Soil-adjusted Total Vegetation Index	$\frac{(B11-B4)}{(B11+B4+L)} * (1+L) - \frac{B12}{2}$	[63]
	Vegetation and landscape Water content	NDII	Normalized Difference Infrared Index	$\frac{B8a-B11}{B8a+B11}$	[64]
		NDWI	Normalized difference water index	$\frac{B8a-B12}{B8a+B12}$	[65]
	Senescent vegetation and soil surfaces indices	PSRI	Plant Senescence Reflectance Index	$\frac{B8a-B12}{B8a+B12}$	[66]
		BI2	Second Brightness Index	$\frac{\sqrt{(B4*B4)+(B3*B3)+(B8*B8)}}{2}$	[67]
		SWIR32	Shortwave infrared Reflectance 3/2 ratio	$\frac{B12}{B11}$	[68]
Textural and Diversity Indices	Co-occurrence matrix	GLCM_M	Mean	Calculated using the first principal component (PC1) with a 3 × 3 pixels spatial moving window in all directions (0°, 45°, 90°, and 135°)	[69]
		GLCM_Cor	Correlation		
		GLCM_Con	Contrast		
		GLCM_D	Dissimilarity		
		GLCM_E	Entropy		
		GLCM H	Homogeneity		
		GLCM S	Second Moment		
		GLCM_V	Variance		
	Diversity index	Rao's Q	Rao's quadratic entropy	Calculated using the NDVI with a 3 × 3 pixels spatial moving window	[70]

Table 1. Sentinel-2-derived predictors. The SR abbreviation indicates for which band was applied in the rescaling approach, namely for the Red edge 1, Red edge 2, Red edge 3, the NIR2, SWIR1, and SWIR2 (20m). L = 0.5 was applied in SATVI index.

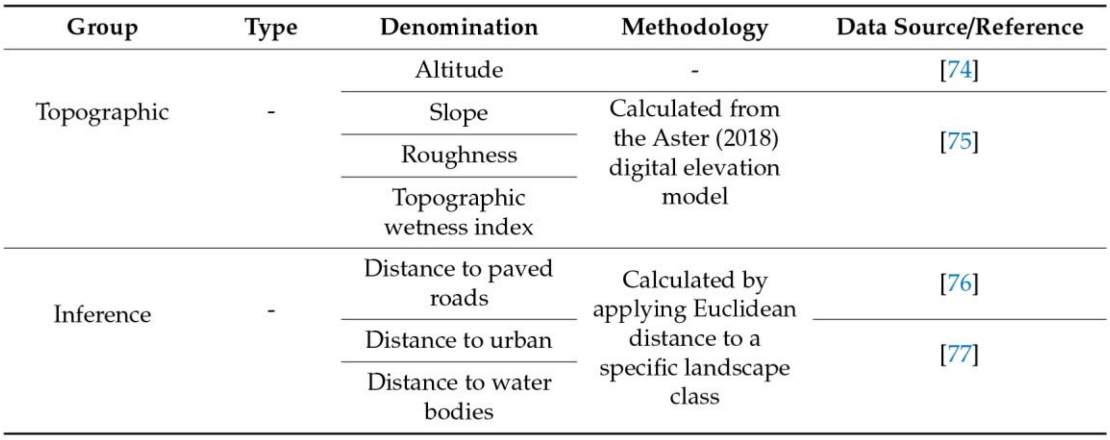
In order to increase the spatial resolution of the 20 m spectral bands, a super-resolution enhancement method was applied, whereby high-resolution bands (10 m) were able to reconstruct coarser (20 m) at the given resolution while maintaining the associated spectral reflectance, as demonstrated by Brodu [54]. Super-resolved (SR) bands were computed using the Sen2res SNAP plugin (http://step.esa.int/main/third-party-plugins-2/sen2res/).

In order to capture different habitat features that are ecologically relevant to predict suitable areas for the Cabrera vole, three groups of spectral indices were computed: (1) Vegetation biomass indices (NDVI, NDRE1, NDRE2, NDRE3, NDI45, and SATVI), (2) senescent vegetation and soil surface indices (PSRI, SWIR32, and BI2), and (3) vegetation and landscape water content indices (NDII and NDWI) (**Table 1**). These indices have been successfully used in retrieving different key biophysical vegetation information in semi-arid tree-grass ecosystems such as the one here addressed (montado) [55,56,57,58,59].

To describe the montado vegetation and landscape structural and diversity properties, the grey-level co-occurrence matrix (GLCM) [69] and the Rao's Q index [36,70] were calculated, respectively. Prior to the textural calculation, the previously selected spectral bands underwent a Principal Component Analysis (PCA) fusion technique with the aim of obtaining a single Sentinel-2 image incorporating all bands' information [71]. The principal component image accounting for over the 90% of bands spectral variability was subsequently used to compute eight GLCM variables, namely, mean, correlation, contrast, Dissimilarity, entropy, homogeneity, second moment, and variance (**Table 1**). The selected textural variables were calculated using the glcm package (v.1.6.1) [72] implemented in the R (v. 3.5.2) [73], and following the same parametrization settings described in Godinho et al. [57]. The Rao's Q diversity index, which accounts for both the abundance and the pairwise spectral distance among pixels [70], and thus is useful to assess spatial diversity, was calculated by using NDVI as input data and a moving window size of 3 × 3 pixels

Topographical Predictor Variables

Four topographical variables (altitude, slope, roughness, and topographic wetness index; **Table 2**) were derived from a digital elevation model [74] using RSAGA R package (v.1.0.0) [75].



Group	Type	Denomination	Methodology	Data Source/Reference
Topographic	-	Altitude	-	[74]
		Slope	Calculated from the Aster (2018) digital elevation model	[75]
		Roughness		
		Topographic wetness index		
Inference	-	Distance to paved roads	Calculated by applying Euclidean distance to a specific landscape class	[76]
		Distance to urban		[77]
		Distance to water bodies		

Table 2. Dataset not involving Sentinel-2A images and representing candidate static predictors.

Distance to Landscape Elements

In order to quantify the potential influence of key landscape elements on Cabrera vole spatial distribution (e.g., [14,52]), distances to paved roads, built-up areas, and water bodies were calculated (Table 2). A shapefile containing the information about paved roads was produced using OpenStreetMap data source [76]. Built-up areas and water bodies shapefiles were obtained from the imperviousness and the water and wetness high-resolution layers of the Copernicus Land Monitoring Service [77].

Habitat Suitability Model

The habitat suitability model was built using all previously described predictors using Cabrera vole presence/absence as response variable. The relationship between the predictors and the spatial distribution of Cabrera vole was evaluated in a three-step statistical approach. The first step consisted in selecting the relevant variables from a set of 67 candidate predictors using the Boruta algorithm [44,78,79]. Basically, Boruta algorithm relies on an extension of the random forest (RF) [80,81] method by introducing an iterative procedure to compare the relative importance of the original variables with the importance of their randomized copies [44]. After running iteratively a large number of random forest models, the Boruta algorithm computes the mean Z-score value to classify all the variables as confirmed, rejected, or tentative at a predefined threshold of statistical significance (p) and a maximum number of times the algorithm is run (maxRuns) [79]. In this study, the Boruta R package (v.6.0.0) [44] was used to execute the algorithm with maxRuns = 2000, ntree = 2000, and p value = 0.01. The second step consisted of running a Pearson’s correlation analysis to determine pairwise correlations within the variables classified as confirmed in the previous step to remove highly correlated ($r > |0.7|$) ones. Finally, in the third step, and employing only the uncorrelated most important variables, an RF analysis was used to predict the spatial distribution of Cabrera vole in the study area. For the RF model, the number of trees (ntree) was fixed to 2000 and number of variables randomly tested on each split (mtry) to the square root of the number of variables. A 10-fold cross-validation resampling method was used to build the RF model. These analyses were done with the ggRandomForest R package (v.2.0.1) [82]. Each variable relative importance for the model was assessed and partial dependence plots [81] were used to explore interaction effects between variables on Cabrera vole presence probability. Model performance was verified using the area under the curve (AUC) of the Receiver Operator Characteristic (ROC), as well as the proportion of correctly predicted presences and absences [83].

3.3 **Results**

Model Performance

The Boruta screening procedure resulted in a considerable reduction of possible explanatory variables, as only 26 predictors were confirmed (38.8% of all the candidate features set, **Figure**

S1). From these, only 11 showed no strong correlation among them ($r < |0.7|$) and were retained for the multivariate analysis (**Figure S2**; for more details regarding all pairwise correlation results, see **Table S1**). The results derived by the 10-fold cross-validation indicated that the RF model developed was robust given the low estimated error rate percentage, (19.15%), determining a high explanatory power of included predictors on the occurrence of the endangered Cabrera vole in our study area (about 80% of variance explained). Results also showed a 'high' AUC score (area under the curve) of 0.904, a sensitivity (true positive rate) of 0.73, and a specificity (true negative rate) of 0.778, therefore a higher performance for correctly predicted absences than presences was noticed.

Variable Importance

Following the multivariate analysis, the "Sentinel-2" variables group showed the highest contribution (65.7%) in explaining Cabrera vole habitat suitability, comprising 10 variables (**Figure 2**). The variables from the group "Distance to landscape elements" contributed to explain 34.22% of the variance, comprising only the distance to paved roads (**Figure 2**). None of the "Topographic" variables were retained in the final model. Half of "Sentinel-2" variables concerned the Spring period and another half to the Autumn period (**Figure 2**). The highest significant contributors from the "Sentinel-2" group were "NDI45 (Spring)" (14.9%), "SWIR1 (Autumn)" (10.4%), and "Rao's Q (Spring)" (9.9%) (**Figure 2**), meaning these variables incorporated most of the relevant habitat information from remote-sensing data. The habitat suitability for Cabrera vole increased when the spectral vegetation index NDI45 had low-medium values in Spring, and the spectral band SWIR1 and the metric Rao's Q showed intermediate values in Autumn and Spring, respectively (**Figure 3**c,d). Response curves for "Distance to paved roads" showed that suitability of Cabrera vole steeply decreased with the increase in distance from roads (**Figure 3**a). The habitat suitability map shows that the occurrence locations fell in high-probability areas in the final habitat suitability model (**Figure 4**).

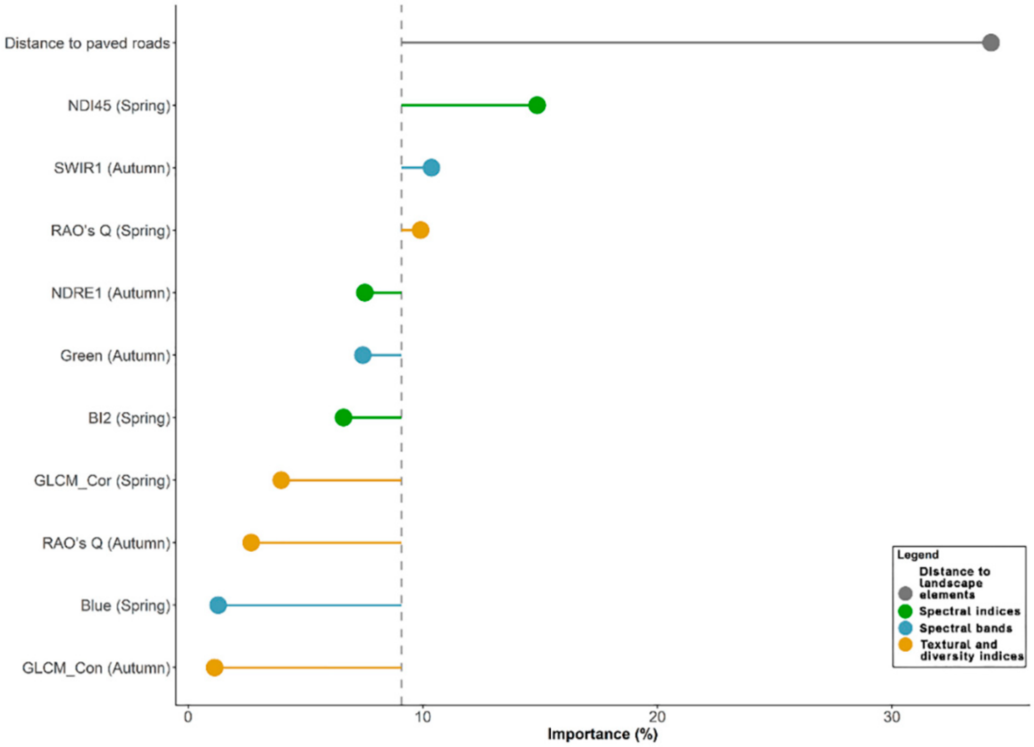


Figure 2. The relative contribution of retained variables (%) in the final habitat suitability model, layered with respective groups (grey dot: Distance to landscape element; green dots: Spectral indices; cyan dots: Spectral bands; orange dots: Textural and diversity indices) and overlapped with a dashed line representing mean importance value.

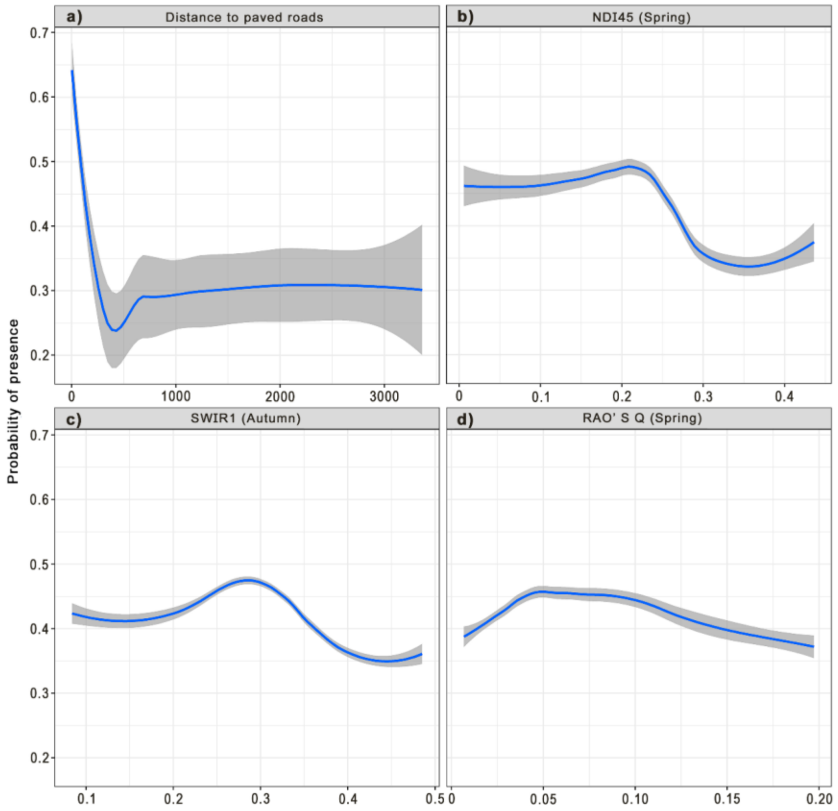


Figure 3. Interactive effects (partial dependence curves) of most important variables: (a) “Distance to paved roads”, (b) “NDI45 (Spring)”, (c) “SWIR1 (Autumn)”, and (d) “RAO’s Q (Spring)”, on probability of Cabrera vole occurrence. The average 10-fold cross-validation results are depicted by the blue lines. The grey area limits ± standard error.

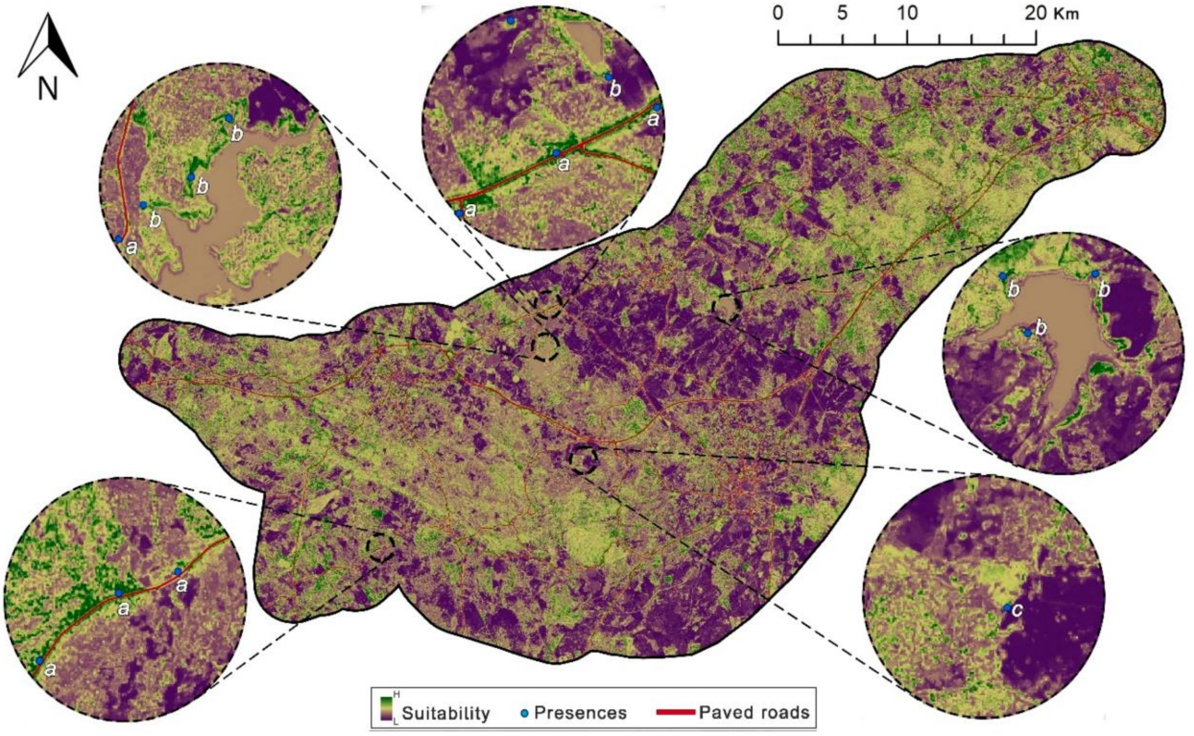


Figure 4. High-resolution Cabrera vole habitat suitability map in Southern part of Portugal, layered with paved roads and presences (blue dots). Zoomed areas are depicted as examples of identified sites of conservation interest namely (**a**) road verges, (**b**) pond banks, and (**c**) field margins. Purple areas: Low suitability; Green areas: high suitability).

3.4 *Discussion*

Results yielded evidence that fine-scale remote-sensing data may be useful to predict favorable habitats for the occurrence of small-sized species, with small home-ranges and specialized niches in spatially and temporally heterogeneous environments (e.g., [84]). Using ground-data from vole surveys across different periods, we are able to demonstrate that spectral, spatial, and temporal information from Sentinel-2 (Sentinel-2A MSI Level-1C) multispectral images analysis is significantly important to predict the Cabrera vole occurrence.

Results show that NDI45 vegetation index describing areas characterized with low-medium chlorophylls is the most important Sentinel-2-derived proxy for Cabrera vole habitat. High values of this index photosynthetically indicate higher biomass activity, i.e., dense canopies and crops linked to intensified agriculture practices, which are not suitable for the species. On the other hand, very low values of NDI45 indicate increasingly lower soil vegetation cover, which is also not suitable for the species occurrence. Reasons for a higher importance of this index during the ‘Spring’ should be related to increased wetness and mild temperature conditions during this period, which promotes annual grasses growth, ensuring higher vegetation cover, hence more available resources and improved habitat quality for Cabrera vole [13,14,52]. Multispectral satellite remote-sensing indices (e.g., NDVI) have been proven to successfully explain small mammal species distribution through the use of Landsat 7 [37] and Sentinel-2 data [42].

However, the present study showed that NDI45 is a better predictor than NDVI because it uses spectral information from the red-edge region, which has been recognized to provide more sensitive measurements of vegetation biophysical properties [60,85].

The SWIR1 spectral band obtained from the autumn season was ranked as the third most important variable in predicting Cabrera vole spatial distribution, and, during this season (in particular in 2018; see Section 2.2), the grasslands over the study area were extremely dry due to the exceptional high temperatures and lack of rain. This is noteworthy because in the shortwave infrared region, the reflectance reduces as the amount of water content increases in vegetation [32] such that SWIR1 can be sensitive to the existing senescent vegetation in the study area because it reaches a peak in terms of spectral reflectance [65]. Hence, it is reasonable to interpret grassy areas with some moisture conditions as associated with medium values of SWIR1. More specifically, a possible ecological explanation for the better support of SWIR1 during dryer periods is that the Cabrera vole might temporally respond to the leaf senescence spectral signals of perennial grasslands, which may help individuals' survival during most adverse environmental conditions (e.g., [30,52]).

Rao's Q metric is a measure of landscape beta diversity and can be a surrogate for landscape heterogeneity [70]. In the context of study area, the species occurs mainly in small marginal patches embedded in or surrounded by larger forest or agricultural areas, or on road verges [14]. The Rao's Q metric seems to be capturing this landscape diversity signal by showing that the species occurrence is favored in moderately heterogeneous landscapes. This pattern was particularly marked in Spring, when grasses become abundant, vegetation heterogeneity is higher, and vole populations increase given the higher availability of resources [12,16]. By contrast, a low suitability for homogeneous areas emerged from our analysis, suggesting vulnerability to habitat simplification, derived for instance from agricultural intensification or grazing pressure [86], which are known to have major impacts on small mammal habitat specialists [87] and for the Cabrera vole in particular [11]. Reasons for the slight decline in species probability of occurrence at the most heterogeneous areas (higher Rao's Q) are unclear, but may be related to the existence of shrubby areas where predation risk might be greater [11].

Apart from Sentinel-2, Cabrera vole occurrence probability peaks on close proximity to roads. This agrees with previous studies showing that the species often occurs on vegetated road verges, particularly in intensive agricultural or grazed areas [11,13,52]. This result does not necessarily suggest that the species is resilient to the negative effects that roads may exert on wildlife [88]. Instead, it emphasizes the compelling role of road verges in providing refuge habitats and corridors for small mammals, particularly where the surrounding matrix is mostly inhospitable

[11,15,89,90]. Nevertheless, a major drawback of road verge habitats is that they may induce road-related mortality [91], which should be duly considered when the goal is to promote the use of verges as habitat and/or corridors for biodiversity.

Interestingly, along with the identification of suitable road verges, other semi-natural infrequently managed areas such as banks and field margins were identified in the habitat suitability model (Figure 4). The conservation value of such areas is remarkable, as they usually support high levels of biodiversity, being key elements of High Nature Value farmland [92]. In addition, suitable areas for the Cabrera vole are often associated with Mediterranean temporary ponds [13], which are priority habitats under the EU Habitats Directive. Protecting such areas may be strategic for the conservation of the Cabrera vole, as well other species in human-dominated landscapes with limited availability of suitable habitats. Also, given the spatially limited and scattered distribution of those habitats, proper identification of priority conservation areas to ensure vole' populations viability, can potentially rely on landscape connectivity assessments (e.g., [93]). Once those areas are identified, conservation actions should consider the implementation of agri-environmental schemes, namely in the context of the European Union's Common Agricultural Policy, through which farmers are paid for restoring habitats, for instance by reducing the grazing pressure [11,15,94].

Earlier SDMs developed for Cabrera vole were carried out at broad scales and relied mostly on bioclimatic variables [10,24]. Despite the conservation value of macro ecological approaches for mapping environmental suitability at large scales [95], such models do not allow identifying, predicting, and mapping small key habitats [96], and thus are insufficient for defining concrete conservation actions. The use of fine-scale remote-sensing variables may thus provide a cost-effective tool to better support conservation planning with reduced survey costs [36], which may be crucial for rare and vulnerable species [97,98]. Higher mapping accuracy, especially when identifying grassland and linear land cover features, could be increased with images possessing very-high spectral and spatial resolutions, namely from data having a resolution spanning around 5m of detail, as suggested by Thornton et al. [99] and Rapinel et al. [100], possibly fulfilled through fusion of Sentinel 2 data [101]. Nevertheless, the use of very-high resolution data may be prohibitive for SDMs applications over larger areas due to its acquisitions costs. In this context, the use of Sentinel-2 data for habitat suitability mapping should be viewed as an effective compromise between spatial (10 m) and temporal resolution (5–6 days), as well as its open-data policy. Regarding the statistical methods inherent to SDMs, further studies are recommended in this research field in order to understand the best robustness of approaches able to handle high dimensional data [102], as well addressed to examine the predictive performances of multiple algorithms, especially when concomitantly integrated into an ensemble modeling framework

[18,43]. This would be particularly interesting when evaluating how sub-sampled group of variables (remote-sensing products, topography, landscape variables) may singularly impact on the performance of species distribution models.

Our findings support the potential of remote sensing for mapping microhabitat suitability of rare small species, which until recently, was largely impracticable due to resource limitations [103]. Sentinel-2 is an open-access resource that provides spatial data at a resolution useful and necessary for this task, and, despite its relatively recent release, effective long-term ecosystem monitoring at local, regional, and national levels is planned to be continuously ensured by this satellite. As such, considering the increasing Sentinel-2 temporal span, future studies on conservation planning incorporating information for longer periods, as it is actually done with other satellites [104], may be valuable because they more likely minimize the common pitfall of assuming stable environmental suitability, and therefore populations persistence, over time [105,106].

3.5 *Conclusions*

Wildlife habitat selection is increasingly understood through the lens of earth observation remote-sensing instruments, either commercial or open-access. We demonstrated that the use of Sentinel-2–derived habitat variables, incorporating biophysical, spectral, and structural landscape information at fine-scales in different seasons, when integrated into RF machine learning methods, may support the identification of potential favorable areas for small and elusive species in dynamic landscapes. Overall, our study highlights that super-resolved remote-sensing data may provide an important tool for identifying linear habitat features (e.g., [99]). Sentinel-2 may provide high-quality and open-access data for fine-scale conservation planning and population monitoring, which may be particularly adequate when considering patchily distributed, small, rare, and elusive species. Finally, our study supports the view that the integration of detailed remote-sensing data into species distribution models is the next stage for linking species occurrences to environmental conditions at functionally relevant spatio-temporal scales, which is a central issue in ecology and conservation.

3.6 *Acknowledgements*

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AUTHORS' CONTRIBUTIONS

Conceptualization, F.V. and S.G.; data curation, F.V., E.F. and N.F.; formal analysis, F.V.; funding acquisition, A.M. and S.S.; investigation, F.V., S.G. and S.S.; methodology, F.V. and S.G.; project administration, S.S.; supervision, S.G., R.P., A.M. and S.S.; visualization, F.V.; writing—original draft, F.V.; writing—review and editing, F.V., S.G., Ricardo Pita, A.M. and S.S. All authors have read and agreed to the published version of the manuscript.

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3.8 *Supplementary material*

Figure S1. Variable explanation power as calculated by the Boruta feature selection algorithm. Rejected and confirmed features are evidenced by red and green boxplots, respectively. No tentative features were achieved.

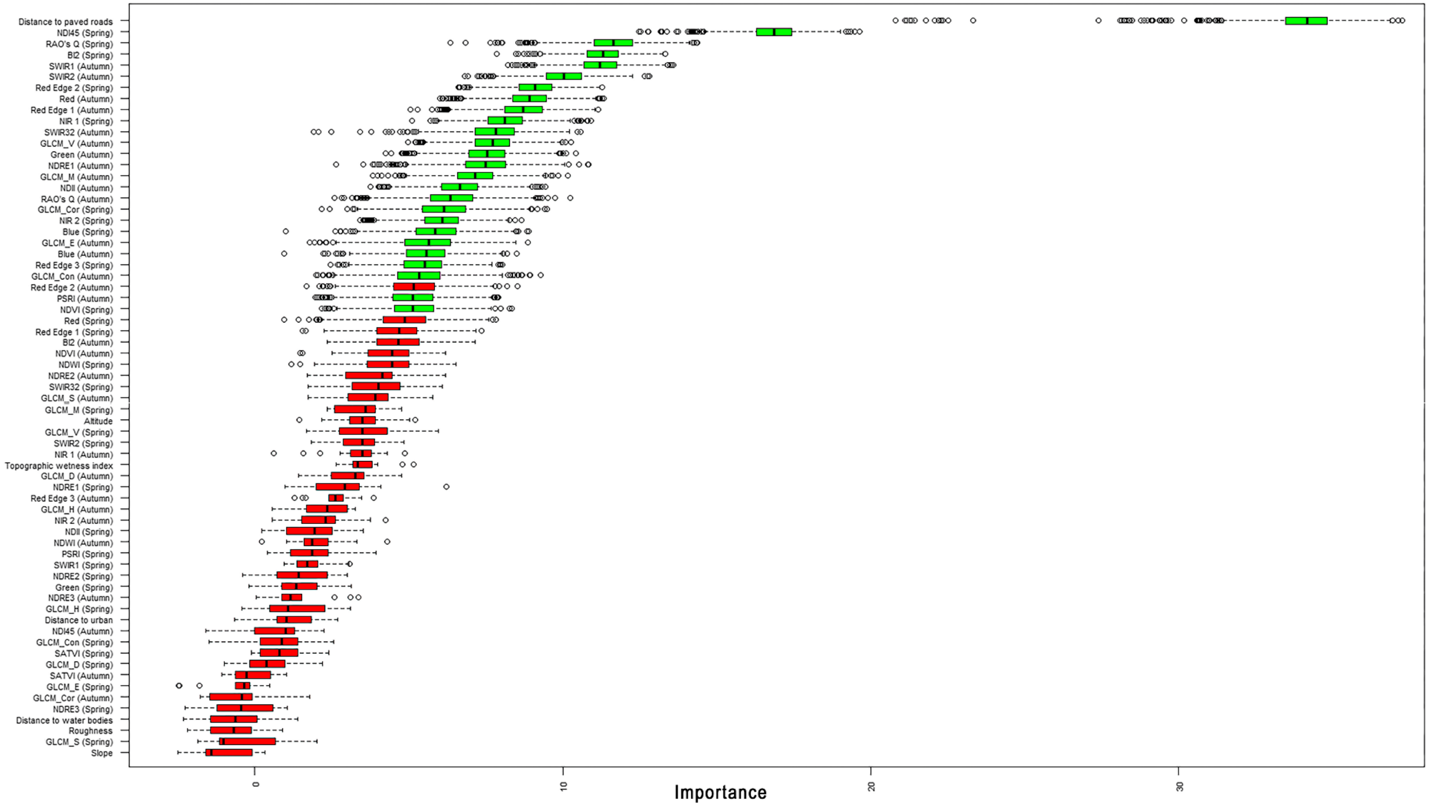

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Figure S2. List of retained confirmed variables after the Boruta screening procedure, with pairwise correlation coefficients <|0.7|.

	NDRE1 (Autumn)	Distance to paved roads	NDI45 (Spring)	Green (Autumn)	BI2 (Spring)	SWIR1 (Autumn)	GLCM_Cor (Spring)	RAO's Q (Spring)	Blue (Spring)	RAO's Q (Autumn)	GLCM_Con (Autumn)
GLCM_Con (Autumn)	-0.192	0.118	-0.248	0.177	-0.138	-0.106	0.042	0.374	0.199	0.447	1
RAO's Q (Autumn)	0.19	-0.121	-0.369	-0.141	-0.327	-0.507	-0.03	0.433	0.173	1	
Blue (Spring)	-0.327	-0.124	-0.695	0.536	0.162	0.081	0.014	0.622	1		
RAO's Q (Spring)	-0.347	-0.03	-0.603	0.274	-0.091	-0.133	0.035	1			
GLCM_Cor (Spring)	-0.069	-0.006	-0.05	0.032	-0.072	0.047	1				
SWIR1 (Autumn)	-0.438	0.183	0.346	0.684	0.691	1					
BI2 (Spring)	-0.126	0.083	0.362	0.599	1						
Green (Autumn)	-0.555	-0.006	-0.106	1							
NDI45 (Spring)	0.11	0.154	1								
Distance to paved roads	-0.09	1									
NDRE1 (Autumn)	1										

Table S1. Pairwise correlation scores between all *Confirmed* features. Correlations scores greater than 0.7 are signaled in bold.

	Blue (Spring)	Blue (Autumn)	Green (Autumn)	Red (Autumn)	NIR 1 (Spring)	BI2 (Spring)	NDI45 (Spring)	NDII (Autumn)	NDRE1 (Autumn)	NDVI (Spring)	PSRI (Autumn)	SWIR1 (Autumn)	Red Edge 1 (Autumn)	Red Edge 2 (Spring)	Red Edge 3 (Spring)	NIR 2 (Spring)	SWIR2 (Autumn)	GLCM_Con (Autumn)	GLCM_E (Autumn)	GLCM_M (Autumn)	GLCM_V (Autumn)	RAO's Q (Spring)	RAO's Q (Autumn)	Distance to paved roads	GLCM_Cor (Spring)	SWIR32 (Autumn)
Blue (Spring)	1.000	0.590	0.536	0.376	0.001	0.162	**−0.695**	−0.087	−0.327	−0.698	0.048	0.081	0.303	0.081	−0.004	−0.022	0.266	0.199	0.164	−0.191	−0.204	0.622	0.173	−0.124	0.014	0.565
Blue (Autumn)	0.590	1.000	**0.985**	**0.926**	0.485	0.552	−0.153	−0.395	−0.563	−0.123	0.515	0.643	**0.850**	0.516	0.472	0.449	**0.764**	0.152	0.036	**−0.728**	**−0.738**	0.292	−0.132	−0.024	0.030	0.693
Green (Autumn)	0.536	**0.985**	1.000	**0.945**	0.540	0.599	−0.106	−0.389	−0.555	−0.072	0.516	0.684	**0.887**	0.565	0.527	0.503	**0.793**	0.177	0.039	**−0.774**	**−0.786**	0.274	−0.141	−0.006	0.032	0.672
Red (Autumn)	0.376	**0.926**	**0.945**	1.000	0.596	**0.623**	0.014	−0.526	−0.572	0.076	**0.736**	0.801	**0.920**	0.605	0.575	0.563	**0.846**	0.051	0.003	**−0.843**	**−0.848**	0.134	−0.296	0.047	0.029	**0.710**
NIR 1 (Spring)	0.001	0.485	0.540	0.596	1.000	**0.946**	0.483	−0.192	−0.057	0.619	0.504	0.651	0.628	**0.953**	**0.963**	**0.962**	0.599	−0.194	−0.220	**−0.728**	**−0.727**	−0.182	−0.368	0.098	−0.064	0.168
BI2 (Spring)	0.162	0.552	0.599	0.623	**0.946**	1.000	0.362	−0.234	−0.126	0.502	0.458	0.691	0.689	**0.982**	**0.975**	**0.974**	0.669	−0.138	−0.178	**−0.769**	**−0.773**	−0.091	−0.327	0.083	−0.072	0.262
NDI45 (Spring)	**−0.695**	−0.153	−0.106	0.014	0.483	0.362	1.000	−0.183	0.110	**0.883**	0.181	0.346	0.127	0.432	0.469	0.485	0.193	−0.248	−0.273	−0.248	−0.242	−0.603	−0.369	0.154	−0.050	−0.261
NDII (Autumn)	−0.087	−0.395	−0.389	−0.526	−0.192	−0.234	−0.183	1.000	**0.741**	−0.064	−0.562	**−0.721**	−0.507	−0.253	−0.210	−0.219	−0.720	0.073	0.158	0.515	0.506	0.005	0.502	−0.218	−0.071	−0.435
NDRE1 (Autumn)	−0.327	−0.563	−0.555	−0.572	−0.057	−0.126	0.110	**0.741**	1.000	0.291	−0.276	−0.438	−0.572	−0.126	−0.084	−0.054	−0.568	−0.192	0.001	0.350	0.351	−0.347	0.190	−0.090	−0.069	−0.692
NDVI (Spring)	−0.698	−0.123	−0.072	0.076	0.619	0.502	**0.883**	−0.064	0.291	1.000	0.308	0.381	0.162	0.565	0.624	0.649	0.194	−0.334	−0.280	−0.331	−0.325	−0.653	−0.401	0.094	−0.073	−0.357
PSRI (Autumn)	0.048	0.515	0.516	**0.736**	0.504	0.458	0.181	−0.562	−0.276	0.308	1.000	**0.716**	0.618	0.473	0.449	0.471	0.641	−0.233	−0.102	−0.669	−0.672	−0.188	−0.555	0.140	0.013	0.199
SWIR1 (Autumn)	0.081	0.643	0.684	**0.801**	0.651	0.691	0.346	**−0.721**	−0.438	0.381	**0.716**	1.000	**0.860**	**0.703**	0.681	0.690	**0.945**	−0.106	−0.158	**−0.934**	**−0.937**	−0.133	−0.507	0.183	0.047	0.356
Red Edge 1 (Autumn)	0.303	**0.850**	**0.887**	**0.920**	0.628	0.689	0.127	−0.507	−0.572	0.162	0.618	**0.860**	1.000	0.681	0.658	0.639	**0.898**	0.083	−0.006	**−0.903**	**−0.923**	0.108	−0.303	0.080	0.038	0.562
Red Edge 2 (Spring)	0.081	0.516	0.565	0.605	**0.953**	**0.982**	0.432	−0.253	−0.126	0.565	0.473	**0.703**	0.681	1.000	**0.989**	**0.986**	0.666	−0.164	−0.212	**−0.761**	**−0.766**	−0.155	−0.364	0.101	−0.067	0.240
Red Edge 3 (Spring)	−0.004	0.472	0.527	0.575	**0.963**	**0.975**	0.469	−0.210	−0.084	0.624	0.449	0.681	0.658	**0.989**	1.000	**0.995**	0.628	−0.179	−0.217	**−0.745**	**−0.747**	−0.194	−0.359	0.090	−0.068	0.178
NIR 2 (Spring)	−0.022	0.449	0.503	0.563	**0.962**	**0.974**	0.485	−0.219	−0.054	0.649	0.471	0.690	0.639	**0.986**	**0.995**	1.000	0.627	−0.203	−0.226	**−0.749**	**−0.748**	−0.224	−0.376	0.097	−0.073	0.149
SWIR2 (Autumn)	0.266	**0.764**	**0.793**	**0.846**	0.599	0.669	0.193	**−0.720**	−0.568	0.194	0.641	**0.945**	**0.898**	0.666	0.628	0.627	1.000	−0.226	−0.103	**−0.910**	**−0.920**	0.060	−0.398	0.162	0.027	0.612
GLCM_Con (Autumn)	0.199	0.152	0.177	0.051	−0.194	−0.138	−0.248	0.073	−0.192	−0.334	−0.233	−0.106	0.083	−0.164	−0.179	−0.203	0.006	1.000	**−0.727**	0.109	0.061	0.374	0.447	0.118	0.042	0.255
GLCM_E (Autumn)	0.164	0.036	0.039	0.003	−0.220	−0.178	−0.273	0.158	0.001	−0.280	−0.102	−0.158	−0.006	−0.212	−0.217	−0.226	−0.103	**−0.727**	1.000	0.132	0.110	0.309	0.540	−0.089	−0.075	0.065
GLCM_M (Autumn)	−0.191	**−0.728**	**−0.774**	**−0.843**	**−0.728**	**−0.769**	−0.248	0.515	0.350	−0.331	−0.669	**−0.934**	**−0.903**	**−0.761**	**−0.745**	**−0.749**	**−0.910**	0.109	0.132	1.000	**0.992**	0.050	0.411	−0.100	−0.029	−0.393
GLCM_V (Autumn)	−0.204	−0.738	**−0.786**	**−0.848**	**−0.727**	**−0.773**	−0.242	0.506	0.351	−0.325	−0.672	**−0.937**	**−0.923**	**−0.766**	**−0.747**	**−0.748**	**−0.920**	0.061	0.110	**0.992**	1.000	0.037	0.398	−0.099	−0.025	−0.415
RAO's Q (Spring)	0.622	0.292	0.274	0.134	−0.182	−0.091	−0.603	0.005	−0.347	−0.653	−0.188	−0.133	0.108	−0.155	−0.194	−0.224	0.060	0.374	0.309	0.050	0.037	1.000	0.433	−0.030	0.035	0.469
RAO's Q (Autumn)	0.173	−0.132	−0.141	−0.296	−0.368	−0.327	−0.369	0.502	0.190	−0.401	−0.555	−0.507	−0.303	−0.364	−0.359	−0.376	−0.398	0.447	0.540	0.411	0.398	0.433	1.000	−0.121	−0.030	0.010
Distance to paved roads	−0.124	−0.024	−0.006	0.047	0.098	0.083	0.154	−0.218	−0.090	0.094	0.140	0.183	0.080	0.101	0.090	0.097	0.162	0.118	−0.089	−0.100	−0.099	−0.030	−0.121	1.000	−0.006	0.015
GLCM_Cor (Spring)	0.014	0.030	0.032	0.029	−0.064	−0.072	−0.050	−0.071	−0.069	−0.073	0.013	0.047	0.038	−0.067	−0.068	−0.073	0.027	0.042	−0.075	−0.029	−0.025	0.035	−0.030	−0.006	1.000	−0.025
SWIR32 (Autumn)	0.565	0.693	0.672	**0.710**	0.168	0.262	−0.261	−0.435	−0.692	−0.357	0.199	0.356	0.562	0.240	0.178	0.149	0.612	0.255	0.065	−0.393	−0.415	0.469	0.010	0.015	−0.025	1.000

Chapter 4

Using remote sensing-based connectivity to optimise multitaxa roadkill mitigation plans: a 15 year case study

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Abstract

1. The dramatic impacts of roads on biodiversity are expected to grow globally, demanding the search for optimal roadkill mitigation strategies. Despite a growing body of research assuming habitat suitability and functional connectivity as proxies for mortality, scant attempts have been made to integrate such information in road mitigation plans for multiple species. In this study, we pinpoint high-risk road locations for 19 woodland vertebrate species from three taxonomic groups (non-flying mammals, birds and bats), with the ultimate intent of prioritising and optimising road mortality mitigation plans.

2. To unveil road planning units, in the Alentejo region (Southern Portugal), we make use of a long-term monitoring study (15-year dataset) composed of species occurrence data, roadkill records, and high-resolution satellite imageries. We differ from traditional risk assessments by identifying remotely sensed habitat metrics for each group, then weighting their independent effects together with state-of-the-art functional connectivity models and road metrics, to estimate the roadkill vulnerability of each species. By these means, the roadkill likelihood variation is further estimated within and between each group to check prediction consistency, which ensures flexibility in mitigation planning.

3. Remote sensing predictors thoroughly describe wildlife habitat suitability, identifying similar metrics within each group, with some differences detected in environmental tolerance across species. Functional connectivity and habitat suitability significantly explain roadkill patterns across species, highlighting connected woodlands and neighbouring matrices. We could prioritise road planning units with high accuracy in predicting mortality for multiple species, and acceptable probability variation within each group. Some discrepancies in prediction consistency yet emerge after group comparisons regarding bats.

4. *Synthesis and applications.* Overall, this study demonstrates that spatial convergence in mortality patterns for multiple species is attainable by habitat suitability modeling and identification of dispersal pathways. Both tools, developed utilising remote sensing information, enabled us to highlight persistent corridors intersecting roads that are transversal to species with different ecological traits and movement capacity. The applicability of this study will facilitate the definition of roadway planning units most pruned to multispecies collision with vehicles, endowing road agencies and practitioners to strategically define long-term, flexible and cost-efficient multitaxa mitigation plans.

Keywords

multispecies mortality, remote sensing, landscape connectivity, species distribution models, road ecology

4.1 *Introduction*

The pervasiveness of roads and their continued expansion are seriously affecting the resilience of ecosystems and ecological communities around the world, contributing significantly to the impoverishment of terrestrial biodiversity (Laurance et al., 2014). This global proliferation of the infrastructure network can lead to increased fragmentation and traffic volumes, thereby exacerbating wildlife movement constraints and direct mortality through collisions, commonly known as roadkill (Forman & Alexander, 1998). Over the past few decades, alarming mortality rates have been reported, prompting unprecedented research efforts to understand and mitigate road-related wildlife casualties (Pagany, 2020). Still, on European roads alone, hundreds of millions of birds and mammals are killed each year, a significant loss possibly threatening the survival of some wildlife species (Oddone Aquino, 2021; van der Grift, 2017). To reduce road barrier effects and mortality, but also to restore connectivity ("the degree to which the landscape facilitates or impedes movements"; Taylor et al., 1993), mitigation structures (e.g., over and underpasses; fences) are being designed and planned (Clevenger & Huijser, 2011; van der Grift, 2017), though they are routinely criticised to have limited effectiveness for multiple species (Rytwinski et al., 2016).

Empirical quantitative approaches, such as roadkill risk models (RRMs), are utilised as management guidance to apply mitigation measures at mortality hotspots and/or movement corridors of target species (Gunson et al., 2011; Fabrizio et al., 2019). Decisions about site selection significantly determine the effectiveness of mitigation structures to implement but leading to a challenge when targeting multiple species with distinct habitat requirements and movement abilities (Polak et al., 2019). A key habitat, as well as functional connectivity, may be approximated for a wildlife group without depending on ecological information, through a so-called *species-agnostic* framework (*sensu* Marrec et al., 2020). In road ecology, this analytical approach may offer advantages over species-based connectivity models whenever a wide range of species is examined for planning initiatives (Koen et al., 2014). On the other hand, it has long been argued that functional connectivity may diverge across species (Zeller et al., 2012), with research efforts rarely addressing multiple habitats and corridors hitherto (Brennan et al., 2020). Undoubtedly, wildlife is unevenly distributed due to their variety of ecological requirements and movement capabilities, outlining multifunctional corridors as a demanding task, yet deserving greater consideration in spatial conservation prioritisation (Brennan et al., 2020; Salgueiro et al., 2021), especially for multiple taxa (Marrec et al., 2020). Furthermore, a neglected issue in RRMs is that studies have merely focused on habitat elements as main mortality drivers (Gunson et al. 2011; Pagany, 2020), even if road-related mortality can also occur from dispersal movements in non-habitat matrix (Vasudev et al., 2015). For this reason, when relating roadkill to multifunctional corridors, various movement costs should be accommodated, since species' willingness to movements within the matrix could differ during dispersal (Zeller et al., 2012). To bridge this gap and improve the efficiency of mitigation measures, it is crucial to approach functional connectivity with a focus on dispersal capabilities (Diniz et al, 2020; Vasudev et al., 2015), as well as to disentangle the effects of habitat and functional connectivity on RRMs (e.g., Fabrizio et al, 2019).

In addition to effectiveness in covering multiple species, a relevant aspect for mitigation structures concerns their ability to keep pace with landscape changes over long periods (Clevenger & Huijser, 2011). From an ecological standpoint, dealing with landscape dynamics has been gradually recognised as an important step in conservation planning and management, although scarcely integrated into road ecology (Oddone Aquino, 2021), considering for instance, that most RRMs-based studies have focused on a spatiotemporally limited representation of a landscape, namely through categorical land cover classes (Gunson et al. 2011; Pagany, 2020). On this issue, some authors have also questioned the reliability of categorical landscape products as being unable to represent the complexity of biological communities (Cushman et al., 2010), while others have pointed to an underestimation of unique habitat elements, which may be crucial in particular for smaller species (Kerr & Ostrovsky 2003). To overcome this drawback and incorporate landscape dynamics over time, a turning point is attributed to continuous and more informative landscape descriptors from satellite remote sensing data (Coops & Wulder, 2019), and to pixel-based methods preserving unique spectral/radar information (Schulte to Bühne & Pettorelli, 2017). However, despite these advantages and increasingly available information (Gorelick et al., 2017), there remains a limitation in how to combine satellite against field data to determine key habitat elements, resulting into an interdisciplinary area with little consensus (Pettorelli et al., 2014).

There is still little empirical evidence on whether similar ecological responses to satellite-derived habitat metrics can be expressed across a group of wildlife species with similar characteristics, which could be beneficial in RRM approaches. This is because a road impact may similarly occur across species, resulting in shared ecological responses (Polak et al., 2019; Santos et al., 2016a). Implications for connectivity, which are typically considered species-specific (Zeller et al., 2012), may also arise, potentially converging for a group of species with similar environmental preferences and characteristics, as well as dispersal capabilities. The major advantage here lies in prioritising conservation measures on planning units (e.g., pixels; see Margules & Pressey,

2000) along roads (road planning units; RPUs), by capitalising on the capacity for 'mortality prevention' within a wildlife group, though this condition may not hold for different groups are considered concomitantly. More specifically, to ensure the effectiveness of mitigation measures, in addition to understanding the relative importance of habitat, functional connectivity and anthropogenic pressures (e.g., road traffic) influencing road mortality, it is also imperative to ascertain whether quantitative models can accurately represent this exposure risk for various species from different groups, as their comprehensive integration into management plans have been mostly neglected (Polak et al., 2019).

In this study, we make use of long-term (15 years) monitoring datasets on species occurrences and road mortality to optimise RRMs that offer support and guidance in identifying high-priority road locations to implement concrete and effective mitigation of multiple species mortality. We selected 19 vertebrate species belonging to three taxonomic groups: non-flying mammals, birds, and bats. Specifically, we aimed to: (1) evaluate the utility of remotely sensed habitat metrics in describing species occurrence; (2) quantify the relative contribution of species-specific habitat, functional connectivity and road metrics in explaining spatial patterns of road mortality; and (3) identify road locations with the highest agreement in long-term mortality predictions, for the greatest number of species in each group and across groups.

4.2 ***Materials and Methods***

Study Area

The study area is located in the Évora district (≅110 km E from Lisbon, southern Portugal; Figure 1) and it is bisected by a major transportation corridor linking Lisbon to Madrid. The area, characterised by a Mediterranean climate, encompasses more than 621,000 ha of gentle terrain, with a landscape dominated by the so-called *montado* agroforestry system, here composed of a mosaic of open areas (pastures) and evergreen forests (mostly *Quercus suber* and *Quercus rotundifolia*) (Pinto-Correia & Godinho, 2013; Godinho et al., 2018). The *montado* is classified as "High Nature Value farming system" meaning that it holds a high biodiversity, while also being of remarkable socioeconomic value (Pinto-Correia & Godinho, 2013). Other land uses present in the study area include olive groves, vineyards, while pine and eucalyptus wood forestry occur sparsely.

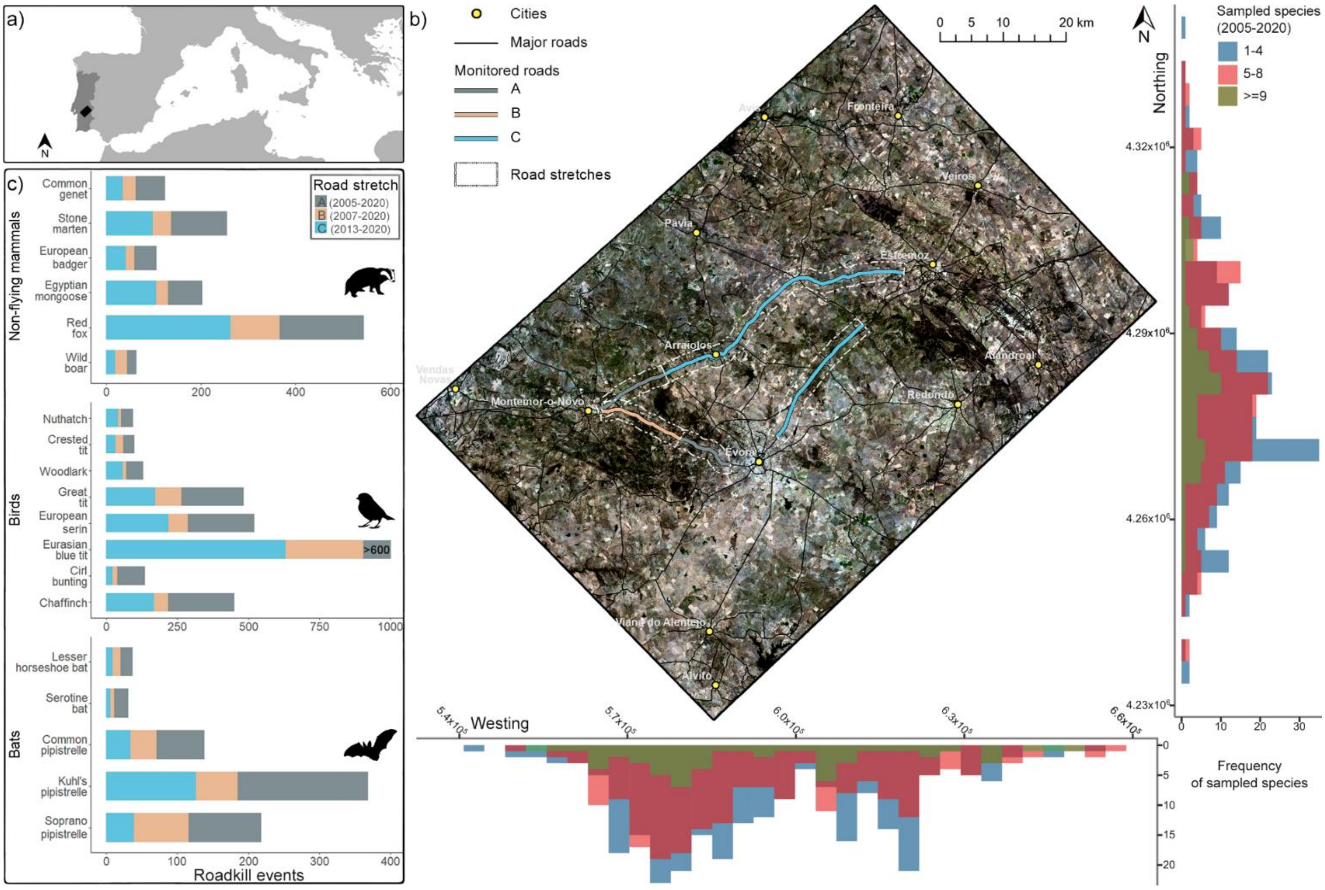

Figure 1. Study area location (a) in SW Europe, Alentejo region (southern Portugal); and (b) a true-colour satellite image composition (Landsat path 203 rows 33 and 34, and path 204 row 33 footprints) combining RGB bands from Landsat (7/8) images (May 2015), layered with cities, major roads and monitored roads. The number of sampled species (species richness) is illustrated in the marginal histograms, summarising the sampling effort in terms of frequency and distribution along the longitudinal and latitudinal axes. The three left panels (c), differentiated per taxonomic group, depict roadkill survey effort for selected species on monitored road stretches.

Major threats to the *montado* system are attributed to the intensification of agricultural practices, but also inappropriate forest harvesting, all of which is leading to increased disturbance dynamics fragmenting the landscape and compromising functional connectivity, jointly with roads (Carvalho & Mira, 2011; Pinto-Correia & Godinho, 2013; Machado et al., 2020; Valerio et al. 2020).

Methodological framework

A framework conceived the prime methodology (Figure 2).

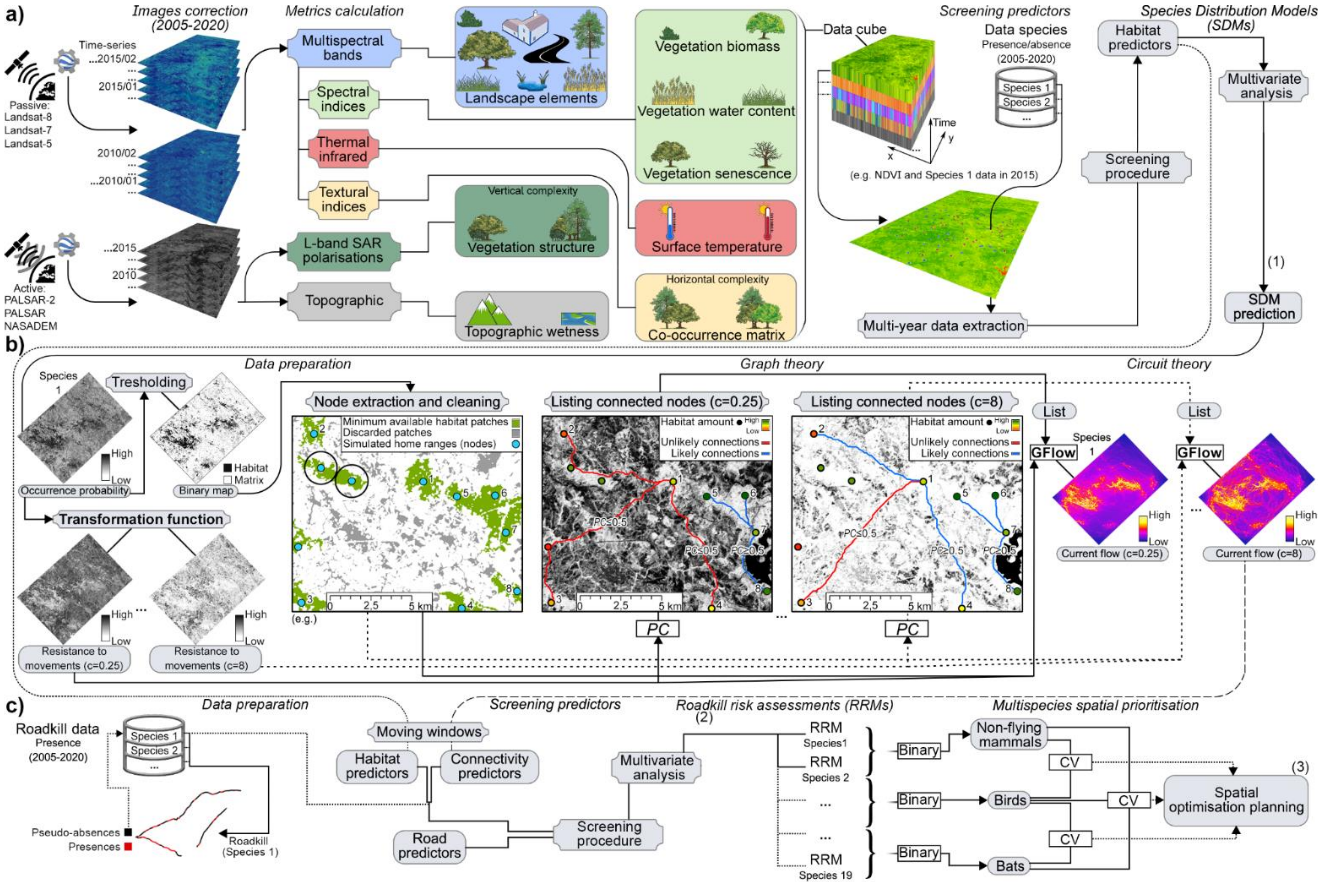

Figure 2. The schematic methodological framework, with arrows guiding the development of the three main sub-tasks (a, b, c). The upper scheme (a) illustrates the task inherently collection and correction of imagery data collected from different earth observation satellites, then how the calculated habitat metrics were organised, here depicted by different color on the basis of biophysical parameters they describe. All this information was then structured into intra-annual time series and archived in a data cube, which allowed the occurrence multi-year data extraction for SDMs, and predictive analysis. The central scheme (b) concisely illustrates the task pertaining the preparation of input data for functional connectivity models, namely the nodes and resistance surfaces generation through SDMs, as well is exemplified how the list of likely connections between pairwise nodes varied following changes in probability of connectivity (*PC*), derived by transformed resistance surfaces. So, functional connectivity models were computed for each species through GFlow (circuit theory). The lower scheme illustrates the final task, namely the preparation of roadkill data and RRMs analysis including species-specific habitat, functional connectivity and road predictors; then, the processing of roadkill probability variation (*CV*) within and between taxonomic groups. The objectives of the study are represented (1, 2, 3), whereas the description of each abbreviation is detailed in the following paragraphs.

Data acquisition and primary processing

Environmental information for the study relied on high resolution satellite remote sensing imagery (Figure 2a). Wildlife occurrence data were gathered for the entire study area encompassing the roads on which roads were monitored, and the surrounding landscape (Figure 1b). Collection of each data source (remotely sensed data, species occurrences and roadkill) was carried out over a long-time span, from 2005 to 2020. We targeted different wildlife taxonomic groups (non-flying mammals, birds, and bats), corresponding to 19 focal forest-dwelling species (Appendix S1.1; Table S1.1), with varying degrees of habitat specialisation and dispersal capabilities.

Cloud-free Landsat satellite imagery was collected and pre-processed in Google Earth Engine (GEE) (https://earthengine.google.com; Gorelick et al., 2017). The Landsat collection 1 Tier 1 was selected, including surface reflectance datasets from Landsat-5 (TM), Landsat-7 (ETM) and Landsat-8 (OLI/TIRS) (USGS; https://www.usgs.gov), at 30m resolution. Selected spectral bands, corresponding to blue, green, red, near infrared (NIR), short-wave infrared 1 (SWIR1) and short-wave infrared 2 (SWIR2) were retained for each of the 645 imageries collected (μ≅41 per year; Table S1.2), which were prior subject to careful verifications, mainly quality corrections and calibrations (Appendix S1.2; Figure 2a). Radar imagery data at 30m resolution, namely the digital elevation model NASADEM and the Phased Array type L-Band Synthetic Aperture Radar (PALSAR), specifically PALSAR-1 and PALSAR-2, was also retrieved from the Advanced Land Observing Satellite ALOS and ALOS-2 missions (ESA; https://earth.esa.int), followed by data conversion (Appendix S1.2; Figure 2a).

Species occurrence data were compiled from several projects conducted in the study area by the University of Évora team, all following best practices for systematic surveys (Appendix S1.3). Non-flying mammals occurrence data was obtained from linear transects targeting species' presence signs (e.g., footprints; Appendix S1.3; Herrera et al., 2016). Bird occurrences were obtained from 10-minute point counts conducted during the breeding season at early dawn and with suitable weather conditions (Salgueiro et al., 2018). Bat activity data came from sample surveys that combined linear transects, point counts conducted after dusk, and roost surveys (see details in Medinas et al., 2013; Medinas et al., 2021; Appendix S1.3). Bat detectors were used in these campaigns, followed by a review of echolocation calls for species identification. For all species, spatially or qualitatively inaccurate data was discarded, as well as redundant data from overlapping surveys, by using a data thinning procedure, where a distance filter was set by an average of home-range sizes to downweight co-dependent observations (Valerio et al., 2019). In this procedure the initial dataset was reduced from 67237 into 5725 records (μ=125.9 presences, μ=174.4 absences per species; Appendix S1.4 and S1.5; Tables S1.3 and S1.4).

The roadkill dataset was obtained from a long-term standardised road survey protocol for all species, that included road sections (N114, N4 and N18 national roads), totalling up to 102 km. Surveys were conducted by a single observer at reduced car speed (μ≅30 km/h) to maximise species detectability, at sunrise to minimise carcass removal by scavengers (Santos et al., 2011). Some road sections were not surveyed for the entire sampling period (Figure 1b; roads B and C). However, roadkill numbers were relatively similar along all road stretches, softening the bias from differences in sampling effort between road stretches (Figure 1b). Roadkill monitoring was performed daily for most of the years, although there were periods with weekly surveys (2006-2008, 2014). However, as we were working with the sum of roadkill (and no year-to-year comparisons are made), that should not affect our conclusions. A total of 5794 roadkill records were considered for the analysis.

Remote sensing-based habitat metrics

Intra-annual time series for habitat metrics were developed to secure consistent spatiotemporal information on landscape patterns (Figure 2a). Metrics were pooled into six predictor groups: "Spectral bands" previously mentioned, given the efficiency in optical spectral properties for identifying landscape elements at high spatial resolution (Valerio et al., 2020); "Spectral indices", describing phenological patterns of vegetation such as biomass (BNDVI, NDVI, EVI, MSAVI2), water content (NDII, NDWI, MSI) and senescence (PSRI, SWIR32); and "Thermal infrared", utilised to infer landscape surface temperature (LST) (Table S1.5). Also, horizontal vegetation complexity was characterised in the form of second-order “Textural indices” (GLCM_H, GLCM_E, GLCM_M; Table S1.5; Haralick, 1979), and parameterised according to Godinho et al., (2018). Finally, radar-derived predictors were identified as "Topographic", describing wetness index (TWI), as well "L-band SAR polarisations" through the PALSAR predictors (HH, HV), that allow to penetrate the vegetation overstorey to infer vertical vegetation complexity (Table S1.5; Le Toan et al., 1992). This portfolio dataset covered prime biogeophysical aspects of the *montado* agroforest ecosystem (Godinho et al 2018; Valerio et al., 2020), including ecological inferences on tackled wildlife species, in geographically close areas (Medinas et al., 2021; Regos et al., 2020; Santos et al., 2016b). Time series were repeatedly collated within each year using the median across metrics, resulting in 316 fine-grained predictors (21 metrics per year), developed in GEE and stored as data cube (Gorelick et al., 2017; Figure 2a). Metric details are provided in Supporting information (Appendix S1.6).

Determining significant habitat metrics for species and taxonomic groups

Quantifying meaningful metrics describing habitat suitability involved building species distribution models (SDMs; Figure 2a; Elith & Leathwick, 2009) with Random Forests classifiers (Breiman, 2001). These were performed in R statistical environment (v.3.6.3; R Core Team, 2016) using "*Boruta*" (v.7.0.0) and "*randomForest*" (v.4.6-14) packages. The response variable (species occurrence data, namely presence/absence set with 0.5 of prevalence; Appendix S1.7) was organised to temporally match annual habitat metrics (remote sensing data) (Figure 2a). Then, Boruta calculation was applied to 'confirm' or 'reject' predictors (Kursa & Rudnicki, 2010), through 2000 runs with as many trees, and by a mean Z-score (0.01 p-value threshold; Valerio et al., 2020). Predictors' significance was assessed by comparison with their randomised copies (Kursa & Rudnicki, 2010). Multicollinearity problems were reduced by discarding predictors until values of Variance Inflation Factor (VIF) were negligible ($VIF \leq 5$), and Pearson correlation coefficient indicated no strong correlation among predictors ($|r|<0.9$) (Millard & Richardson, 2015). Following a parameter tuning for SDMs (detailed in Supplementary material; Figure 2a), sensitivity, specificity, error rate (%) and area under the receiver operating characteristic curve (AUC; Fielding & Bell, 1997) were estimated through 10-fold cross-validation runs, to assess the performance of the multivariate Random Forests models. We employed AUC threshold scores of $0.6 \geq AUC \geq 0.7$, $0.7 \geq AUC > \geq 0.8$, and $AUC \geq 0.9$, to define model discrimination capacity as 'acceptable', 'excellent' and 'outstanding', respectively. The relative contribution was obtained from each SDM to determine the top-performing predictors influencing species occurrence. In addition, we selected the average of relative contribution between predictors to identify those potentially useful for further analysis (RRMs). Partial dependence plots were also computed, to assess the species' ecological responses to predictors.

Functional connectivity analysis

To incorporate the environmental variability into our predictive analysis, we performed inter-annual collations of habitat metric time series, allowing to each of SDMs, a spatiotemporal interpolation over the entire 15-yr period. Remote sensing was incorporated into connectivity via SDMs, from whose resistance surfaces and nodes were constructed (Figure 2b). Linear and non-linear transformation functions (Eq. S1.1; Trainor et al., 2013) were applied on the SDMs at 3-degree scores (c=0.25, c=0.5, c=8; Valerio et al., 2019) to simulate movement cost scenarios by progressively reducing costs on resistance surfaces. The connectivity analysis for each species was performed in a two-step process. First, a graph-based approach analysed the landscape spatial configuration, identifying the strength of connections between each pair of nodes (Diniz et al., 2020). Nodes were selected as habitat patches by converting each SDM into a categorical, species-specific binary (habitat/non-habitat) classification (Appendix S1.8) (Moilanen, 2011; Salgueiro et al., 2018). The probability of connectivity (PC) (Equations S1.2; Saura & Pascual-Hortal, 2007) between each pair of nodes was calculated in Conefor-Sensinode (v.2.2; Saura & Torné, 2009), by weighing the habitat amount within a node and species dispersal distances (Table S1.6; Appendix S1.9), and the functional distance between nodes, in turn dependent on the selected resistance surface (Figure 2b; e.g., Salgueiro et al., 2021). Using this procedure, only resulting linkages with $PC > 50\%$ were retained for further analyses, and compiled as a list of pairwise linked nodes. Second, following the omniscape method, with circuit-theoretic analysis (McRae et al., 2008) we evaluated multiple alternative routes as current flow between the list of linking nodes (e.g., Salgueiro et al., 2021). Current was set to flow between each set of linked nodes subjected to the resistance surface (Figure 2b) using GFlow (v.0.1.7; Leonard et al., 2016). In total we produced 57 fine-grain potential connectivity models (19 species * 3 movement cost scenarios).

Quantifying intra and inter-group roadkill risk optimisation efficacy

RRMs multiscale models were performed to spatially prioritise RPUs, and examine the agreement in mortality predictions within and between taxonomic groups. First, roadkill occurrences were used as a response variable (presences and pseudo-absences; Appendix S1.10) to quantify the relative contribution of species-specific "Habitat predictors", "Connectivity predictors" and "Road predictors" in explaining spatial mortality patterns. "Road predictors" (Figure 2c) included road and culvert features (traffic density percentage, culverts density percentage, distance from culverts, and road width) (Carvalho & Mira, 2011; Medinas et al., 2013; Appendix S1.11). Multiple scales were systematically applied through moving windows over "Habitat predictors" and "Connectivity predictors" (Figure 2c), with windows of 3X3 (≈100m) and 16X16 (≈500m) grid cells (following Carvalho et al. 2011; Medinas et al., 2013). The modeling procedure was performed using Boruta, previously mentioned. Multivariate Random forests classifications to perform RRMs were conducted with "randomForest" (v.4.6-14) package. (Figure 2c). We determined the top-performing predictors, and correspondent predictor group, by calculating their relative contribution to the models. Predictors were then screened for multicollinearity and model evaluation was performed as for SDMs (Figure 2c). Within each taxonomic group (Figure 2c), RRM binary maps (see Appendix S1.10 for the thresholding procedure) underwent an overlay to obtain a cumulative distribution frequency of RPUs to spatially inform about the maximum number of species subjected to high mortality exposure, here intended as mortality richness and utilised as priority ranking method. Secondly, by using the previous threshold, high risk probability values from RRMs were aggregated to analyse RPUs agreement in covering mortality for multiple species and taxa (e.g., intra- and inter-groups). For this purpose, we only considered RPUs where at least one species of each taxonomic group was identified at high risk of mortality. To ensure more flexibility in spatial planning, the variation in risk probability (RRMs), within each group, was assessed by using the coefficient of variation percentage (%CV; Figure 2c). The lower the variability of RRMs probabilities, the higher is the agreement among species and the higher is the intra-group flexibility of mitigating impacts beyond prioritised RPUs. That said, for this study we considered a CV of 30% as an 'acceptable' flexibility threshold. Statistical differences in %CV of RRMs probabilities within RPUs were further investigated for inter-group flexibility in spatial mitigation planning, determined utilising the D'AD test (Feltz & Miller, 1996) in the R package “cvequality” (v.0.2.0, Marwick & Krishnamoorthy, 2018), wherein a $P \leq 0.05$ was set to reject the hypothesis of no difference in variance between groups.

4.3 ***Results***

Habitat suitability drivers among wildlife taxonomic groups

Habitat metrics based on remotely sensed data, and analysed with SDMs, resulted in good performances for most of the selected species. Non-flying mammals achieved 'excellent' accuracy scores (AUC μ=0.75; error rate μ=31.42%), together with birds (AUC μ=0.7; error rate μ=35%), whereas bats obtained the lowest, though 'acceptable' scores (AUC μ=0.67; error rate μ=38.16%) (Appendix S2.1). "Spectral indices" predictors (MSAVI2, EVI, NDVI, BNDVI, NDWI, NDII) were all identified as top-performing for describing occurrence patterns in non-flying mammals, followed by "L-band SAR polarisations" predictors (HV) (66.6% of species). The latter group was also the most representative for birds (62.5% of species), followed by "Spectral indices" (PSRI and SWIR32) (62.5% of species)."Thermal infrared" (LST) was the top-performing predictor for bats (Figure 3) (80% of species), followed by "Spectral indices" (PSRI and SWIR32) (80% of species).

Figure 3. The relative contribution (%) of predictors in explaining species occurrence: non-flying mammal, bird and bat species. Bar colours indicate the predictor group, while the vertical dashed lines (averaged importance values) were used to discriminate predictors to use for further RRMs analysis (the ones at the right side of the line).

We found similar ecological responses among species in each group (Figure 4).

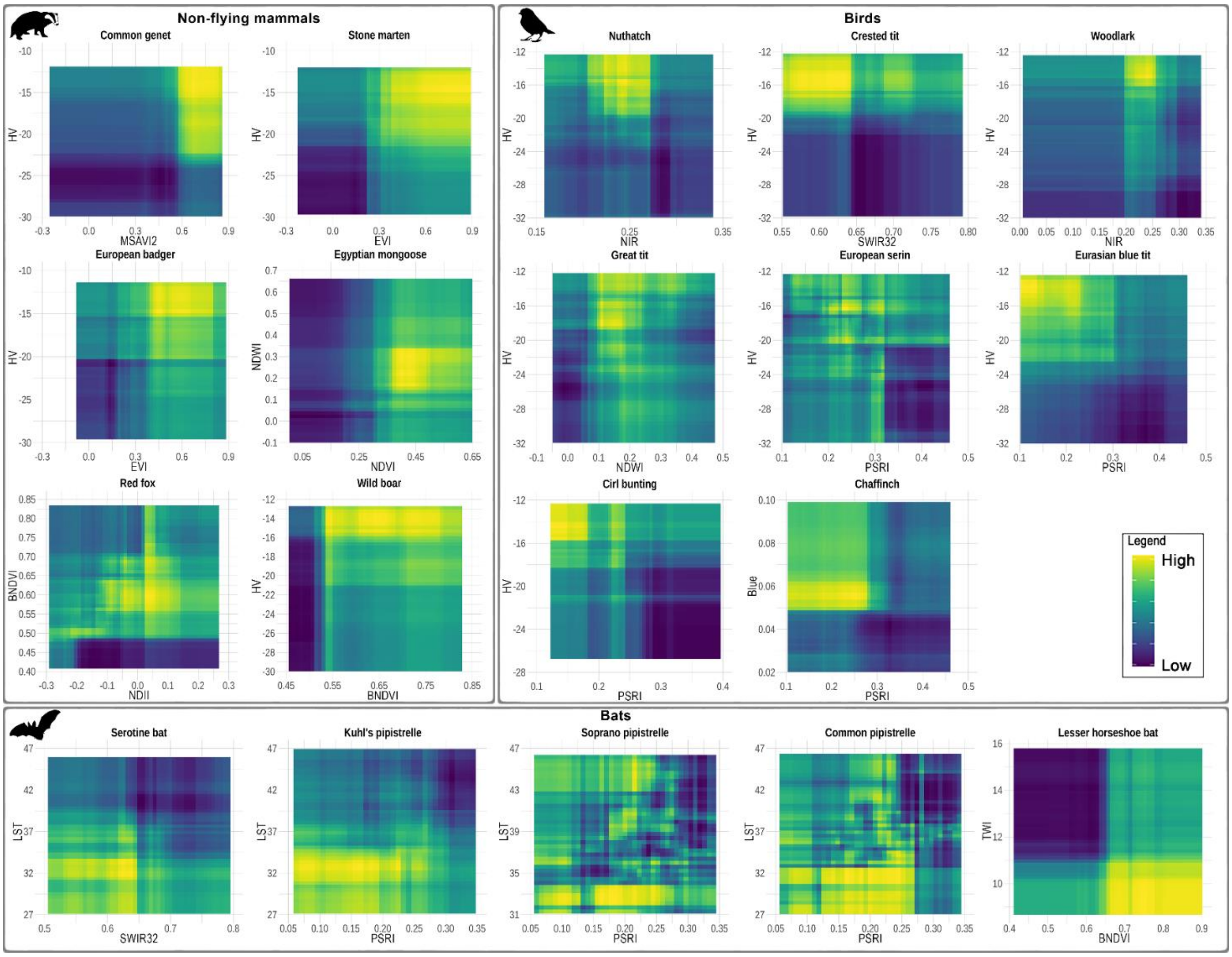

Figure 4. Partial dependence plots, scaled on occurrence probability (SDMs), showing interactions between the two most important habitat metrics for non-flying mammal, bird and bat species. High probabilities of species occurrence are coded in yellow, while low probabilities are coded in dark blue. Further details on the response curves for relevant predictors pertaining SDMs are supplied in Appendix S2.1.

Non-flying mammals held positive relationships with higher vegetation biomass (MSAVI2, EVI, NDVI, and BNDVI) and structure (HV), while higher habitat suitability was observed with areas of low vegetation water stress (lower values of NDWI and NDII). The occurrence of birds responded positively to vegetation structure (HV), as well to low vegetation stress and senescence (lower values of NDWI, PSRI and SWIR32). Bats responded negatively to high temperatures (LST), while again showing a similar response to non-flying mammals and birds for stressed areas (PSRI and SWIR32). Also, for analogous metrics detected in each group, differences in environmental tolerance (amplitude) are observable, showing varying habitat specialisations.

Disentangling habitat, connectivity and anthropogenic influence on roadkills

After retaining the best scales from uncorrelated predictors (see Appendix S2.2 in the RRMs section), evaluation metrics for the RRMs models indicated 'excellent' performances in predicting roadkills, with slight differences detected among species (AUC μ=0.84 and error rate μ=22.3; further details in Appendix S2.2). "Habitat predictors", which were selected as influencing species occurrence (SDMs), resulted significant also for predicting roadkill patterns (RRMs) (in

Appendix S2.2). Similar ecological responses were also detected in RRMs to those observed in SDMs, along with positive responses for most important "Connectivity predictors” and "Road predictors" (Appendix S2.2). As depicted in Figure 5, "Connectivity predictors" were top-performing in explaining roadkills for non-flying mammals (66.6% of species), while for bird and bat species "Connectivity predictors” and "Habitat predictors" shared the same importance.

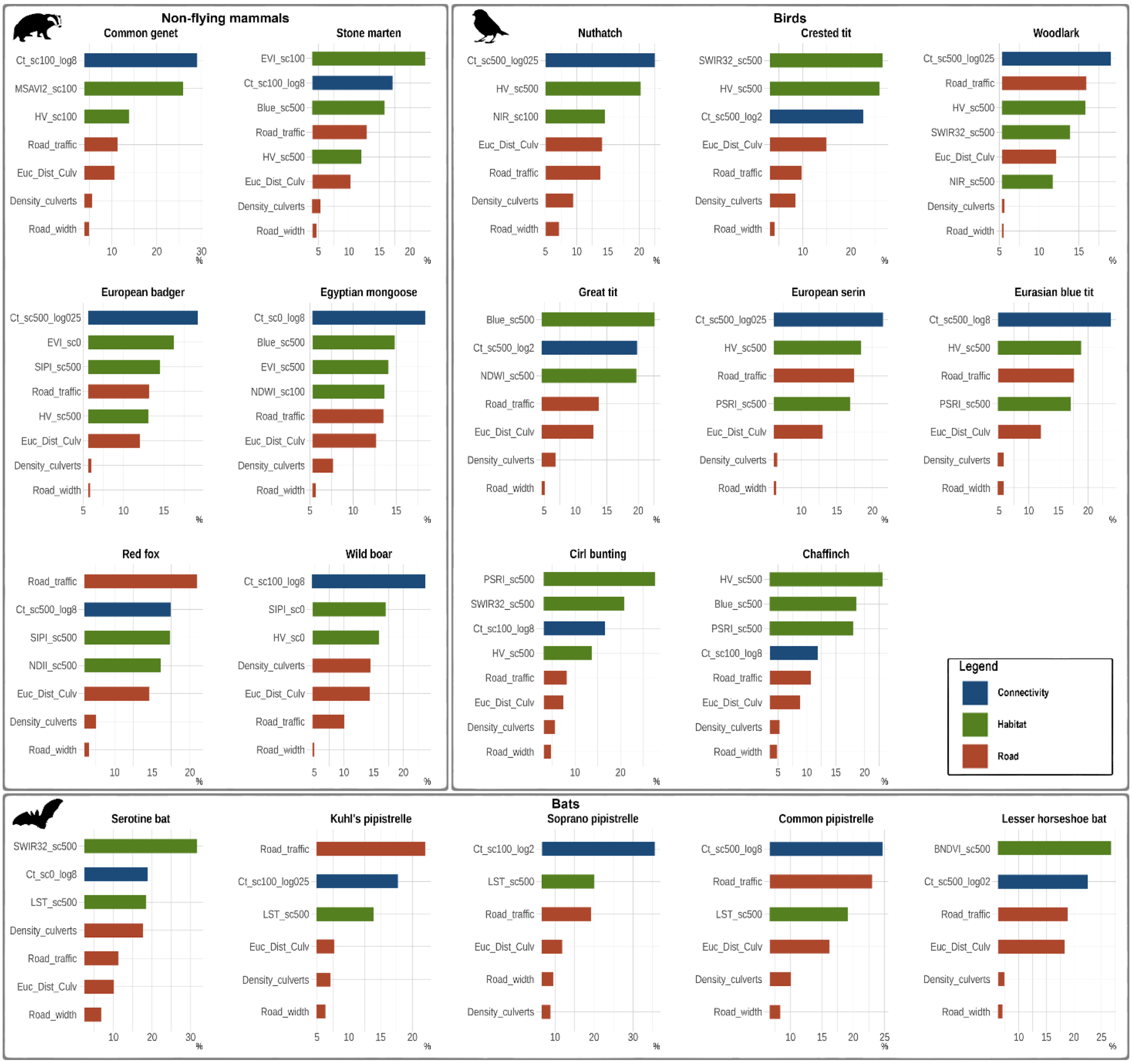

Figure 5. The relative contribution (%) of Habitat, Connectivity and Road predictors for explaining roadkill patterns (RRMs) of non-flying mammal, bird and bat species. Bar colours indicate the typology of the predictors. The "*sc*" suffix is relative to the retained scale (0, 100, 500), with "*Ct*" indicating the species-specific connectivity scenario used, and the score of the "*log*" suffix corresponding to the applied degree of the transformation function (0.25, 2, 8).

Functional connectivity performed better when high function scores (c=8; lower movement cost) were applied for non-flying mammals (75% of species), while for birds and bats the function scores that perform better were low (c=0.25; higher movement cost) (60% of species) and medium-high (c=2 and c=8) (50% of species), respectively. Regarding the importance of "Road predictors", traffic density percentage, was the most important in explaining mortality only for two species, red fox and Kuhls' pipistrelle.

RPUs prioritisation and roadkill risk uncertainties assessment between wildlife taxonomic groups

RPUs with the highest richness scores, to identify risky mortality locations across taxonomic groups, were predominantly found in the southern part of the study area (road N114), as well as in the south-western and partly northern areas (road N4), with scarce risk found in the eastern area (road N18) (Figure 6a).

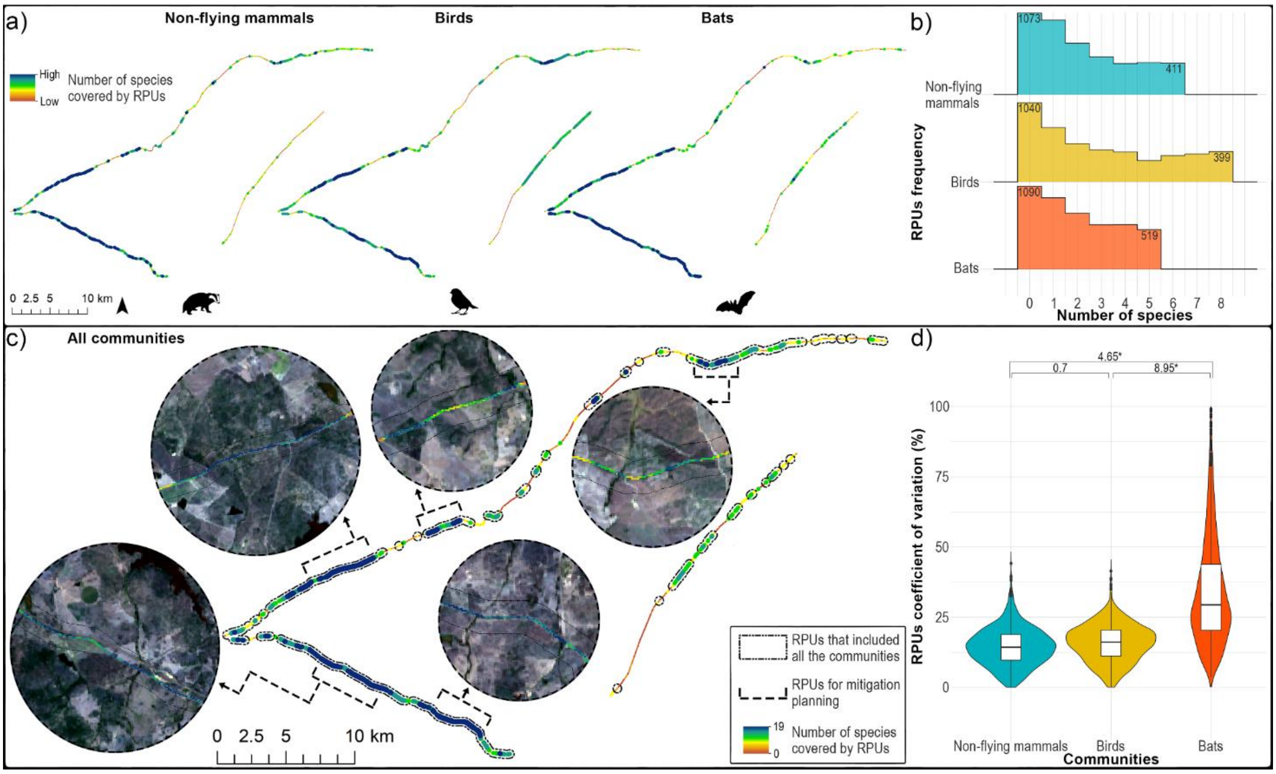

Figure 6. RPUs with number of species (richness) subject to high mortality risk (Figure 6a), and histograms showing the RPUs frequency according to richness scores, for each group (Figure 6b). RPUs covering all the taxonomic groups indicating multispecies mortality risk for mitigation planning, layered with RGB bands from Landsat (7/8) images. Darker areas, notably vegetated areas (e.g., forests, riparian habitats), corresponds to higher roadkill risk locations for multiple species (dark violet) (Figure 6c). For each group, %CV boxplots are depicted with the interquartile range, minimum, maximum, median and outliers, together with D'AD scores, wherein the * symbol indicates $P \leq 0.05$, hence no agreement in variation between groups (Figure 6d).

All groups showed a decreasing trend in RPUs frequency as richness increases, where RPUs with maximum species richness represented a remarkable small proportion compared to others (9.1% for non-flying mammals, 8.9% for birds, whilst 11.6% for bats) (Figure 6b), corresponding to prioritised RPUs with ameliorated focus for allocating resources for mitigation. Within RPUs representing the three groups with at least one species, priority locations for multitaxa mitigation planning were identified by considering the overall species richness across all groups (Figure 6c). Also, the median %CV showed a relatively low variability for non-flying mammals and birds

(%CV≅14), whilst higher for bats (%CV≅27) (Figure 6d), generally indicating an 'acceptable' score, hence intra-group flexibility beyond prioritised RPUs for resource allocation. Between taxonomic groups, there were significant differences in variability (%CV) of RRMs probabilities between non-flying mammals and bats, and between birds and bats. Variability differences were not significant between non-flying mammals and birds, here supporting an inter-group flexibility for allocating resources beyond prioritised RPUs (Figure 6d).

4.4 ***Discussion/Conclusions***

Usefulness of remotely sensed habitat metrics in describing species occurrence

By combining remote sensing information with wildlife occurrences, this study showed that SDMs can greatly benefit from the use of complementary habitat metrics derived from optical multispectral and radar data. The potentialities in using optical and radar data fusion approaches for mapping species distributions have recently been highlighted in the literature, yet their use is still in its infancy (Schulte to Bühne & Pettorelli, 2017). Here, for non-flying mammals, the use of spectral indices related to ecosystem productivity (MSAVI2, EVI, NDVI, and BNDVI) has been shown to play a primary role in explaining species occurrence. In general, higher values of MSAVI2, EVI, NDVI and BNDVI are related to environments with high levels of productivity, which may reflect greater vegetation cover and resources availability (e.g., shelter and food; Kerr & Ostrovsky 2003; Pettorelli et al., 2011). Nevertheless, the relative importance of each vegetation index varied across species. For the common genet, a forest specialist, the low occurrence probabilities predicted over a low range of MSAVI2 values, represents the reluctance of this species to occur in areas with scarce vegetation cover (Valerio et al. 2019), which was better captured by the MSAVI2 index than by EVI, NDVI or BNDVI, likely due to its better ability to minimise the influence of soil on the spectral signal from the sparse vegetation cover component (Qi et al., 1994). The observed better EVI index association with stone marten and European badger occurrences, is consistent with previous work (Santos et al., 2016b), and can be explained by their similarities in complementing forests with high canopy cover conditions, jointly with open habitat mosaics of lower cover conditions (Santos & Santos-Reis, 2010). On this issue, species with more opportunistic habits, namely Egyptian mongoose, red fox, and wild boar, showed an even higher tolerance to sparse vegetation conditions as evidenced by the importance of NDVI and BNDVI indices to explain these species occurrences, where both indices are characterised by a weaker sensitivity to canopy density when compared to EVI (Pettorelli et al., 2011). Forests vertical structural attributes, as described by the L-band SAR HV polarisation, showed a relevant predictive capacity for non-flying mammals. The positive relationship observed between HV backscatter values and the presence of these species may reflect a general favourability of mature forests, in the sense that the higher the HV values, the higher the trees, canopy cover, as well as aboveground biomass levels (Flores-Anderson et al., 2019, Pettorelli et al., 2014; Yu and Saatchi, 2016). Birds occurrence is also greatly influenced by the L-band SAR HV index, showing a stronger forest dependence. This is particularly observed in forest specialists (e.g., nuthatch, crested tits) when compared to more generalist species (e.g., great tit, European serin), having a higher tolerance towards lower HV values. These findings suggest that forest vertical structure relates to habitat requirements, demonstrating that the inclusion of radar information, in particular L-band HV polarisation, is useful in predicting the occurrence of bird species (Bergen et al., 2007). Even though this study was focused on using PALSAR's L-band backscatter data for SDMs, the obtained results highlight the relevance of the upcoming NASA Indian Space Research Organisation (ISRO) Synthetic Aperture Radar (NISAR) mission (to be launched in 2022), which will provide L-band data at a higher spatial and temporal resolution (NISAR, 2020). Unlike Regos et al. (2020), we could not report a significant influence, by LST, for describing bird species occurrences. Importantly, the current findings additionally highlighted a general avoidance of stress areas (NDWI, PSRI, SWIR32), possibly attributable to drought stress in Mediterranean ecosystems (Santos et al., 2016b). Besides water-deficient areas, bats also avoided those prohibitively warm (LST), for which the most generalist species (e.g., soprano, common pipistrelle) exhibited the greatest thermal tolerance. This raises the hypotheses that the warmest areas identified by LST might hold scarcer resources (Friedl, 2002), and in Mediterranean regions, a higher favourability towards cooler areas may relate with greater water availability and woodland cover, but also with foraging opportunities and proximity to riparian habitats (Dietz, 2016; Medinas et al., 2021). Remotely sensed metrics from SDMs explained most of the species occurrences, providing useful insight, and observational evidence of similar ecological responses for species within the same taxonomic group.

Contributing factors to roadkill

We could demonstrate that roadkill patterns are not randomly distributed and can be predicted by the different types of metrics. Top-performing predictors of roadkill, were more associated to functional connectivity than habitat for non-flying mammals, whereas both connectivity and habitat were equally important among bird and bat species. Moreover, many of the predictors retained from SDMs are also important for explaining roadkill risk. For non-flying mammals, the higher performances of low movement cost scenarios, from RRMs, can be associated with periods characterised by greater mobility, such as during dispersal (Grilo et al., 2009), with higher landscape permeability, and roadkills additionally occurring in non-habitat matrix (Diniz et al., 2020; Vasudev et al., 2015). In Mediterranean systems, this is explained for ungulates and mesocarnivores by a high roadkill risk not only circumscribed to forest areas, but jointly to the surrounding matrix of more open areas (Malo et al., 2004). Birds showed better performances associated with higher movement costs scenarios, which may derive from a more pronounced matrix avoidance during road-crossing events, while for some species (e.g., crested tit, great tit, chaffinch), the higher performance of habitat metrics may be explained by a foraging behaviour strategy surrounding roads, namely foliage/bark gleaning, which was previously described as among the strongest roadkill drivers (Santos et al., 2016a). Regarding bats, for opportunistic species (e.g., common pipistrelle) we found roadkill related to movements in non-habitat matrix (given the low movement cost scenarios), including open areas, also used for hunting (Dietz, 2016). Conversely, for less generalist species (e.g., Soprano pipistrelle), mortality was more pronounced in areas of high connectivity closer to woodlands, as well as riparian corridors, being both elements leveraged as landmarks during flight orientation, and representing areas where bat activity is more pronounced (Dietz, 2016; Medinas et al., 2021). For cases of higher mortality risk associated with habitats, this might be due to habitat elements influencing movements along roads, which may be the case of edge-adapted species (e.g., serotine bat) (Tink et al., 2014), and/or deriving by the difficult representation, at the landscape level, of suitable roosting conditions (e.g., lesser horseshoe bat; Dietz, 2016). Road predictors (road traffic) were important in explaining roadkills for some species (e.g., for red fox, Kuhls' pipistrelle), and despite this is an aspect deserving further investigation, it is possible that some characteristics, such as being habitat generalists and common species with a wide distribution, may have diluted the influence of landscape patterns in driving roadkill (e.g.; Santos et al., 2013). Here we clarify the various effects shaping wildlife road mortality according to the taxonomic group, landscape characteristics and ecologically relevant scales analysed, offering new findings, as well as in agreement with previous research (Carvalho et al. 2011; Gunson et al. 2011; Medinas et al., 2013).

Mitigation optimisation of RPUs over long-term

To our knowledge, this is the first attempt to predict road mortality across multiple taxa, while considering a high resolution (30X30m pixels) and a long study period. Results for each taxonomic group showed that for RPUs integrating both high mortality risk and richness scores, represent 9 to 12% (depending on the group) of all RPUs, and hence conveniently unveiling risky areas for multiple species with different degrees of habitat specialisation, home-ranges and dispersal abilities that should be a priority for mitigation. Additionally, to optimise resource allocation in mitigation structures and maximise financial return, an overlap across all taxonomic groups identified RPUs potentially offering a comprehensive mortality protection, including species from all the three studied taxonomic groups. This represents a further step in environmental impact assessment, which is most relevant when considering that the need for broad biodiversity representation in conservation planning and action is paramount (Margules & Pressey, 2000), to effectively apply the scarcely available resources (Polak et al., 2019). Besides, when investigating the mortality risk prediction ability of RPUs, results revealed relatively low variation scores within each group. Moreover, no significant differences in variation were found between non-flying mammal and bird groups. Our findings suggest an intra and inter-group agreement in mortality prediction, that may be explained by an overlap between habitats, which is common in Mediterranean forest ecosystems (Santos & Santos-Reis, 2010), jointly with a woodland connectivity that similarly accommodates species, at least with 'medium' dispersal abilities (see Machado et al., 2020). For the sake of practicality, the current results give decision-makers more flexibility in implementing mitigation measures beyond best RPU sites, ensuring facilitated decision agreements between conservationists and road managers during the strategic planning phase (Rytwinski et al., 2015; van der Ree et al., 2015). This may be relevant when allocating conservation resources, as unexpected factors can often influence the application of designed structures on desirable sites (van der Grift, 2017), for example due to overlooked topographic conditions, or when it is more costly-efficient to adapt existing structures, such as culverts (Rytwinski et al., 2015; van der Ree et al., 2015). Interestingly, although the adaptation of culverts to multi-use is thought to benefit mostly generalist species in human-dominated landscapes (Clevenger & Huijser, 2011), an optimisation still seems feasible given the present findings for RPUs encompassing both specialist and generalist species. Culverts are often supplemented with fencing for non-flying mammal species (e.g., mesocarnivores; Clevenger & Huijser, 2011), and results suggest that strategically applied fences can simultaneously help to reduce mortality for birds as well, resulting into so-called *opportunity costs* for applied measures (Armsworth, 2014). Nevertheless, other frameworks at the structure and design level are needed to provide further support for post-hoc mitigation effectiveness regarding multiple taxa (e.g., BACI; Rytwinski et al., 2016). Bats show greater, even though acceptable, intra-group variation in mortality risk, but significant divergence emerged when compared to other groups. The latter may derive from the larger home-range sizes and travelled distances (Dietz, 2016), which may have led to divergent mortality patterns. Our method can be used to spatially define the most efficient measures that benefit the greater and more diverse number of species, with results also pointing at some possible flexibility in the location of the roadkill mitigation measures for single or multi taxonomic groups without hindering the main goals of the conservation plan. Nevertheless, it should be stressed out that in specific cases when mismatches occur across species or taxonomic groups, prioritisation of RPUs with highest predicted richness is preferable, even at the expense of flexibility towards those sub-optimal. We further advise caution towards *species-agnostic* connectivity frameworks (e.g., Koen et al., 2014), as it may not always be generalisable as a mitigation guidance for multiple taxa.

The roadkill sampling campaigns on which our study was based covered a longer time period than the norm, and while a consequent limitation may lie in delayed implementation of conservation actions, on the other hand, it allowed for supporting a higher efficacy of mitigation measures over extended study periods (Rytwinski et al., 2016). Such a larger temporal scale also allowed to minimise data gaps, which may be determinant in the strategic planning phase (Armsworth, 2014). To secure the reliability of conservation planning efforts over long-term, remote sensing information can help to address the problem of stochasticity in landscape dynamism, while its integration into fine-grain connectivity can enable the characterisation of habitats sufficiently large and persistent to ensure immigration/emigration rates between populations, thus viability. Across the landscape, by inferring structural, functional and demographic connectivity components (e.g., habitat amount), we contextualise RPUs into a larger corridor network composed of multiple meaningful and resilient pathways, which are key elements for optimal mitigation planning along roads (Clevenger & Huijser, 2011), while to promote the prevention of populations genetic drift for multiple generations (Cosgrove et al., 2018). Biodiversity alone can constitute a solid guideline for determining the *conservation value* of a planning unit during prioritisation (Margules & Pressey, 2000), and while we do not explicitly include theoretical costs associated with RPUs, the proposed pixel-based framework is designed to represent an original baseline for forward-looking cost-effectiveness formulations (e.g., *B*/*C*; Kujala et al., 2018), heightening its applicability in real-world contexts. We recommend conservation planners consider top-performing habitat metrics here selected for each studied taxon, along with connectivity and road predictors for comprehensive RRMs evaluation. We also advocate for scrutiny of spatial predictions and evidence-based optimisation for long-term planning strategies targeting multiple taxa, along with the consideration of trans-disciplinary research for solving conservation problems (Pressey et al., 2017; Rose et al., 2019).

4.5 ***Acknowledgements***

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AUTHORS' CONTRIBUTIONS

F.V.; S.G., A.M., and S.S. conceptualised the research; A.M., and S.S. supported with resources; F.V., S.G., G.M. and S.S. developed the framework; F.V., P.S., D.M., N.P., A.M., S.G., J.C., P.C., and E.F. collected the data; F.V. analysed the data; F.V. led the manuscript writing. All authors significantly supported to the writing and reviewing, and agreed for the definitive manuscript version to be eligible for publication.

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3.7 *Supplementary material S1*

1.1 Selected species.

Prior analysis, migratory behaviour was selected as a species exclusion criterion. Such a step was considered necessary to avoid confounding intra-year ecological responses, while for non-migratory species and in specific bats, minimising variations in phenological traits was checked, by including only species including only species that also frequent hibernation areas as for breeding (Smeraldo et al., 2017).

Supplementary table S1.1. Selected species and representative taxonomic group.

Common name	Binominal name	Group
Common genet	*Genetta genetta* (L. 1758)	Non-flying mammals
Stone marten	*Martes foina* (Erxleben 1777)	
European badger	*Meles meles* (L. 1758)	
Egyptian mongoose	*Herpestes ichneumon* (L.1758)	
Red fox	*Vulpes vulpes* (L. 1758)	
Wild boar	*Sus scrofa* (L. 1758)	
Nuthatch	*Sitta europaea* (L. 1758)	Birds
Crested tit	*Lophophanes cristatus* (L. 1758**)**	
Woodlark	*Lullula arborea* (L. 1758)	
Great tit	*Parus major* (L. 1758)	
European serin	*Serinus serinus* (L. 1766)	
Eurasian blue tit	*Cyanistes caeruleus* (L. 1758)	
Cirl bunting	*Emberiza cirlus* (L. 1766)	
Chaffinch	*Fringilla coelebs* (L. 1758)	
Lesser horseshoe bat	*Rhinolophus hipposideros* (Bechstein 1800)	Bats
Serotine bat	*Eptesicus serotinus* (Schreber 1774)	
Common pipistrelle	*Pipistrellus pipistrellus* (Schreber 1774)	
Kuhls' pipistrelle	*Pipistrellus kuhlii* (Kuhl 1817)	
Soprano pipistrelle	*Pipistrellus pygmaeus* (Leach 1825)	

1.2 Remote sensing data correction and calibration.

Landsat scenes (Landsat-5, Landsat-7, Landsat-8), considering the case study, constituted the non-commercial solution for satellite imageries holding the highest spectral, radiometric and spatiotemporal detail (16-day repetition and 30m spatial resolution; Wulder et al., 2019). In order to avoid biases, each image, belonging to the Landsat-5 and Landsat-7 satellites, was subjected to the Landsat Ecosystem Disturbance Adaptive Processing System protocol (LEDAPS; Masek et al., 2006), as well the Land Surface Reflectance Code (LaSRC; Vermote et al., 2016) regarding Landsat-8, which was required for both calibration and atmospheric correction to surface reflectance. Regression coefficients were utilised to harmonise spectral and radiometric data from different sensors (Roy et al. 2016). Then, once images were calibrated, artifact pixels suffering from cloud contamination, shadows and other anomalies (e.g., oversaturation) were masked out from each scene throughout quality assessment bands (QA), these created utilising the CFmask (C version of "Function of mask" algorithm; Foga et al., 2017; Zhu & Woodcock, 2012; Zhu et al., 2015). The missing data was filled with the "Phase-2 gap-filling" algorithm (USGS, 2004), applied to correct the notorious failure of the scan-line corrector pertaining the Landsat-7 sensor. In addition, as striping data from Landsat-7 scenes was markedly pronounced, such information was incorporated only under conditions of complete absence of data from other satellites, namely during the year 2012 (Table S1.2).

Likewise, the same approach was applied for the whole collection regarding problematic meteorological periods in the study area, namely during the predominant cloud cover in January and February, whose temporally coincident scenes were filtered out.

The missing data also involved PALSAR/PALSAR2 information (Shimada et al., 2014), being notably available only for the following years: 2007, 2008, 2009, 2010 for PALSAR, while 2015, 2016, 2017 for PALSAR2. Therefore, to obtain an approximation for each missing year between 2005 and 2020, we simply utilised the most temporally closed image data before and after the missing year, and calculated the median. Subsequently, the values were converted from unsigned 16-bit digital numbers (DN) values into gamma-naught (γ^0) backscatter values (unit: decibel) because less likely dependent on the incidence angle of the radar beam. This solution was adopted to basically improve the incorporation of terrain variations (Small; 2011). The conversion was applied following the formula proposed by Rosenqvist et al. (Rosenqvist et al., 2007), for which DN is the digital number and CF is a calibration factor equal to "- 83" for PALSAR/PALSAR2 data:

$$\gamma^0(dB) = 10\log_{10}(\mathrm{DN})^2 + \mathrm{CF}$$

The NASADEM digital elevation model was also collected, and derives from an interpolation of ASTER GDEM, ICESat, and PRISM data (Crippen et al., 2016).

Supplementary table S1.2. Imageries acquired according to satellite sensors (Landsat-5, Landsat-7, Landsat-8), years, the type of path/rows pertaining spatial coverage, and the total of those adequate collected each year (in bold). Overall, 625 imageries ($\mu \cong 41$ per year) were collected.

path/row	Landsat-5 TM							Landsat-7 ETM+
	2005-2006	2006-2007	2007-2008	2008-2009	2009-2010	2010-2011	2011-2012	2012-2013
203/33	11	9	13	10	12	11	10	10
203/34	11	10	14	9	16	11	10	10
204/33	8	10	12	11	12	6	10	10
Total	**30**	**29**	**39**	**30**	**40**	**28**	**30**	**30**
path/row	Landsat-8 OLI							
	2013-2014	2014-2015	2015-2016	2016-2017	2017-2018	2018-2019	2019-2020	
203/33	14	19	19	19	20	17	19	
203/34	12	17	17	19	20	18	19	
204/33	16	15	18	18	20	15	18	
Total	**42**	**51**	**54**	**56**	**60**	**50**	**56**	

1.3 Species field survey details: presences and absences.

Data records were compiled by the same surveyors, when applicable, and conducted between 2005 and 2020. For all sampling strategies, sites were rarely surveyed more than once (excluding the 3-times point visit regarding bat surveys from Medinas et al., 2021, as well the 5-times roosts visits for bats from Medinas et al., unpublished), as generally more effort was given to homogenously cover the whole study area (e.g., Loos et al. 2015).

For non-flying mammals (medium-sized carnivores plus wild boar), all surveys included monitored tracks coupled with camera traps. Track surveys were carried out in two 750m long transects, with a minimum distance of 500m each other. Here, signs of species presences were inspected and recorded, with predominant attention paid to footprints, and less to faeces and latrines, these recorded only in exceptional cases of suitable conditions (e.g., recent droppings with unmistakable shape or scent (Pita et al., 2009)). All track surveys were complemented with fixed camera traps (Reconyx HC600 Hyperfire™), which were placed at the beginning, as well end of transects, while at roughly 1.5m above the ground. The two methodologies were combined maximise the detection of meso-mammals, and previous studies in the area supported such approach in covering most of detectable species (Herrera et al. 2017).

For bird species, all representing passerines, censuses consisted on point counts, each distant 100m from others, where species were surveyed for 10min by direct sight or indirectly by vocalization (Bibby et al. 2000). The breeding season was selected as the best period, specifically between April and May, and in favourable weather conditions, given the higher detectability (6:00–11:00 a.m.; Bibby et al. 2000). This monitoring technique was also selected because it is commonly applied for birds, as well in various studies conducted in the area (Herrera et al., 2017; Salgueiro et al., 2018a; Salgueiro et al., 2018b)

For bat species, censuses involved point counts, transects, and roost surveys where presences were registered, and bat echolocation calls identified. All sessions took place between April and October, generally coinciding with activity peak bat activity in Mediterranean areas (Raino, 2007), with best detectability ensured by selecting only calm, dry weather conditions (Dixon, 2012). Bat detectors (Petterson D500x and D240x, Petterson Elektronik AB, Sweden, BATLOGGER, Elekon AG, Lucerne, Switzerland) were primarily set with a microphone sensitivity spanning 10 to 200 kHz, while located at 1.5m above ground, together with digital recorders (Archos AV 500 mobile digital video recorder). Bat call sequences were processed using Kaleidoscope and Audacity software (Wildlife Acoustics, Inc., v.3.1.1; Audacity v.1.3.10 Beta software) with default settings (Medinas et al. 2019; Medinas et al., 2021; Herrera et al., 2017). The dataset with regards to point counts was derived from Medinas et al. (2021), with points falling 1000m apart, and sessions during 15min. Point count data was derived additionally from Medinas et al. (2019), here associated with transects, with an approximation distance of 500m each other, hence resembling the approach of Herrera et al., (2017) for bats and carnivores surveys. Finally, roosts inspections were conducted mostly for the lesser horseshoe bat, each consisting of 5 visits, in which suitable conditions for colonies (in buildings, mines, cave etc.) were searched, followed by presence (and absence) verification (Medinas et al., unpublished).

Notably, absences were generally considered, but when unavailable, their derivation as pseudo-absences depended on non-detections of the species, according to the type of survey of the corresponding community. Such a method was considered appropriate for common species, while for rarer (e.g., the lesser horseshoe bat), absences were systematically collected, thus kept wherever possible to avoid omission errors (Tyre et al., 2003).

1.4 Deriving species home-range sizes for each species.

Home-range sizes were derived through the literature giving priority to studies located in Portugal, then in the Iberian Peninsula, and if still missing, in Southern Europe. In case of two or more references, we estimated the home-range value using the median of the different sources. For scarce or no information, exceptional decisions were taken by the surveyors responsible for each community (NP and SS for non-flying mammals, PS for birds, while DM for bats), each time depending on the specific case of study.

Supplementary table S1.3. Home-range dimension (ha) for each species according to literature, followed by correspondent conversion into diameter (m), and references used. The * symbol indicated for which species exceptional considerations were taken into account for calculation.

Species	Home range size (ha)	Hectares to diameter (m)	References
Genet	308	≅ 2000	Carvalho et al 2018; Santos-Reis et al 2005
Stone marten	260	≅ 1800	Santos-Reis et al 2005
Badger	501	≅ 2500	Revilla and Palomares 2002; Rosalino et al 2004
Mongoose	300	≅ 2000	Palomares and Delibes 1991
Fox	167	≅ 1500	Servin et al. 1991; Travaini et al. 1993
Wild boar	551	≅ 2600	Barasona et al 2014
Nuthatch	1.87	≅ 150	Nilsson, 1976; Enoksson and Nilsson, 1983; Cramp and Perrins, 1993; Paradis et al., 1998
Crested tit*	0.96	≅ 100	
Woodlark	4.7	≅ 250	Cramp, 1988; Patzold, 1986 in Bowden, 1990; Langston et al., 2007; Sirami et al., 2011; Bosco, 2014
Great tit	1.2	≅ 100	Krebs, 1971; Cramp and Perrins, 1993; Paradis et al., 1998; Storch, 1998
Serinus	1	≅ 100	Cramp and Perrins, 1994; Paradis et al., 1998
Blue tit	0.82	≅ 100	Cramp and Perrins, 1993; Paradis et al., 1998; Storch, 1998
Cirl bunting	0.94	≅ 100	Cramp and Perrins, 1994; Paradis et al., 1998; Dale et al., 2005
Chaffinch	0.48	≅ 70	Marler, 1956; Paradis et al., 1998; Storch, 1998
Lesser horseshoe bat*	590	≅ 2750	Dietz 2016; Charles-Dominique 2001
Serotine bat*	1980	≅ 5000	Dietz 2016; Charles-Dominique 2001
Common pipistrelle*	185	≅ 1500	Dietz 2016; Charles-Dominique 2001
Kuhls pipistrelle	290	≅ 2000	Andriollo (2015); Greenfeld, A. (2012)
Soprano pipistrelle*	239	≅ 1700	Dietz 2016; Charles-Dominique 2001; Nicholls, B. and Racey, P. 2006

We take into consideration the following observations:

- **Crested tit**: Home range size pooled (median) from other tits: *Parus major*, *Cyanistes caeruleus*.
- **Lesser horseshoe bat**: We considered feeding areas estimated up to 1200 ha. In Bavaria, the individual home range is estimated between 6.8 to 62.8 ha (μ=25.2 ha). The distance to maternities roosts from the feeding areas ranges between few hundred meters up to 8 km, usually around 2.5 km.
- **Serotine bat**: Distance to foraging sites can be up 5-7 km; often this also involved spending most of the foraging time at distance smaller than 2 km.
- **Common pipistrelle**: The distance between maternities roosts and feeding areas is estimated about 850 meters, while from 1,5 to 5 km in Germany, and Scotland 1.5 km.

- **Soprano pipistrelle**: In Scotland, the Soprano pipistrelle may travel an average of 700 meters to feeding areas, whereas in another study this corresponded to around 1.5 km, and generally more than common pipistrelle.

1.5 Thinning presences and absences.

The presence and absence datasets were filtered separately using a spatial-thinning algorithm (Aiello-Lammens et al., 2015) within the spThin package (v.0.2.0), implemented in the R statistical environment (v.3.6.3; R Core Team, 2016). The algorithm calculates, based on a user-defined thinning distance, all possible distances between points, progressively removing random subsamples to reach the maximum number of points respecting the distance set between them (Aiello-Lammens et al., 2015). Such distances were established utilising home-range dimensions (ha), which were translated into distinct diameters (m) (Table S1.3).

Supplementary table S1.4. Number of records for each species after the filtering procedure, for presences and absences.

Species	Presences	Absences
Genet	148	254
Stone marten	160	238
Badger	181	196
Mongoose	104	224
Fox	189	231
Wild boar	124	167
Nuthatch	104	176
Crested tit	34	248
Woodlark	148	129
Great tit	134	143
Serinus	114	162
Blue tit	201	75
Cirl bunting	32	251
Chaffinch	255	53
Lesser horseshoe bat	43	269
Serotine bat	60	172
Common pipistrelle	100	139
Kuhls pipistrelle	139	92
Soprano pipistrelle	122	114

1.6 Habitat metrics calculation.

Prior to calculating predictors, all satellite data was standardised to reflect the same spatial scale, so the nominal 30m grain resolution was maintained for LANDSAT and NASADEM data, while upscaled for the 25m PALSAR/PALSAR2 data. The 30m grain resolution was considered adequate to reflect the minimum habitat area requirements across all species (Table S1.3). Regarding the textural indices, as first step a Principal Component Analysis (PCA) was calculated upon spectral bands (Schulte to Bühne & Pettorelli; 2018). The PC1, accounting for 90% of variance was underwent a calculation to develop GLCM predictors (Table S1.5) through the glcm package (v.1.6.5; Zvoleff, 2020), using a 3 × 3 pixels spatial moving window in all directions (0°, 45°, 90°, and 135°; Godinho et al., 2018). The TWI was calculated from the NASADEM digital elevation model, using the RSAGA package in R (v.1.3.0; Brenning et al., 2018).

Supplementary table S1.5. Remote sensing-derived habitat predictors and respective sensors. Habitat predictors were pooled into types, and then groups, on the basis of their common characteristics in describing landscape environmental aspects. The G = 2.5, C1 = 6, C2 = 7.5 and L = 1 values were applied within the equation to calculate the EVI index, in which: the "L" value helped the background correction, "C1" and "C2" are coefficient utilised to suppress the aerosol influence, while the "G" value corresponded to a gain factor. The "a" and "β" elements for the TWI index corresponds to "Catchment Area" and "Slope" calculations, respectively (see Quinn et al., 1995).

[1] Spectral bands from Landsat-5 and Landsat-7 were renamed to preserve the coherence of bandwidths (wavelength intervals) with those of Landsat-8.

Sensor	Group	Type	Code	Description	Spectral Band or Equation	Reference
LANDSAT 5/7/8	Spectral bands[1]		Blue		B2	Wulder et al., 2019
			Green		B3	
			Red		B4	
			NIR		B5	
			SWIR1		B6	
			SWIR2		B7	
	Spectral indices	Vegetation Biomass Indices	NDVI	Normalized difference vegetation index	$\frac{(B5-B4)}{(B5+B4)}$	Huete et al., 2002
			BNDVI	Blue Normalized difference vegetation index	$\frac{(B5-B2)}{(B5+B2)}$	Yang et al., 2004
			EVI	Enhanced vegetation index	$2.5*\frac{(B5-B4)}{(B5+C1*B4-C2*B2+L)}$	Huete et al., 2002
			MSAVI2	Modified soil-adjusted vegetation index (II)	$\frac{(2*B5+1-\sqrt{(2*B5+1)^2-8*(B5-B4)}}{2}$	Richardson & Wiegand, 1977
		Vegetation and landscape Water content	NDII	Normalized Difference Infrared Index	$\frac{(B5-B7)}{(B5+B7)}$	Hardisky et al., 1983
			NDWI	Normalized difference water index	$\frac{(B5-B6)}{(B5+B6)}$	Gao 1996
			MSI	Moisture Stress Index	$\frac{B6}{B5}$	Hunt & Rock, 1989
		Senescent vegetation and soil surfaces indices	PSRI	Plant Senescence Reflectance Index	$\frac{(B4-B2)}{B5}$	Merzlyak & Gitelson, 1999
			SWIR32	Shortwave infrared Reflectance 3/2 ratio	$\frac{B7}{B6}$	Guerschman et al., 2009
	Thermal infrared	Surface Temperature	LST	Land Surface Temperature	GEE Source code	Ermida et al., 2020
	Textural indices	Co-occurrence matrix	GLCM_M	Mean	Calculated using a moving window (3 × 3 pixels) in all directions (0°, 45°, 90°, and 135°) applied to the first principal component (PC1) from all selected spectral bands	Haralick, 1979
			GLCM_E	Entropy		
			GLCM_H	Homogeneity		

PALSAR/ PALSAR-2	L-band SAR polarisations	Vegetation structure	HH	Horizontal transmitting, horizontal receiving signal		Shimada et al., 2014
			HV	Horizontal transmitting, vertical receiving signal		
NASADEM	DEM	Digital elevation model	TWI	Topographic wetness index	$\ln(a/\tan\beta)$	Quinn et al., 1995

1.7 Parametrisation processes for achieving tuned SDMs and theoretical assumptions.

Each species was subjected to precautions to avoid biases. By selecting RF models, a prevalence value of 0.5 was considered) to balance datasets (Barbet-Massin, 2012). In situations of higher absences frequencies than presences (Table S1.4), the numerical gap was solved by random absence removal, and for opposite situations, through their addition, as pseudo-absences. This latter step followed the random selection (*RS*) methodology in Iturbide et al. (2015). Basically, this methodology means that lacking (pseudo)absences, to reach a 0.5 prevalence, were allocated in the background area, but outside an exclusion buffer from known species-specific presences/absences data, utilising the previously mentioned home-range sizes as distance thresholds (Table S1.3). Once the dataset was balanced, for the predictor selection within the Boruta feature selection, to reduce biases, we fixed 'tentative' predictors by using the "*TentativeRoughFix*" function within the Boruta package (Kursa & Rudnicki, 2010). Furthermore, for multivariate analysis, a RF model tuning procedure identified the best parametrisation that allowed to best performances results (AUC; Fielding & Bell, 1997), relatively the number of trees (*ntree*), here spanning from 1000 to 3000, together with the number of available predictors that can be splitted at each tree node (*mtry*), here ranging from 1 to 6.

Finally, for the analysis, two assumptions were made: (1) that each sample size was sufficient to environmentally cover the ecological niche of a given species, an assumption supported by the capability of machine learning method to identify significant habitat predictors even for low sample size datasets (e.g., 30; Table S1.4) (Smith & Santos, 2020); and (2) species/environment relationships were stationary (species at the equilibrium) along the study period, a problematic that was intended to soften by spatiotemporally matching the field-based data, with remote sensing-based data.

1.8 Functional connectivity assessment details.

The nodes and resistance surface where both extracted from SDMs, reflecting areas of high persistence in terms of habitat conditions, and corridor routes (or barriers) in the matrix.

Regarding the construction of the nodes, SDMs were converted into binary maps. Expert-based thresholds (cutoffs) or hypothetical scores found in the literature were avoided in order to ensure the replicability of the study in other regions. Hence, as optimal cutoff, was selected the optimisation of sensitivity (true positive rate) and specificity (true negative rate), also called the "*SeSpeql*" approach (*sensu* Nenzén & Araújo, 2011), which aim to soften the absolute difference between the sensitivity and specificity (Fielding & Bell, 1997; Nenzén & Araújo, 2011). This identifies a balanced 'cross-over' between classification rates, namely between probability of correctly predicted presences and probability of correctly predicted absences (Fielding & Bell, 1997). Secondly, to enhance discrimination between high-quality areas, and the matrix, the MSPA (Morphological Spatial Pattern Analysis) framework was applied by default over the binary map layer to extract habitat core areas (Salgueiro et al., 2021; Vogt & Riitters, 2017). Third, to extract nodes from core areas, pixels were converted into centroids while retaining the area amount (ha) of the respective original patch. These centroids within core areas constituted the species 'foraging ground' (Finch et al., 2020), and underwent the thinning procedure abovementioned described, with the intention that each node independently represented a species-specific home-range. Finally, for each species, nodes that did not meet the minimum amount of available habitat (ha; Table S1.3), were discarded, as well nodes in the near proximity of major roads (250m for birds and bats, while 500m for mesomammals) to deal with negative effects from roads on wildlife, which may be particularly pronounced within woodlands habitats (Carvalho et al., 2018; Medinas et al., 2019; Reijnen et al., 1995).

Besides, regarding the parametrisation of resistance surfaces, a negative exponential transformation function was employed, following Trainor et al. (2013):

(Equation S1.1) $R = 100 - 99 * \frac{1-e^{-c*H}}{e^{-c}}$

where "R" is the resistance to dispersal, "H" corresponds to occurrence probability from SDMs, while "c" is the degree to the function for the three employed scores, namely c=0.25, c=0.5 and c=8. The first score corresponds to a linear relationship with SDMs, whereas the others indicate a growing exponential non-linear relationship (Trainor et al., 2013).

Once were prepared nodes and resistance surfaces for each species, the probability of connectivity (*PC*; Saura & Pascual-Hortal, 2007) was computed, which in turn required the following components: functional distance between pairwise nodes, the median dispersal threshold associated with each species and amount of habitat within each node. Firstly, a list of effective least-cost distances (*LCD*; m) between pairwise nodes was calculated in the "*gdistance*" R package (v. 1.3-1; van Etten, 2017), which varied each time on the basis of species-specific resistance surface and c score utilised (c=0.25, c=0.5 and c=8). Secondly, the dispersal probability pij calculated the probability of a link between nodes i and j:

$pij = e^{-\alpha\ dij}$

where "pij" is the symmetric probability of direct dispersal between a pair of nodes, "dij" is the pairwise nodes direct functional distance from "*LCD*" (on the basis of the selected resistance surface), "α" is a species-specific constant (decay parameter) reflecting dispersal behaviour and dispersal distance (Saura and Pascual-Hortal, 2007):

$\alpha = \frac{1}{r*d}$

where "r" corresponds to the median cost value of the resistance surface and "d" the dispersal distance of a given species.

It should be noted in the case that a distance threshold is the median of species dispersal, the negative exponential function of "dij" is set in the form that the dispersal probability pij=0.5 (Saura et al., 2011; Saura & Pascual-Hortal, 2007), a condition previously supported in other road ecology studies (e.g., Gurrutxaga and Saura, 2014). The median of dispersal distances was derived from the literature for each species (Table S1.6), adopting the same estimation used when deriving home-ranges (Table S1.3). Then, by selecting the "*Distance*" as connection type within the CONEFOR software (v.2.2; Saura & Torné, 2009), the probability of connectivity "*PC*" was calculated, which is defined as 'the probability that two animals randomly placed within the landscape fall into habitat areas that are reachable from each other' (Saura & Pascual-Hortal, 2007):

(**Equation S1.2**) $PC = \frac{\sum_{i=1}^{n}\sum_{j=1}^{n} a_i a_j p_{ij}^{*}}{A_L^2}$

where "n" is the total number of habitat nodes, "ai" is the attribute (ha) of node "i", "aj" is the attribute of node "j", while "AL" is the total landscape area (habitat and matrix). The "pij *" is the maximum product probability of link scores of the intermediate paths connecting nodes "i" and "j". As mentioned in the manuscript, the internodes connection probability was repeated for each species due to the different resistance surfaces used, hence generating 3 lists of connected pairwise nodes, for which the operative links were considered only for greater probability scores than 0.5 (Salgueiro et al., 2021).

1.9 Deriving dispersal distances for each species.

On the basis of available literature, the dispersal distance for each species was derived, as for home-range estimation, by prioritising studies in Portugal or Iberian Peninsula. For non-flying mammals, the dispersal distances were inferred by utilising species' home-range sizes (Table S1.3), each within the Bowman isometric equation (Bowman et al., 2002). Then, to approximate median dispersal distances, such derived scores were averaged to what found in literature. For the other communities, namely birds and bats, personal observations from surveyors (PS and DM) were taken into account owing to the scarcity of literature on this issue.

Supplementary table S1.6. Median dispersal distances (m) for each species according to literature and other inferences. The * symbol indicated for which species exceptional observations were taken into account for calculation.

Species	Dispersal distances (m)	References on dispersal movements
Genet	≈ 7000	Carvalho et al., 2016
Stone marten	≈ 11500	Wereszczuk and Zalewski, 2018
Badger	≈ 12500	Revilla and Palomares, 2002
Mongoose	≈ 8000	
Fox	≈ 8000	Servin et al. 1991; Travaini et al. 1993
Wild boar	≈ 10000	Prévot and Licoppe, 2013; Keuling et al., 2018
Nuthatch	≈ 6500	Nilsson, 1976; Enoksson and Nilsson, 1983; Cramp and Perrins, 1993; Paradis et al., 1998
Crested tit*	≈ 5300	
Woodlark	≈ 4000	Cramp, 1988; Patzold, 1986 in Bowden, 1990; Langston et al., 2007; Sirami et al., 2011; Bosco, 2014
Great tit	≈ 5300	Krebs, 1971; Cramp and Perrins, 1993; Paradis et al., 1998; Storch, 1998
Serinus*	≈ 5800	Cramp and Perrins, 1994; Paradis et al., 1998
Blue tit	≈ 5300	Cramp and Perrins, 1994; Paradis et al., 1998; Storch, 1998
Cirl bunting*	≈ 5600	Cramp and Perrins, 1994; Paradis et al., 1998; Dale et al., 2005
Chaffinch	≈ 3600	Marler, 1956; Paradis et al., 1998; Storch, 1998
Lesser horseshoe bat	≈50000	Dietz 2016; Charles-Dominique 2001
Serotine bat	≈ 50000	Dietz 2016; Charles-Dominique 2001
Common pipistrelle	≈ 20000	Dietz 2016; Charles-Dominique 2001
Kuhls' pipistrelle	≈ 40000	Andriollo (2015); Greenfeld, A. (2012)
Soprano pipistrelle	≈ 180000	Dietz 2016; Charles-Dominique 2001; Nicholls, B. and Racey, P. 2006

We take into consideration the following observations:

- **Crested tit**: Natal dispersal data pooled (median) from other tits: *Parus major*, *Cyanistes caeruleus*.
- **Serinus:** Natal dispersal data pooled (median) from other true finches: *Fringilla coelebs*, *Chloris chloris*, *Carduelis carduelis*, *Linaria cannabina*.
- **Cirl bunting**: Natal dispersal data pooled (median) from other buntings: *Emberiza citrinella*, *Emberiza schoeniclus* and *Emberiza hortulana*.

1.10 Roadkill analysis details

Univariate and multivariate roadkill analysis followed the same methodology of SDMs, consequently using a dataset composed of a 0.5 prevalence, between presences and absences. However, contrary to presences, roadkills absences were randomly generated on monitored roads (pseudo-absences), but carefully avoiding areas overlapping significant species-specific roadkill hotspots, to soften potential biases. For each species, significant hotspots were derived from kernel density estimations (KDE; Bíl & Andrášik, 2013), adopting a window of 150m bandwidth (Bíl & Andrášik, 2013; Medinas et al., 2021). Calculations were carried out separately for each road sector to not confound spatio-temporal consistence in KDE calculations (Bíl & Andrášik, 2020), resulting into a total of 57 KDE analysis (19 species * 3 road sectors).

1.11 Road-related metrics calculation.

Regarding predictors, a novel experimental approach was also considered in the present study for calculate the "traffic volume percentage" predictor. Here, despite a vast number of studies infer traffic from the typology of road, such studies often lack the integration of traffic variation, being this information attributed to the entire section, and thus, 'diluted'. This is similarly the case when considering data from different municipalities, as information on traffic is averaged from beginning of road section to the end, and as consequence, losing how information varied along a considered road. Also, even when data is available from some municipalities, models extrapolation may be difficult on roads assigned to other municipalities due to divergent approaches in traffic monitoring, or simply because data might be difficult to achieve, or is often inexistent. Given that, a novel data source was selected in this study, which in spite of the limited temporal resolution, possess high coverage in terms of spatial extension, type of sampled roads, and resolution. Data, in specific, was retrieved from TomTom products, namely the "Historical Traffic Stats", containing trillions of measurements (https://www.tomtom.com/products/historical-traffic-stats/). A query was generated for the monitored road sector for the present study, relatively "traffic density" product within the study period, which is a spatial information at high resolution of traffic density from the dual directions of considered roads. Therefore, once probe data on traffic density was converted in pixel and averaged for the dual road directions, the information was subsequently converted into percentage, being this information relative to vehicle with functioning probes, then not an absolute traffic estimation, such as traditional approaches (e.g., volume traffic; Medinas et al. 2021). This information directly originated from historical trips of TomTom users in the area, in the form of georeferenced points transmitted by probes, which frequency is recorded in space and time, and stored within an anonymous database, representing a good spatio-temporal coverage given the 600 million of GPS and floating car data on which the TomTom is relying. Then, "road width" was calculated from PS by georeferencing points along lanes of monitored road, approximately every 15m, which corresponds to half of pixel size employed in this study. Afterwards, point distances between the two lanes were in parallel calculated for each road sector, hence converted into a raster layer. Data on culverts was retrieved from a previous study in the area where functional culverts were registered and georeferenced (Craveiro et al., 2019), allowing the calculation of Euclidean "distance from culverts" predictor, along with "culvert density percentage". The culvert density percentage was initially derived through a kernel density function, which measures the density of points (culverts) per meter, on the basis of bandwidth radius distance from the points. We used as search radius distance the abovementioned 150m bandwidth (Section 1.10). For simplicity, calculated values were converted into percentage, such as the previously calculated traffic predictors, where higher values indicated increases in proportion of culver density.

1.12 Programming languages and hardware employed.

The programming languages employed for the entire framework were: JavaScript with regards to the GEE platform, the C language regarding the Gflow software, and R. Gflow performed into 2 high-performant systems (Ubuntu Oss each carrying 12-core processors with 64GB-RAM) to tackle computational limitations routinely hindering fine-grain connectivity approaches (Moilanen, 2011).

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3.8 *Supplementary material S2*

2.1. Species distribution models (SDMs) results.

The Boruta feature selection confirmed on average 9 to 21 habitat predictors explaining species occurrence (Figure S2.1.1-S2.1.3). Uncorrelated predictors were also identified for each species (Table S2.1.1., Figure S2.1.4.). From the multivariate SDMs analysis, the tuning results from random forests models are shown (Figure S2.1.4), along with accuracy results (Table S2.1.2) and ecological response curves of most important predictors (Figure S2.1.6-Figure S2.1.8). Threshold values for converting SDMs into binary maps were also identified (Figure S2.1.9).

Figure S2.1.1 SDMs habitat predictors explaining species occurrence from the Boruta feature selection. Boxplots of relative importance of predictors are depicted with the interquartile range (box), minimum, maximum, median and outliers, for each non-flying mammal species (common genet, stone marten, European badger, Egyptian mongoose, red fox and wild boar), while comparing habitat metrics with significant (green) against irrelevant (red) contribution.

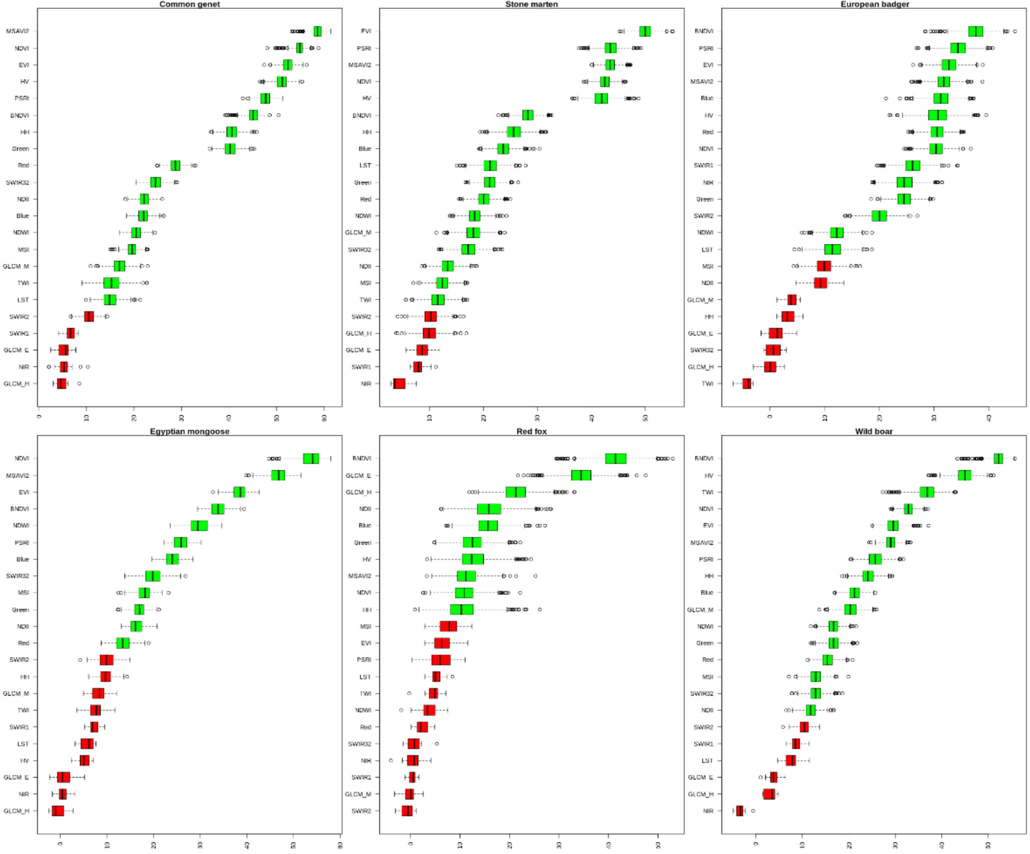


Figure S2.1.2 SDMs habitat predictors explaining species occurrence from the Boruta feature selection. Boxplots of relative importance of predictors are depicted with the interquartile range (box), minimum, maximum, median and outliers, for each bird species (nuthatch, crested tit, woodlark, great tit, European serin, Eurasian blue tit and cirl bunting chaffinch), while comparing habitat metrics with significant (green) against irrelevant (red) contribution.

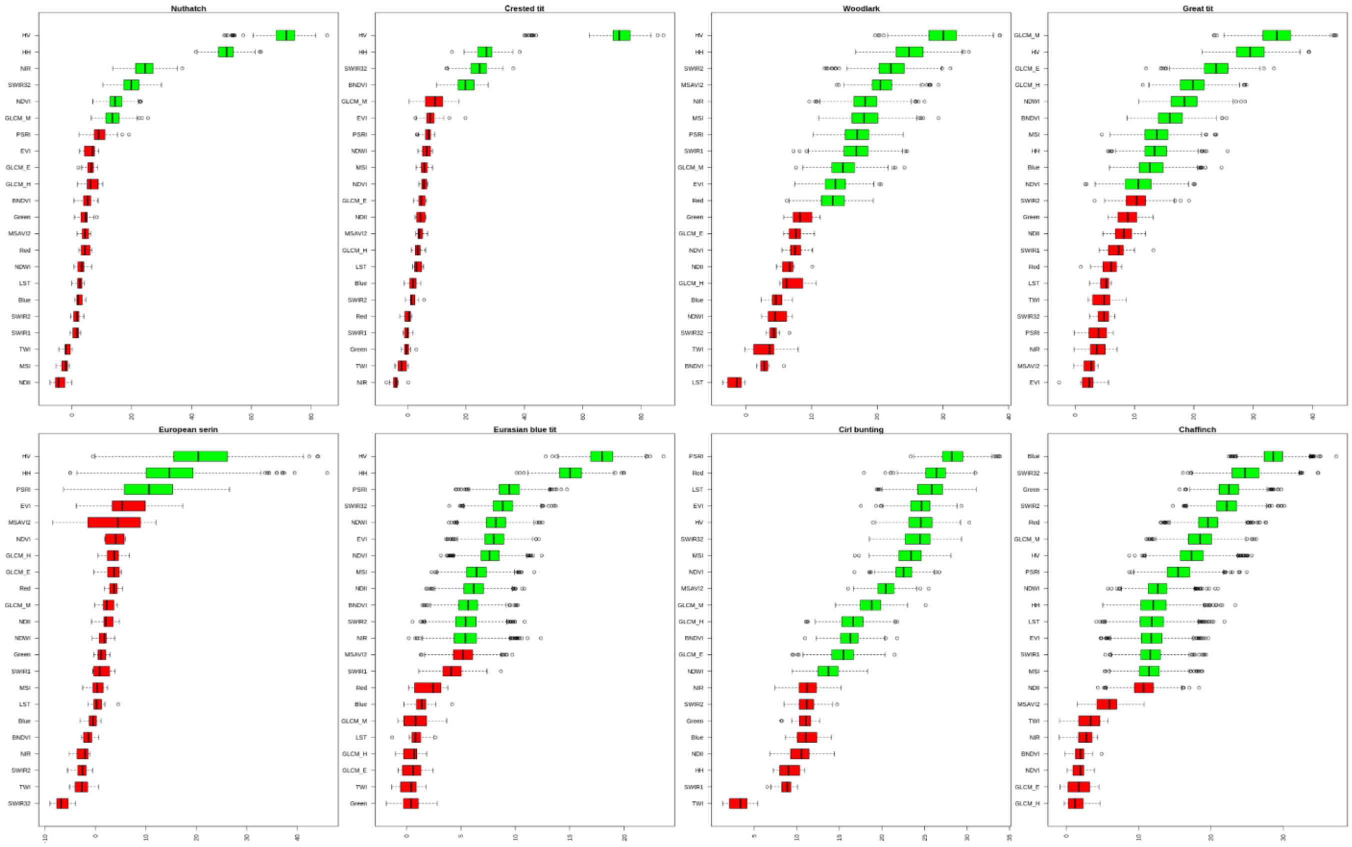


Figure S2.1.3. SDMs habitat predictors explaining species occurrence from the Boruta feature selection. Boxplots of relative importance of predictors are depicted with the interquartile range (box), minimum, maximum, median and outliers, for each bat species (lesser horseshoe bat, serotine bat, common pipistrelle, Kuhls' pipistrelle and soprano pipistrelle), while comparing habitat metrics with significant (green) against irrelevant (red) contribution.

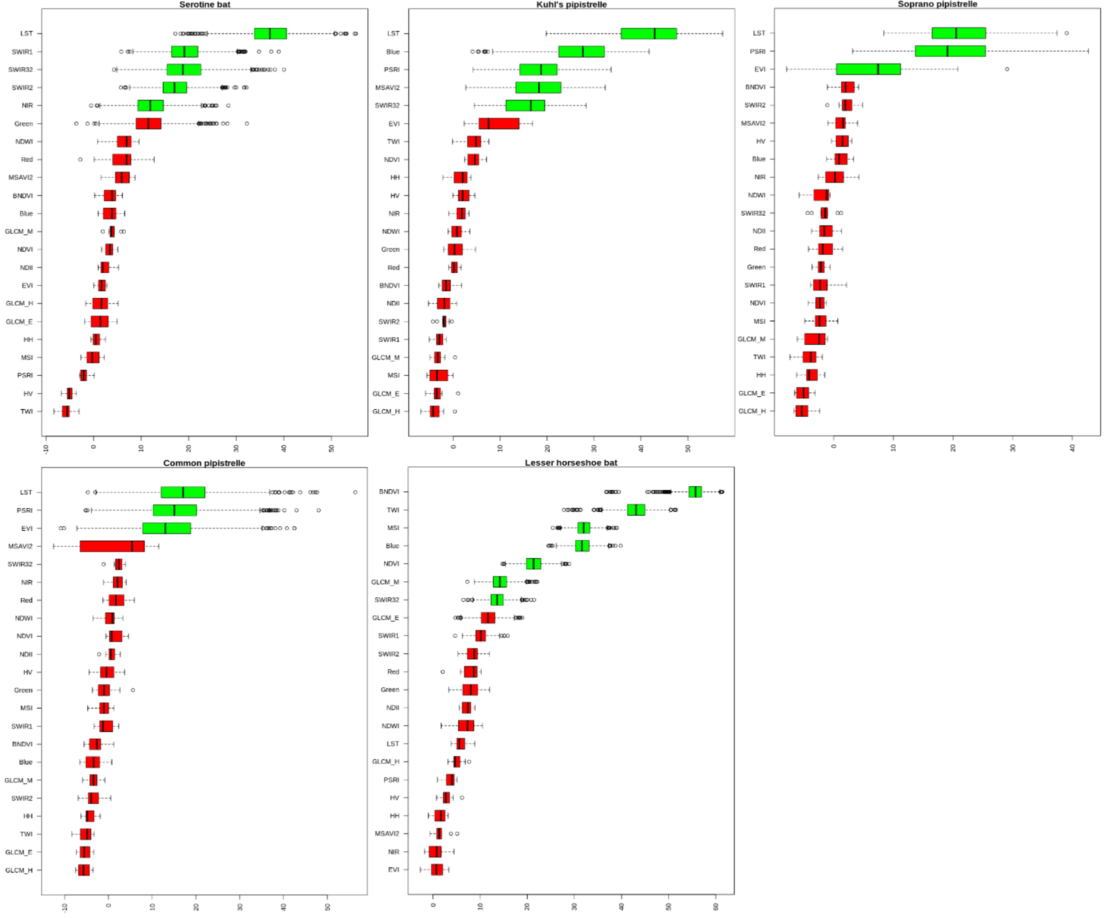


Table S2.1.1. Variance inflation factor (VIF) results for the retained habitat metrics prior to SDMs multivariate analysis.

Species	Common genet	Stone marten	European badger	Egyptian mongoose	Red fox	Wild boar	Nuthatch	Crested tit	Woodlark	Great tit	European serin	Eurasian blue tit	Cirl bunting
Blue		3,01	1,8	2,35						3,28			
Green	3,81												
NIR							1,14	1,1	1,78			1,29	
SWIR1													
SWIR2									3,28				
NDVI				3,75			4,05						
EVI		4,34	2,95										
MSAVI2	3,7								2,63				
NDII	3,21	2,71			3,3								
NDWI			2,85	2,57						2,73		3,59	
MSI						3,74							
PSRI				2,3		3,79			3,76		1,75	3,74	3,29
BNDVI		2,46	1,58		3,07	4,16						3,64	
SWIR32	4,2	2,59		2,5		3,16	2,66	1,2				2,87	2,11
LST	2,18	2,27	1,66										1,73
GLCM_M	2,97	2,55				2,94				3,85			
GLCM_E					1,07					1,13			1,06
HH					1,22			3,58		4,2	4,23		
HV	2,08	1,98	1,65			2	1,93	3,85	1,87	4,55	4,92	1,94	2,03
TWI		1,03											

					1,09
4,99					
4,42					
					1,89
2,08	2,11	2,03	1,13	1,16	
3,15	2,2	2,49			2,72
					3,59
4,65		2,16	1,13	1,16	
4,18					
	3,6				
	1,85				
3,62		2,3			
Chaffinch	Serotine bat	Kuhl's pipistrelle	Soprano pipistrelle	Common pipistrelle	Lesser horseshoe bat

Figure S2.1.4. Upper-triangular correlation matrices of habitat metrics prior to SDMs multivariate analysis, for non-flying mammal (a), bird (b) and bat (c) species. Pearson rank correlation values are depicted for each species, together with colours indicating the degree of correlation.

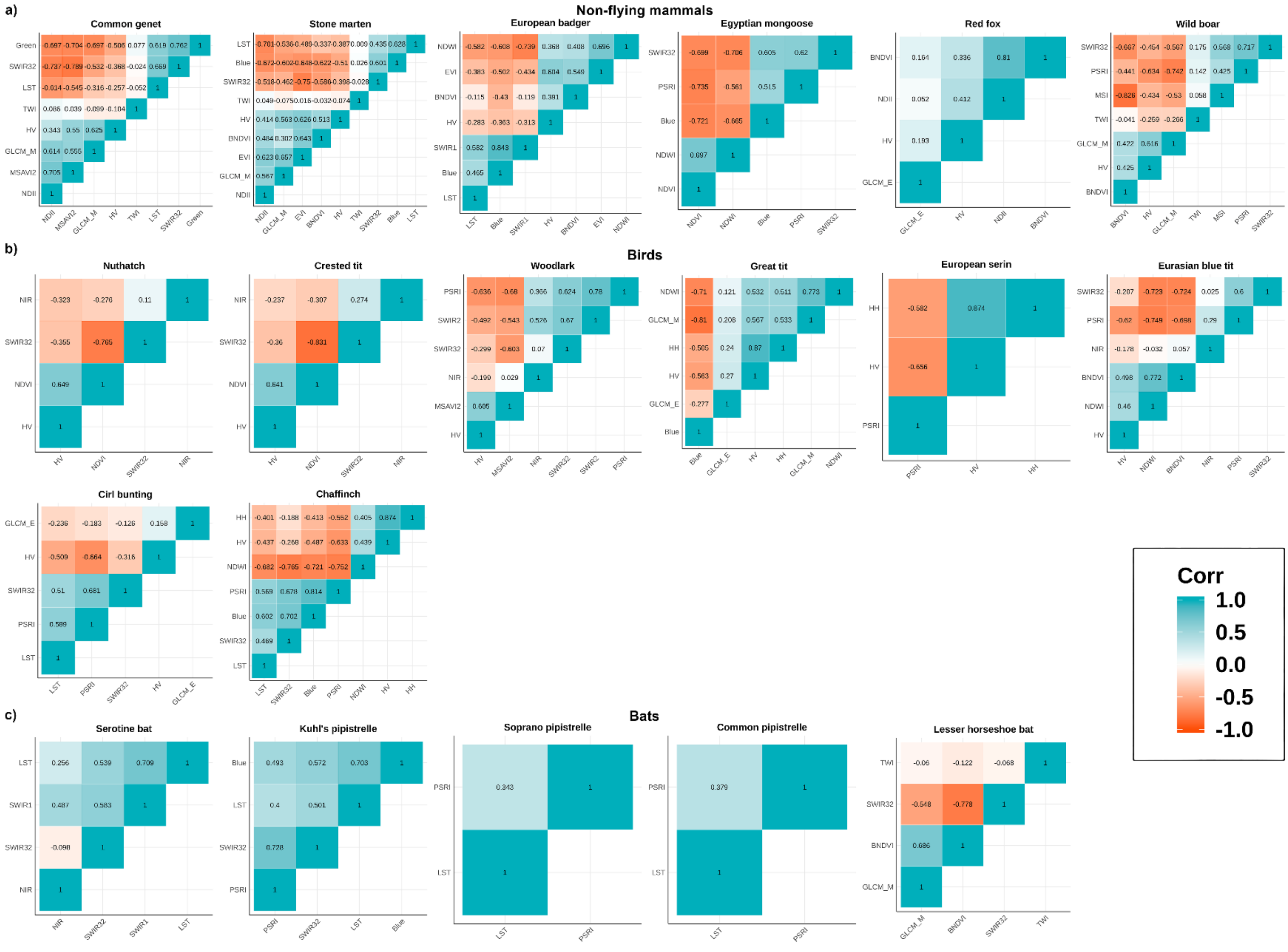


Figure S2.1.5. The AUC values from SDMs on the basis of selected parameter scores, namely under different number of splitted predictors (*mtry*) and number of trees (*ntree*), with respecto to non-flying mammal a), bird b) and bat c) species. The best model is represented by the highest AUC score, according to different combination of *mtry* and *ntree* parameters, or solely the *ntree* parameter in case of *mtry*=1 (see for instance the soprano pipistrelle and commgon pipistrelle results).

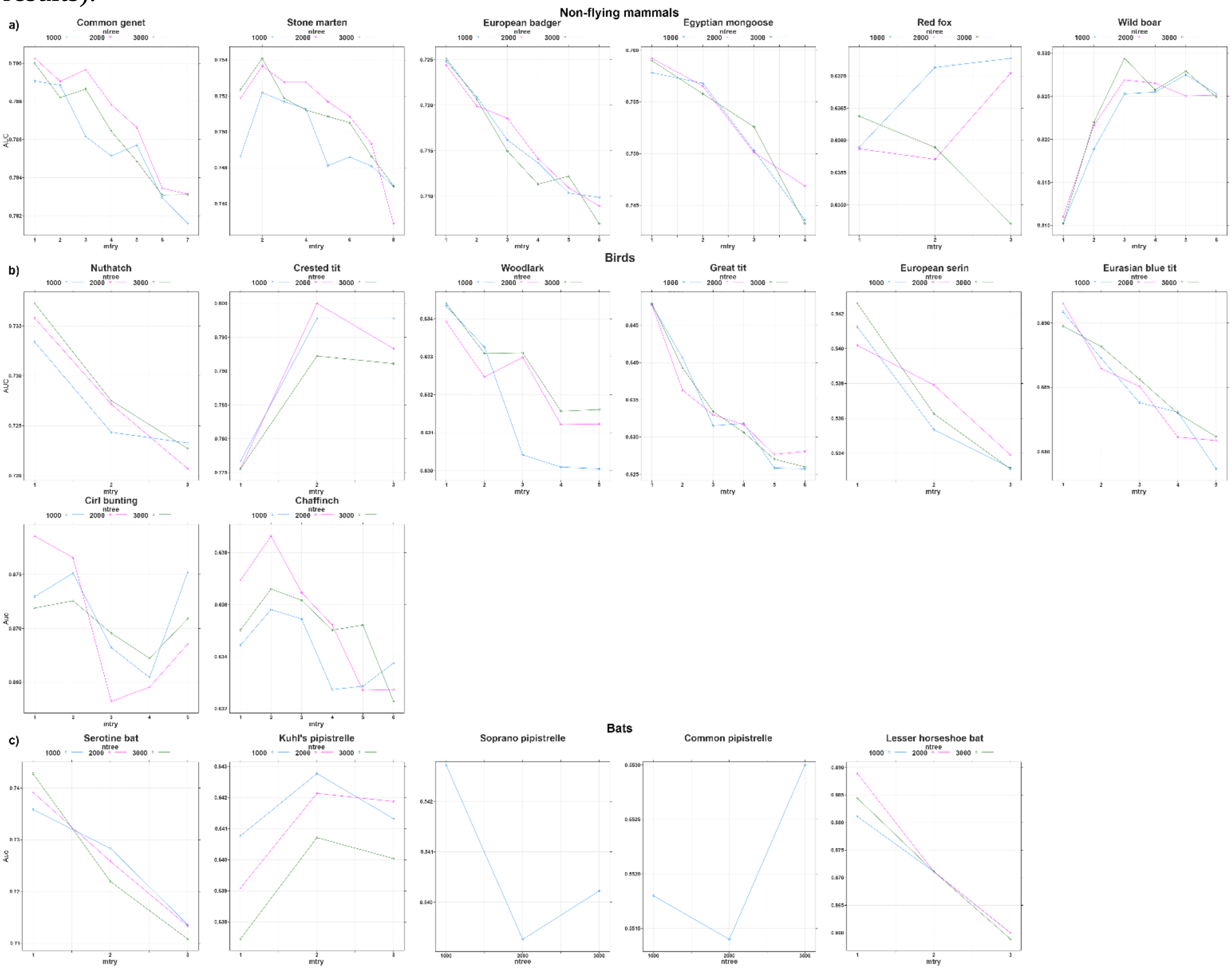


Table S2.1.2. SDMs accuracy scores, for each species, with mean and standard deviation (SD), with respect to the area under the receiver operating characteristic curve (AUC), sensitivity, specificity and error rate percentage.

	AUC		Sensitivity		Specificity		Error rate (%)
Species	Mean	SD	Mean	SD	Mean	SD	
Common genet	0.79	0.07	0.74	0.11	0.73	0.11	26.52
Stone marten	0.75	0.08	0.66	0.1	0.72	0.1	30.68
European badger	0.72	0.07	0.64	0.11	0.68	0.1	33.53
Egyptian mongoose	0.75	0.08	0.67	0.12	0.67	0.14	32.59
Red fox	0.63	0.08	0.54	0.11	0.65	0.12	40.18
Wild boar	0.83	0.09	0.75	0.12	0.74	0.13	25
Nuthatch	0.73	0.1	0.68	0.14	0.65	0.14	32.93
Crested tit	0.79	0.18	0.74	0.25	0.75	0.23	25.33
Woodlark	0.63	0.09	0.59	0.13	0.58	0.12	41.10
Great tit	0.64	0.09	0.58	0.12	0.62	0.12	39.88
European serin	0.55	0.1	0.56	0.15	0.58	0.14	46.15
Eurasian blue tit	0.69	0.07	0.63	0.1	0.64	0.09	35.2

Cirl bunting	0.87	0.13	0.87	0.14	0.71	0.25	18.29
Chaffinch	0.64	0.08	0.58	0.11	0.59	0.09	41.1
Serotine bat	0.74	0.14	0.7	0.18	0.68	0.2	30.25
Kuhl's pipistrelle	0.63	0.1	0.58	0.12	0.57	0.13	45.5
Soprano pipistrelle	0.53	0.1	0.51	0.14	0.49	0.14	49.2
Common pipistrelle	0.55	0.12	0.50	0.16	0.54	0.16	47.4
Lesser horseshoe bat	0.88	0.15	0.85	0.21	0.78	0.24	18.44

Figure S2.1.6. Response curves of most important predictors influencing the probability of occurrence (SDMs) for common genet (a), stone marten (b), European badger (c), Egyptian mongoose (d), red fox (e) and wild boar (f). The result from the averaged models is depicted by dashed lines, whose colours reflect the groups on which habitat metric belong. The grey area corresponds to the standard error.

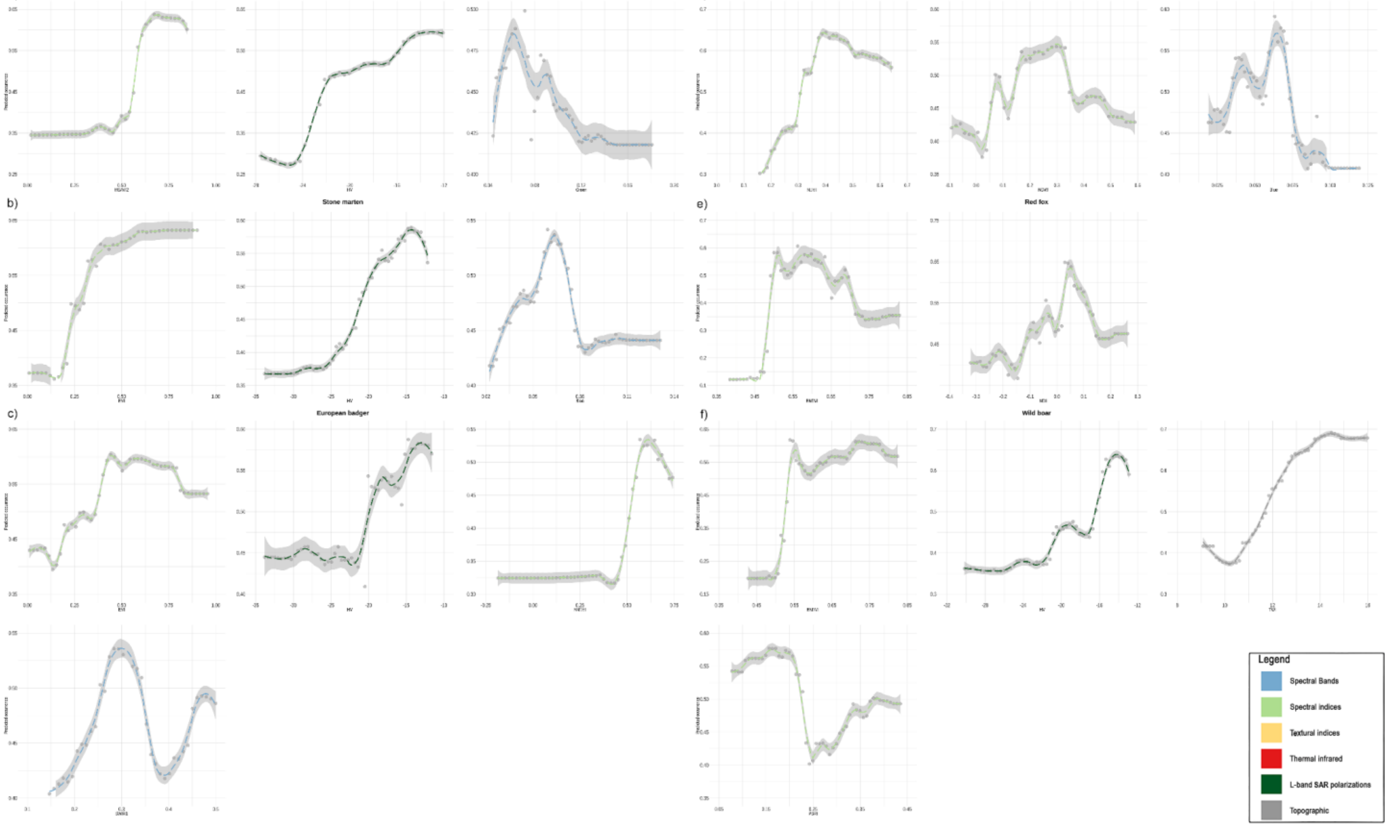


Figure S2.1.7. Response curves of most important predictors influencing the probability of occurrence (SDMs) for nuthatch (a), crested tit (b woodlark (c), great tit (d), European serin (e) Eurasian blue tit (f), cirl bunting (g) and chaffinch (h). The result from the averaged models is depicted by dashed lines, whose colours reflect the groups on which habitat metric belong. The grey area corresponds to the standard error.

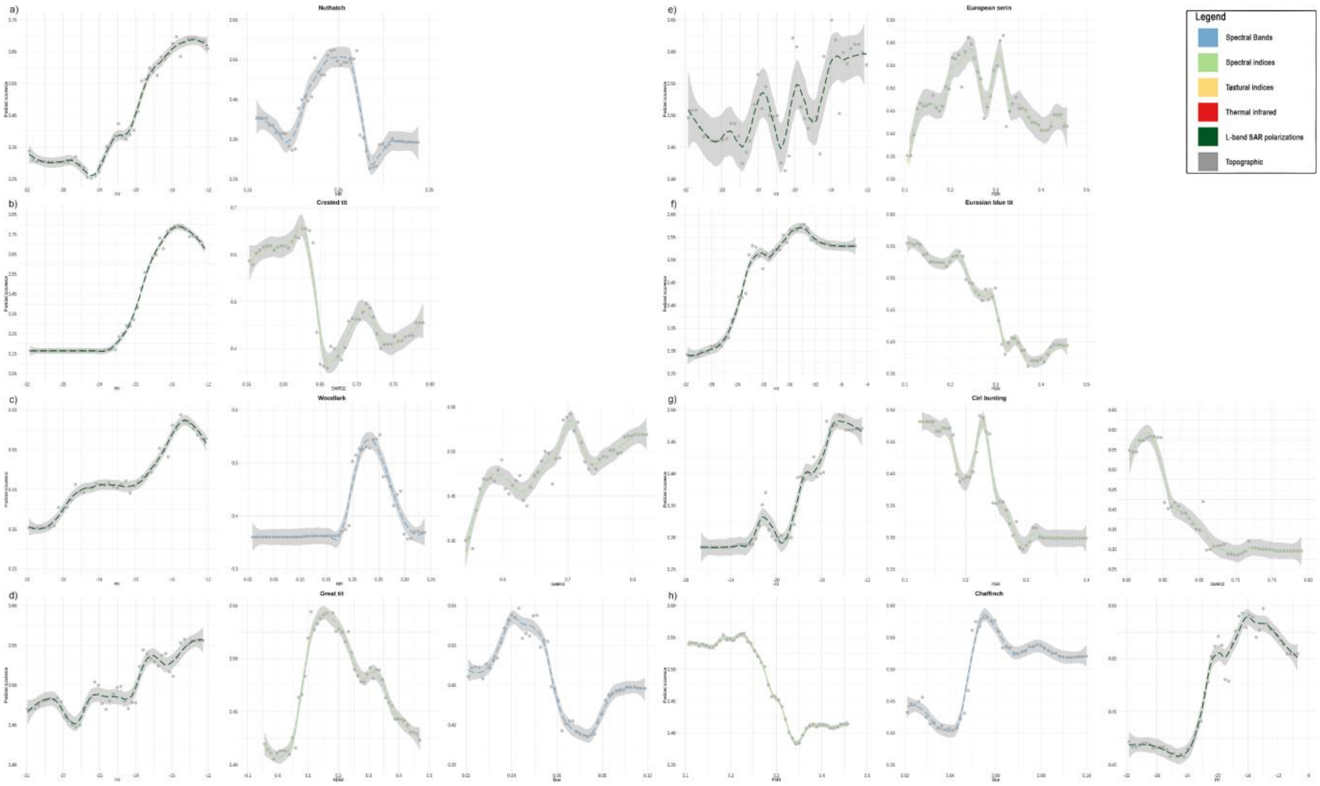


Figure S2.1.8. Response curves of most important predictors influencing occurrence probability (SDMs) for lesser horseshoe bat (a), serotine bat (b), common pipistrelle (c), Kuhls' pipistrelle (d) and soprano pipistrelle (e). The result from the averaged models is depicted by dashed lines, whose colours reflect the groups on which habitat metric belong. The grey area corresponds to the standard error.

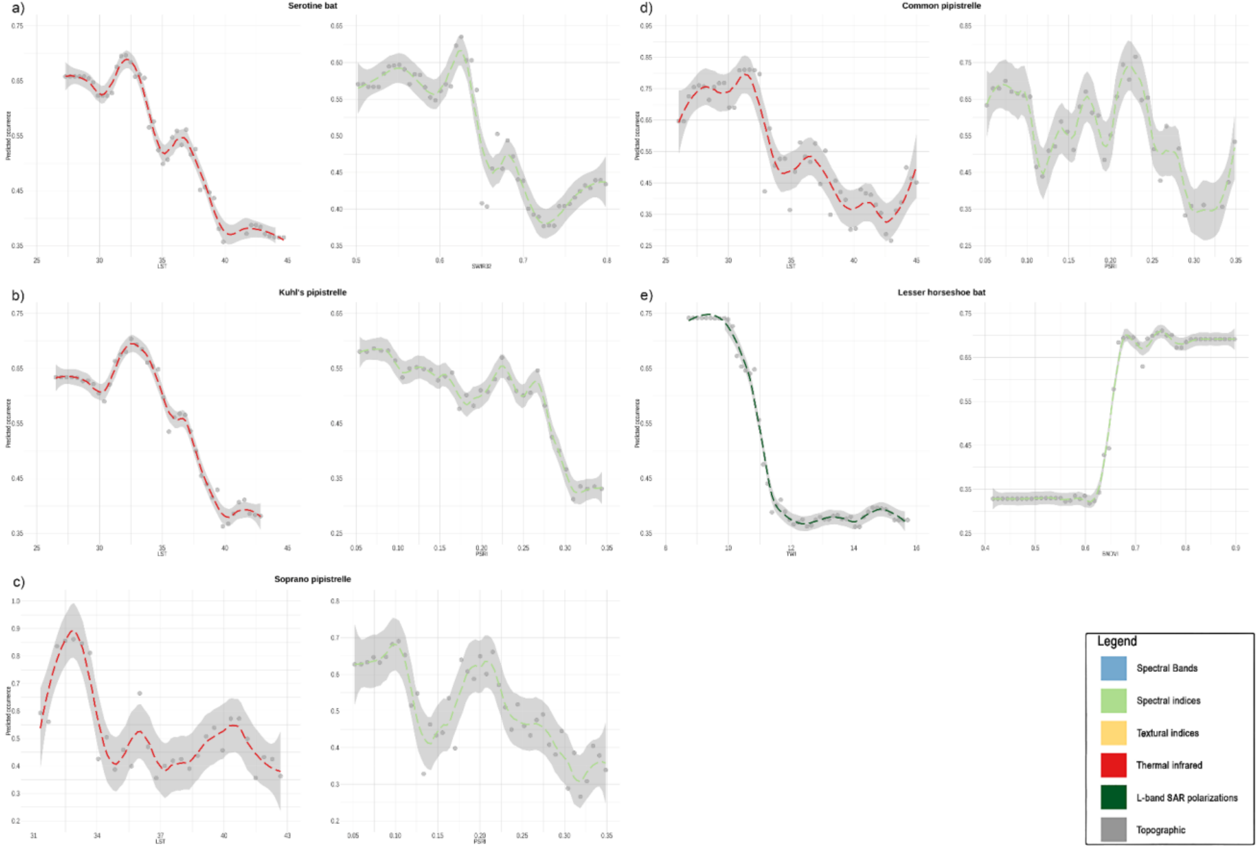
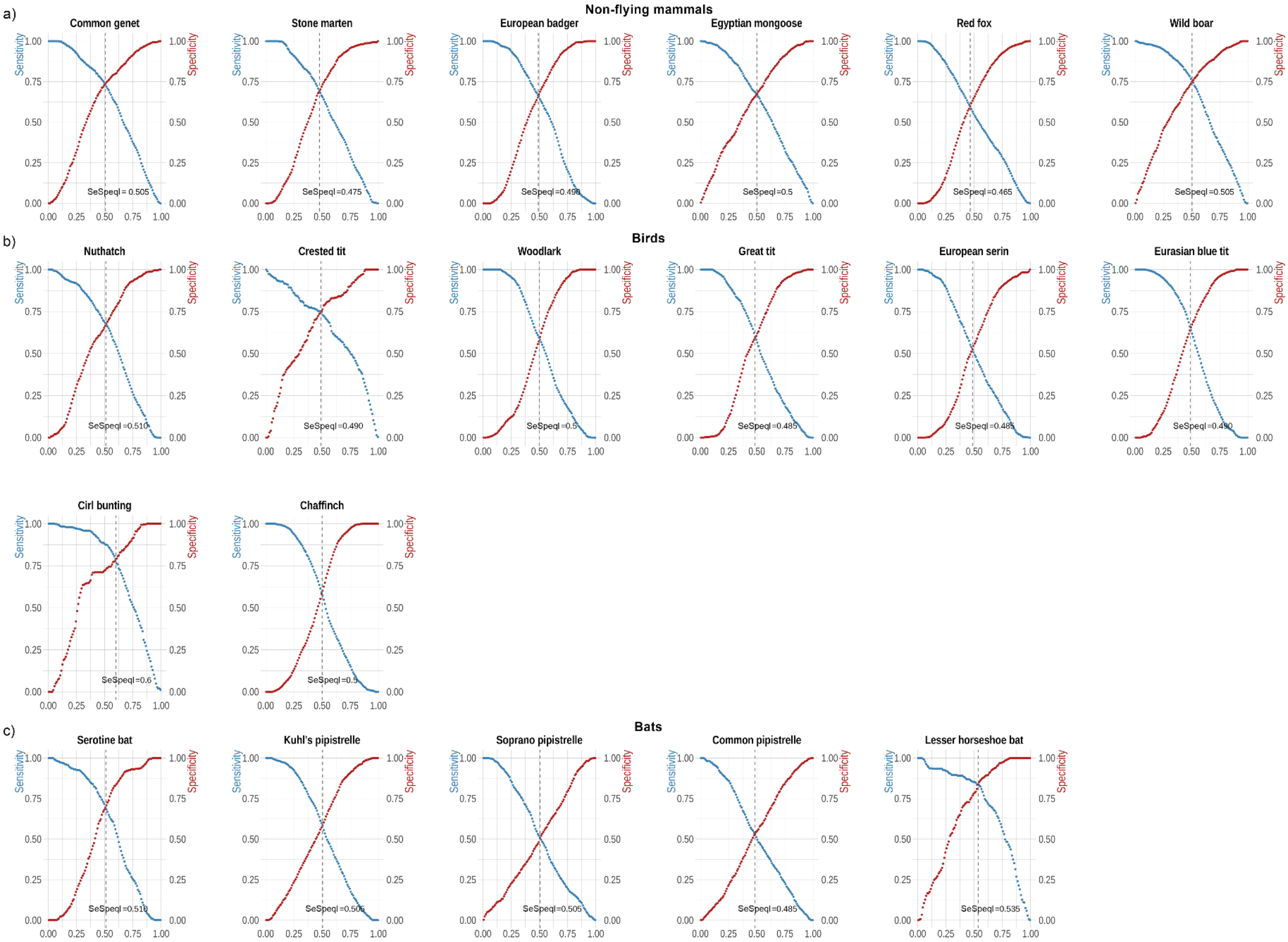


Figure S2.1.9. Vertical dashed line representing the optimal threshold equalizing sensitivity and specificity (from SDMs), for non-flying mammal (a), bird (b) and bat (c) species.



2.2. Roadkill risk models (RRMs) results.

The Boruta feature selection, confirming that retained habitat metrics from SDMs were also significant for RRMs, hence in explaining mortality (Figure S2.2.1, Figure S2.2.2, Figure S2.2.3). For each species, correlation results are showed for predictors, retaining those with the most important scale (Table S2.2.1., Figure S2.2.4.). From RRMs multivariate analysis, accuracy results (AUC, sensitivity, specificity and percentage error rate) are highlighted (Table S2.2.2), along with ecological response curves for connectivity, habitat, and road most important predictors (Figure S2.2.5- S2.1.12). Threshold values for converting RRMs into binary maps were also identified (Figure S2.2.13).

Figure S2.2.1. Connectivity and habitat predictors explaining species-specific roadkill risk (RRMs) from the Boruta selection. Boxplots of relative importance of multi-scale predictors are depicted with the interquartile range (box), minimum, maximum, median and outliers, for non-flying mammal species (common genet, stone marten, European badger, Egyptian mongoose, red fox and wild boar), while comparing habitat metrics with significant (green) against irrelevant (red) contribution. The "*sc*" suffix is relative to the selected scale (0, 100, 500), with "*Ct*" indicating a species-specific circuit-theory connectivity scenario, while "*log*" corresponding to the degree of transformation function (0.25, 2, 8) applied to resistance surfaces.

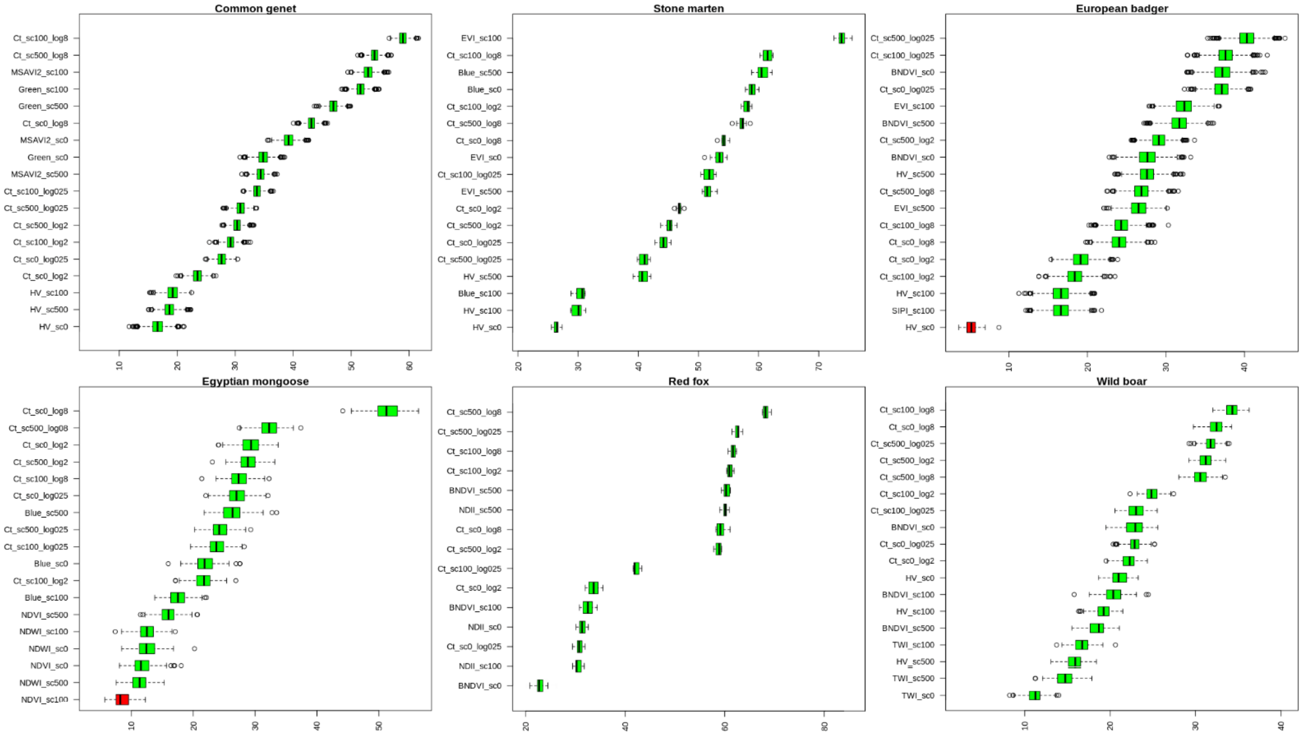


Figure S2.2.2. Connectivity and habitat predictors explaining species-specific roadkill risk (RRMs) from the Boruta selection. Boxplots of relative importance of multi-scale predictors are depicted with the interquartile range (box), minimum, maximum, median and outliers, for each bird species (nuthatch, crested tit, woodlark, great tit, European serin, Eurasian blue tit and cirl bunting chaffinch), while comparing habitat metrics with significant (green) against irrelevant (red) contribution. The "*sc*" suffix is relative to the selected scale (0, 100, 500), with "*Ct*" indicating a species-specific circuit-theory connectivity scenario, while "*log*" corresponding to the degree of transformation function (0.25, 2, 8) applied to resistance surfaces.

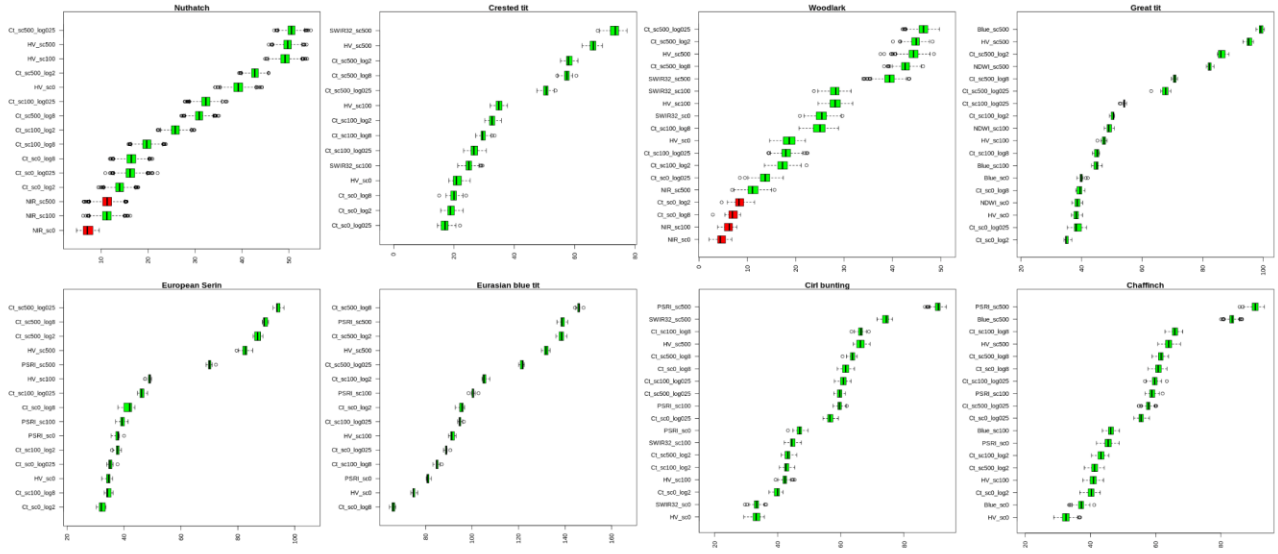


Figure S2.2.3. Connectivity and habitat predictors explaining species-specific roadkill risk (RRMs) from the Boruta selection. Boxplots of relative importance of multi-scale predictors are depicted with the interquartile range (box), minimum, maximum, median and outliers, for each bat species (lesser horseshoe bat, serotine bat, common pipistrelle, Kuhls' pipistrelle and soprano pipistrelle), while comparing habitat metrics with significant (green) against irrelevant (red) contribution. The "*sc*" suffix is relative to the selected scale (0, 100, 500), with "*Ct*" indicating a species-specific circuit-theory connectivity scenario, while "*log*" corresponding to the degree of transformation function (0.25, 2, 8) applied to resistance surfaces.

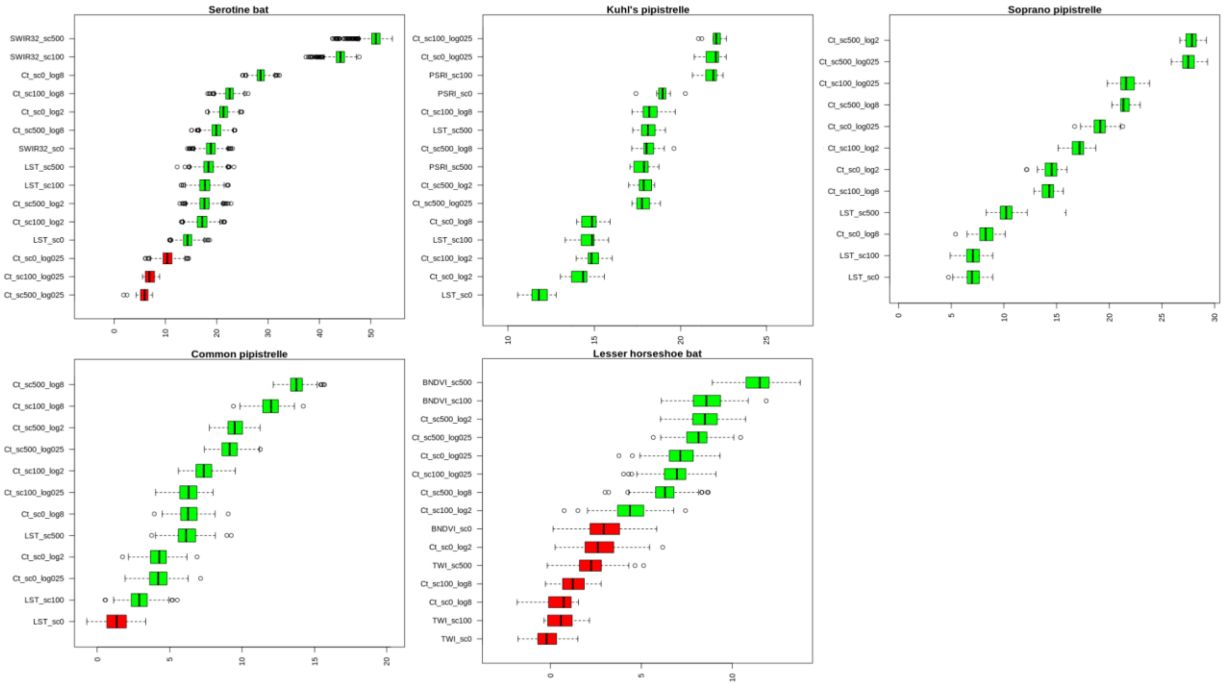


Table S2.2.1. Variance inflation factor (VIF) results for the selected predictors prior to RRMs multivariate analysis.

Species	Blue	NIR	NDVI	EVI	MSAVI2	NDII	NDWI	PSRI	BNDVI	SWIR32	LST	HV	Connectivity	Road_traffic	Road_width	Density_culverts	Euc_Dist_Culv
Common genet					2.56							1.84	1.79	1.21	1.1	1.41	1.45
Stone marten	2.2			2.29								1.82	2.02	1.05	1.02	1.13	1.17
European badger	2.16			1.87								1.74	1.69	1.21	1.17	1.27	1.35
Egyptian mongoose	3.04		3.84				1.74						1.09	1.05	1.03	1.05	1.09
Red fox						2.9			3.37				1.66	1.15	1.02	1.08	1.06
Wild boar						1.68			1.7				1.61	1.16	1.12	1.09	1.12
Nuthatch		1.14										3.12	3	1.28	1.07	1.15	1.17

1.12	1.11	1.08	1.09	1.16	1.18	1.14	1.18	1.17	1.07	1.06	1.59
1.16	1.08	1.11	1.07	1.12	1.06	1.09	1.23	1.17	1.08	1.05	1.06
1.05	1.07	1.01	1.01	1.01	1.1	1.05	1.16	1.17	1.09	1.04	1.04
1.1	1.12	1.15	1.09	1.14	1.33	1.08	1.63	1.08	1.07	1.09	2
4.31	1.65	3.3	4.2	3.45	2.75	1.13	2.36	1.36	1.17	2.97	2.8
3.87	1.97		3.7	4.38	2.3	1.78					
							3.33	2.03	1.18	2.95	
1.5	1.52				1.62		3.06				
			2.5					1.73			3.03
		2.64									
		2.91				1.73					
Crested tit	Woodlark	Great tit	European serin	Eurasian blue tit	Cirl bunting	Chaffinch	Serotine bat	Kuhl's pipistrelle	Soprano pipistrelle	Common pipistrelle	Lesser horseshoe bat

Figure S2.2.4. Upper-triangular correlation matrices of habitat metrics prior to RRMs multivariate analysis, for non-flying mammal (a), bird (b) and bat (c) species. Pearson rank correlation values are depicted for each species, together with colours indicating the degree of correlation.

Table S2.2.2. RRMs accuracy results with mean and standard deviation (SD) relatively the area under the receiver operating characteristic curve (AUC), sensitivity, specificity and error rate percentage, for each species.

	AUC		Sensitivity		Specificity		Error rate (%)
Species	Mean	SD	Mean	SD	Mean	SD	
Common genet	0.88	0.07	0.81	0.12	0.81	0.12	18.61
Stone marten	0.88	0.04	0.79	0.08	0.82	0.08	19.24
European badger	0.81	0.09	0.72	0.12	0.74	0.14	26.69
Egyptian mongoose	0.74	0.07	0.67	0.1	0.71	0.1	30.6
Red fox	0.82	0.03	0.73	0.06	0.77	0.06	24.47
Wild boar	0.86	0.09	0.81	0.14	0.72	0.18	23.28
Nuthatch	0.82	0.09	0.7	0.15	0.77	0.12	23.93
Crested tit	0.89	0.06	0.84	0.1	0.79	0.13	17.77
Woodlark	0.8	0.08	0.66	0.13	0.8	0.12	26.5
Great tit	0.85	0.03	0.73	0.06	0.8	0.05	23.03
European serin	0.82	0.03	0.73	0.05	0.76	0.05	24.96
Eurasian blue tit	0.88	0.07	0.81	0.11	0.81	0.12	18.61
Cirl bunting	0.89	0.07	0.83	0.12	0.85	0.11	15.72
Chaffinch	0.87	0.03	0.77	0.06	0.82	0.05	20.05
Serotine bat	0.88	0.07	0.81	0.11	0.81	0.12	18.61
Kuhl's pipistrelle	0.81	0.04	0.7	0.07	0.78	0.06	25.79
Soprano pipistrelle	0.88	0.04	0.81	0.08	0.77	0.09	28.94
Common pipistrelle	0.79	0.07	0.68	0.11	0.73	0.12	20.59
Lesser horseshoe bat	0.89	0.18	0.87	0.22	0.82	0.26	15

Figure S2.2.5. Response curves of most important predictors influencing roadkill probability (RRMs) for non-flying mammals, namely common genet (a), stone marten (b), European badger (c). The result from the averaged models is depicted by dashed lines, whose colours reflect the groups on which each predictor belong. The grey area corresponds to the standard error.

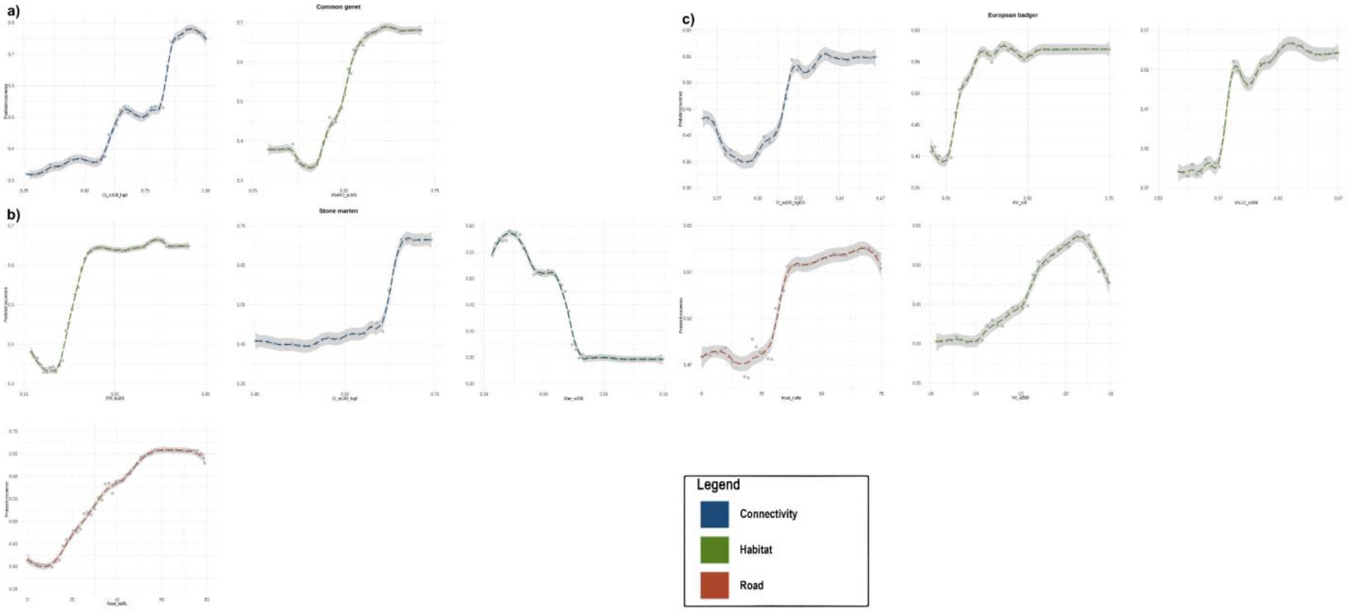


Figure S2.2.6. Response curves of most important predictors influencing roadkill probability (RRMs) for non-flying mammals, namely Egyptian mongoose (d) and red fox (e). The result from the averaged models is depicted by dashed lines, whose colours reflect the groups on which each predictor belong. The grey area corresponds to the standard error.

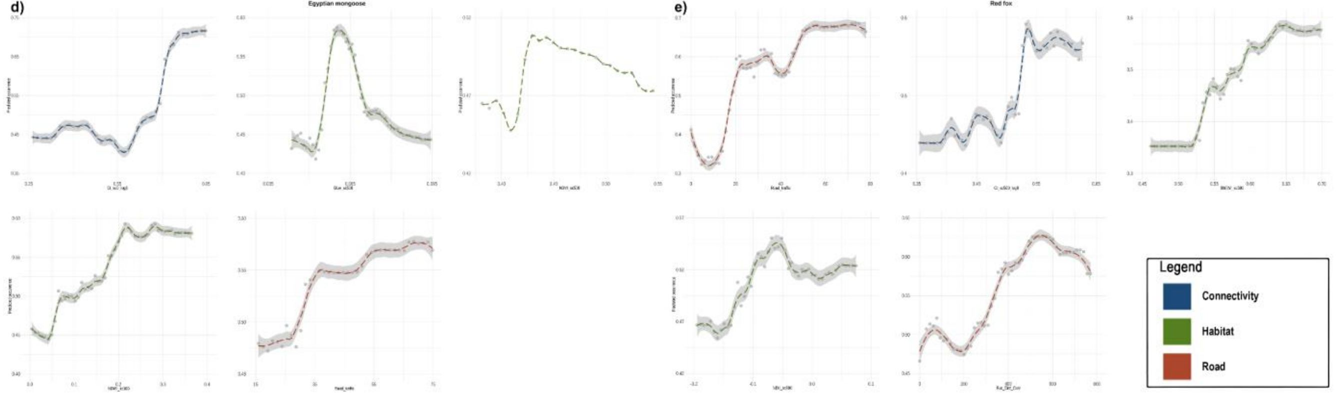


Figure S2.2.7. Response curves of most important predictors influencing roadkill probability (RRMs) for non-flying mammals, namely wild boar (f). The result from the averaged models is depicted by dashed lines, whose colours reflect the groups on which each predictor belong. The grey area corresponds to the standard error.

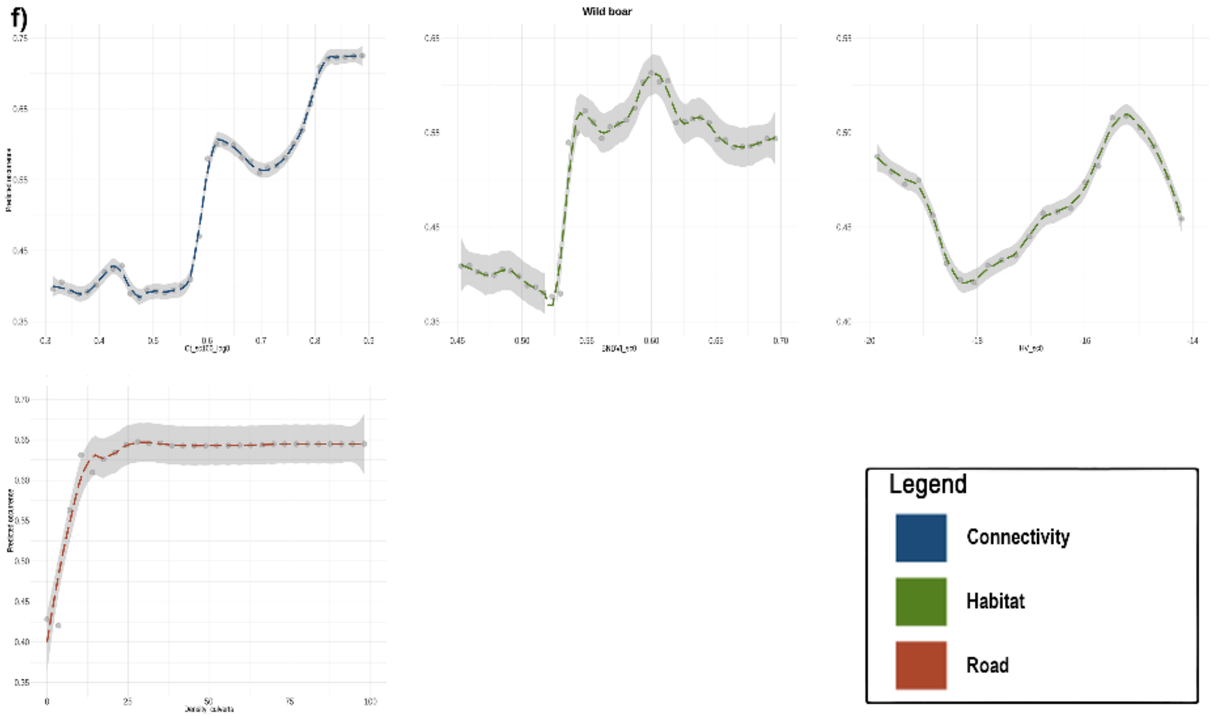


Figure S2.2.8. Response curves of most important predictors influencing roadkill probability (RRMs) for birds, namely nuthatch (a), crested tit (b) and woodlark (c). The result from the averaged models is depicted by dashed lines, whose colours reflect the groups on which each predictor belong. The grey area corresponds to the standard error.

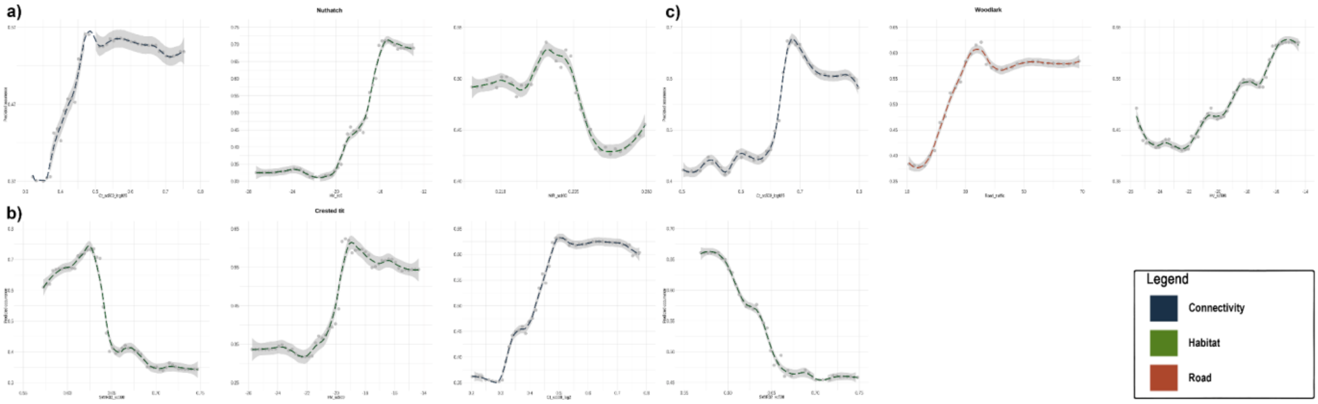


Figure S2.2.9. Response curves of most important predictors influencing roadkill probability (RRMs) for birds, namely great tit (d), European serin (e) and Eurasian blue tit (f). The result from the averaged models is depicted by dashed lines, whose colours reflect the groups on which each predictor belong. The grey area corresponds to the standard error.

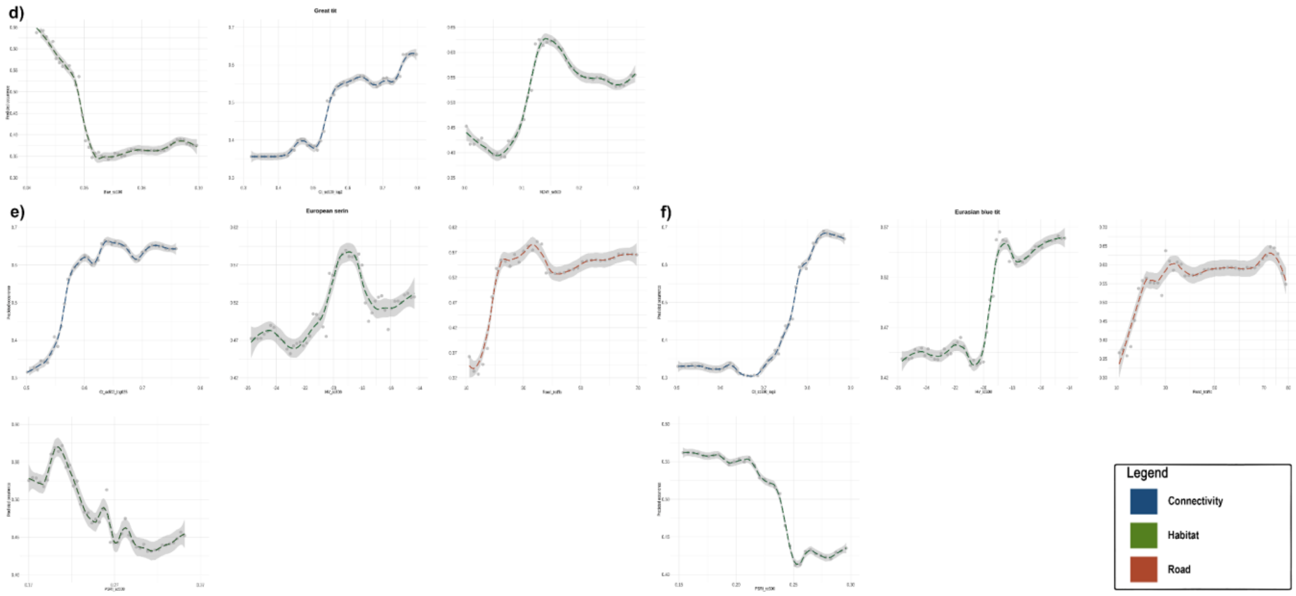


Figure S2.2.10. Response curves of most important predictors influencing roadkill probability (RRMs) for birds, namely cirl bunting (g) and chaffinch (h). The result from the averaged models is depicted by dashed lines, whose colours reflect the groups on which each predictor belong. The grey area corresponds to the standard error.

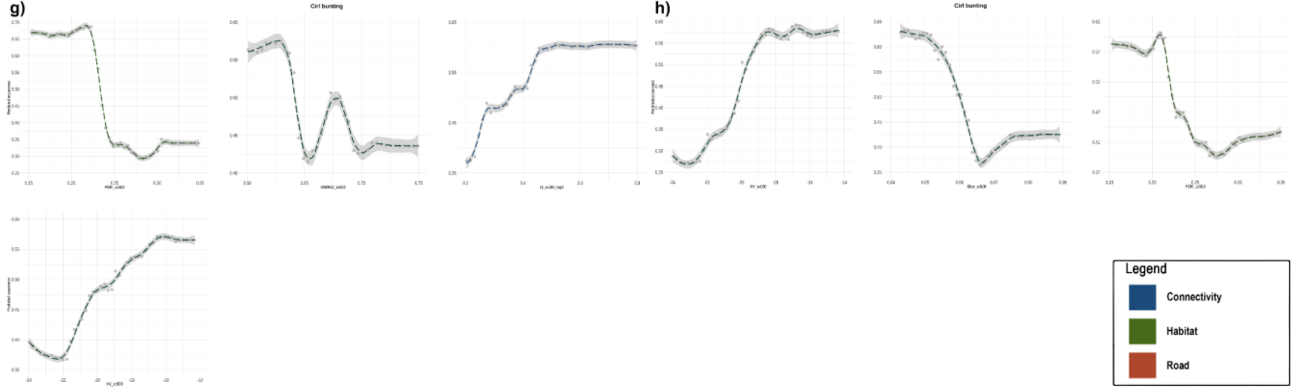


Figure S2.2.11. Response curves of most important predictors influencing roadkill probability (RRMs) for bats, namely lesser horseshoe bat (a), serotine bat (b) and common pipistrelle (c). The result from the averaged models is depicted by dashed lines, whose colours reflect the groups on which each predictor belong. The grey area corresponds to the standard error.

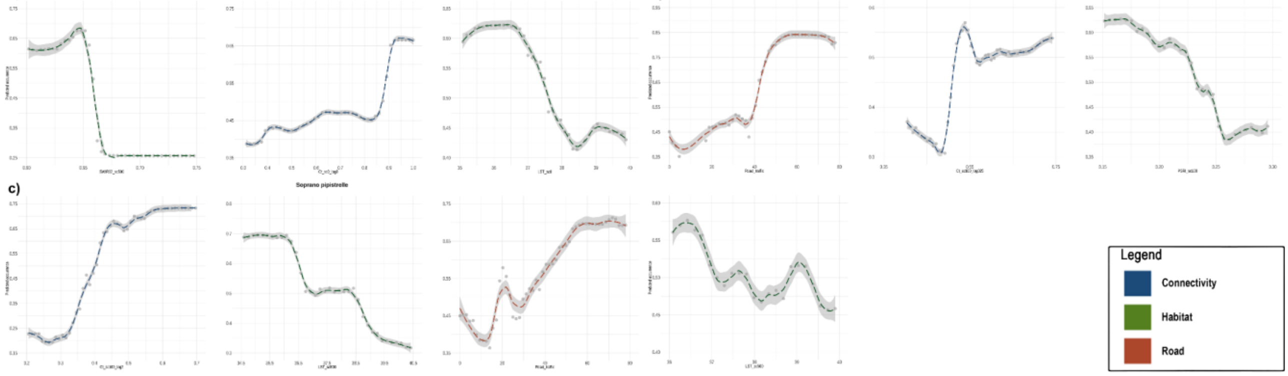


Figure S2.2.12. Response curves of most important predictors influencing roadkill probability (RRMs) for bats, namely Kuhls' pipistrelle (d) and soprano pipistrelle (e). The result from the averaged models is depicted by dashed lines, whose colours reflect the groups on which each predictor belong. The grey area corresponds to the standard error.

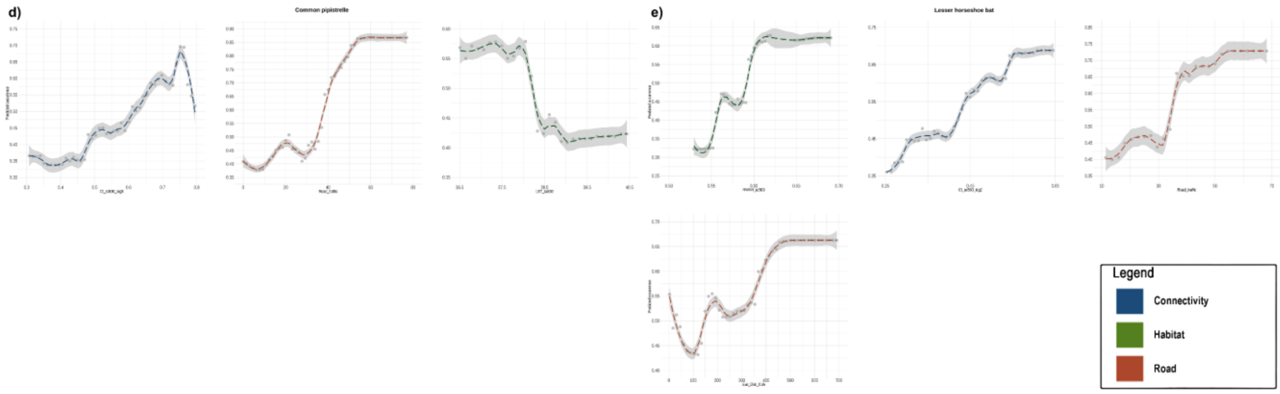
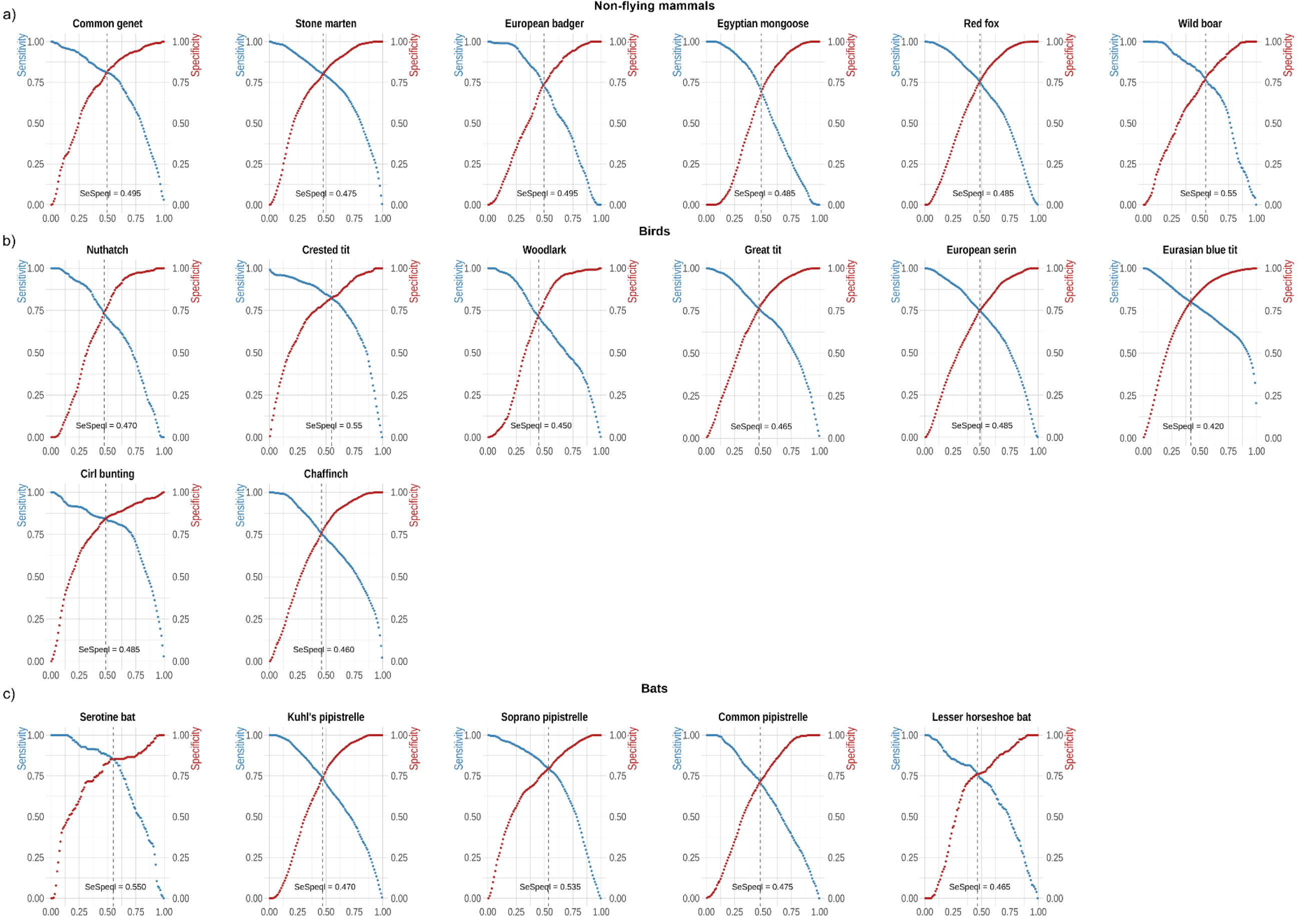


Figure S2.2.6. Vertical dashed line representing the optimal threshold equalizing sensitivity and specificity (from RRMs), for non-flying mammals (a), birds (b) and bats (c).



Chapter 5

Synthesis of the main findings, limitations, and future challenges

The development of human societies, prompted by transportation infrastructures, is well-known to play a major role in the current biodiversity crisis, for which extinction rates are likely expected to accelerate in the near future (Dirzo et al. 2014). To bend this pressure, there is a need for optimising mitigation actions based on planning and resource prioritisation procedures, dealing with limited financial resources (Dalerum, 2014). Such problematics has raised some questions. First of all, how mitigations structures for wildlife can be best distributed along roads, and what kind of movements at those locations could potentially be facilitated. For most of wildlife species exposed to road mortality pressures, these questions are still unanswered or ignored today, with planning decisions mostly resolved through expert opinions rather than through tested models (Pressey et al., 2017). A further complicating factor that exacerbates uncertainties stems from the uneven distribution of wildlife across landscapes, making it challenging their integration into management plans (Polak et al., 2019), as well the consideration of different types of movements (Vasudev et al., 2015). Moreover, it is known that the expansion of roads is directly responsible for landscape fragmentation, as well as facilitating human access and development of many areas, triggering additional resource exploitation, and often intensive practices altering landscapes (Laurance et al., 2014). While representing a secondary aspect, when pronounced spatial alterations occurs, it is necessary to secure the efficiency of conservation plans with respect to landscape dynamism over large periods (Zeller et al., 2020), and to take these effects into consideration for the entire landscape (Clevenger & Huijser, 2011). Besides, landscape dynamics may also naturally occur over the shorter term, for instance in Mediterranean systems where some habitats, namely grasslands associated to *montado*, may be markedly affected in their conditions by cyclical fluctuations associated with different seasons (Mazzoleni et al., 2004). This is of conservation interests where grasslands are relatively undisturbed, such as along linear habitat elements, and in particular roads verges in human-dominated landscapes, for which remote sensing may constitute a resource for detecting wildlife habitats that change spatiotemporally at a fine scale.

The synthesis presented here emphasises the main findings, conclusions, and limitations, useful for optimising the best allocation of resources for strategic mitigation plans on existing roads, (chapters 2 and 4), whereas for systematic conservation planning targeting identification and management plans for biodiversity refuges occurring in areas surrounding roads, (chapter 3). Secondly, the chapter highlights further insights linking all the objectives, along with future research recommendations in road ecology about spatial prioritisation considering some unresolved gaps.

5.1 Performance of two different data type models, and different life history stages in functional connectivity assessment (Research Goal I)

For connectivity assessments, it is fundamental to rely on accurate modeling frameworks, which in turn have their foundation on two data types: animal movements and species occurrence. Gaps are intimately associated to occurrences when those are selected for species-based functional connectivity modeling, because real movements are not really measured (Zeller et. al., 2012), and thereby independent validations, are required (e.g., with roadkills; Laliberté et al., 2020). The results reported in this chapter point to the hypotheses that occurrence data type models have the same potential in their prediction as those from movement for the common genet since occurrence data was as good as movement data for producing accurate predictions of roadkill patterns. In chapter 2 (objective 1), it was discussed the possibility of similarity between resistance surfaces produced by those different approaches, outlining that this condition may not always be expected, but rather be context-dependent. Two major conditions were advanced for explaining this similarity, respectively based on (1) the ecological scale analysed, and (2) the accessibility of habitat resources, meaning how movement decisions varied across sampled individuals within their habitats. The former condition was justified on the basis of what was found by Ziółkowska et al. (2016), namely that movement patterns underlining movement data type models may not diverge from occurrence when using scales within home range. To justify this condition in the road ecology context, findings were compared to studies examining road-crossing events for mesocarnivores, for which best ecological responses were found when applying scales within the species home range (Červinka et al., 2015). The second explanation for the similarity between resistance surfaces is based on the fact that when movement rules are homogenous, and thus resources selection, simpler models such as logistic regressions (e.g., SDMs) may be as capable as sophisticated models in predicting movement patterns (Duchesne & Courbin, 2010). Concisely, as supported in chapter 2, such a similarity in predicted connectivity patterns had likely been owed by a higher constancy in habitat resource selection over the examined species biological cycle, even though this argument does not exclude landscape alterations. This studied aspect have paramount relevance in mitigation planning, as lesser demanding data may be utilised for the strategical mitigation planning phase.

Secondly, following the postulate that "animals move in the same way as they select habitat" is a controversial issue (Zeller et al., 2021), it was investigated whether roadkill prediction performances between PathSFs and SDMs differed with respect to dispersal movements (objective 2). More specifically, besides comparison between PathSFs and SDMs resistance surfaces in predicting roadkill with respect to daily movements, it was also investigated how roadkill performances varied when dispersal was simulated. The relation between roadkill and landscape elements have been amply demonstrated by numerous studies (Gunson et al., 2011), but roadkill locations have rarely been associated with the type of movement. In chapter 2, for the first time it is disentangled at the landscape level the problematic of wildlife roadkill empirically associated to different movement types. Here, it is shown that no statistical difference in predicting roadkill emerged from dispersal movements when compared to daily movements within home range, which may provide further support to reduce spatial uncertainty and to ensure greater congruence on the decisional implementation of road mitigation measures.

The overall finding in chapter 2 is that real connectivity cannot be always measured in a world of limited resources, but can be inferred though the evaluation of potential connectivity which is a useful for robust EIAs (*sensu* Karlson et al., 2014), thereby increasing the cost-efficiency of road planning strategies. The second most important finding is that conservation planners can also target both resident and disperser individuals, which is also important for enhancing the effectiveness of mitigation measures, as those may cover different movements associated to different ecological processes (Tulloch et al., 2016). However, it is worth highlighting that although no divergences have been found in roadkill prediction between daily movements and dispersal within habitats, the study only focused on resistance surface performances ('*continuous*' models; Brudvig et al., 2017), thus purely on functional connectivity without integrating the structural component ('*patch-matrix*' models; Brudvig et al., 2017), and in this sense, dispersal mortality may additionally occur outside habitats in the matrix, at the road interface of communicating networks of populations (Diniz et. al., 2020; Vasudev et al., 2015). This constituted a limitation in chapter 2, which pursued for the need to contextualise site mitigation measures (road units) into a larger ecological network, considering also that Clevenger & Huijser (2011) advocate this as a key principle to increase the effectiveness of mitigation measures. This gap was subsequently accounted in chapter 4, by integrating structural within functional connectivity, to analyse mortality risk. In chapter 2, it was also recommended that the presented approach may be replicated for optimising mitigation measures targeting other mammal species with similar ecological requirements and dispersal abilities, highlighting the possibility of defining similar connectivity paths across species, an aspect subsequently examined in chapter 4.

Highlights of Research Goal I

- The limitations of SDMs are tested compared to PathSFs in predicting roadkills and with respect to different movement types.
- No statistical divergence was found between data type models in predicting roadkill, thereby supporting the reliability of SDMs.
- No statistical divergence also emerged when simulating dispersal movements compared to daily movements, with dispersal mortality having no significant influence on SDMs performances.
- The main implication of this chapter is that SDMs are a costly-effective and simpler solution for functional connectivity assessments, hence for guiding the placement of mitigation measures within woodlands, facilitating different movements and ecological processes.

5.2 *Usefulness of EOS for identifying suitable microhabitats for small species of conservation concern (Research Goal II)*

After having analysed the negative impact of roads, an original contribution to road ecology was to identify linear habitat elements along roads, and how the identification of such microhabitats is influenced by intra-annual variability in landscape conditions. Summarising, in chapter 3 an advanced remote sensing-based methodological approach was conceived to highlight the spatiotemporal attributes of remotely sensed metrics representing different habitat conditions, in order to identify at best some particular habitats, such as open grasslands pertaining to the *montado* system. There is little work in this sense regarding the identification of microhabitats in highly dynamic landscapes, for which natural resources variation may be considered a consequence of dynamism within the *montado* system (Mazzoleni et al., 2004), even though management practices, especially those intensive, constitute a primary factor of changes in the *montado* patterns, often impairing the functioning of the associated ecosystems (Godinho, 2015). This has often caused to linear habitat elements constitute the sole refuge in human-dominated landscapes with increasing intensive practice, which directed towards the purpose of this research, regarding the beneficial effect of linear habitat elements, including road verges, in protecting wildlife species (Pita et al., 2006; Santos et al., 2006). The poor spatial and temporal precision of traditional information on the *montado* land cover characteristics limits the spatial identification of very specific habitat elements, as well as temporal identification associated intra-annual conditions. On this issue, this work constitutes a novelty in the field of both remote sensing and road ecology, as it used advanced remotely sensed spatial information on open grasslands, which helped to unveil habitat elements, including road verges, of valuable status for the delineation of protected areas as microreserves. A more detailed spatial information of the *montado* system is therefore pivotal for understanding its spatiotemporal patterns. Our findings supported that outcome for the objective 3, as highly suitable pattern of distribution at high precision and over large extensions were identified for an endangered species, the Cabrera vole (*Microtus cabrerae*), along patchy areas and linear habitat elements. Moreover, a super-resolution technique was applied on a highly detailed spatiotemporal data easily available, notably from Sentinel-2 sensor, for which there was no example of application in conservation biology overall, and more specifically for identifying habitats surrounding road verges, which may benefit a plethora of species, and may offer protection for some vulnerable (Santos et al., 2006). Sentinel-2 possesses an unprecedent accuracy for a sensor with this "swath" characteristic and at no cost, and in this sense, more precise sensors exist, such as those hyperspectral, though they have a little "swath" and are usually mounted on UAV, demanding highly qualified technicians and highly costly devices (Emilien et al., 2021). Regarding EOS, higher detailed sensors also exist such as panchromatic, with a superior resolution, but they are prohibitively expensive (Kpalma et al., 2014; Zhu et al., 2018), and therefore Sentinel-2 seems an optimal costly-effective compromise according to present findings and conservation goal.

The findings presented in chapter 3 constitute an unprecedent and pioneering outcome in microreserve mapping, as most of studies are used to discover microreserves exclusively through field work. In those areas, microhabitat quality was estimated between periods pertaining the dry and wet season, and the findings pointed out that Cabrera vole distribution was highly determined by conditions during wet periods for which habitat quality was higher (Pita et al., 2006; Pita et al., 2007; Santos et al., 2006), even though favourability, with a lesser contribute, was also determined in dry period, highlighting some microhabitat elements favouring the persistence of populations during adverse conditions. The selection of a multi-seasonal Sentinel-2 models integrating spectral bands and phenological vegetation conditions improved significantly the classification power, allowing to accurately describe the most suitable vegetation conditions at the temporal and spatial level. This ameliorated classification may contribute to reduce the cost in optimising the delineation of microreserve areas by minimising oversimplifications from traditional landscape products (e.g., land cover), as well may optimise the cost in monitoring by better guiding the field campaigns to sample populations of Cabrera vole. Traditional products have underpinned the development of SDMs, but may have great limitations to represent some particular habitats (Coops & Wulder, 2019), especially those with a less contiguous distribution (Cerrejón et al., 2021), and for small species (Kerr & Ostrovsky 2003). These outcomes demonstrated that continuous and highly detailed EOS data may offer various advantages, jointly with "data fusion" SDMs techniques, for producing accurate information on *montado* spatial patterns, which allowed to explore these aspects in an even more challenge in chapter 4, by targeting multiple taxa of different body size (from small to mid-sized species). Interestingly, numerous of habitats identified have been found along linear landscape features, which, jointly with some metrics in the wet period inferring vegetation productivity (e.g., NDI45) and heterogeneity (e.g., Rao's Q), highlight the potential role of vegetated road verges in promoting the persistence of Cabrera vole populations (e.g., Santos et al., 2006). The research here developed has great relevance to support systematic conservation planning and management with precise details on strategic landscape elements, particularly along roads, and for species with low detection and localised distribution. In this respect, road corridors (*sensu* Forman & Alexander, 1998) built primarily to facilitate human transport, can be exceptionally exploited as corridors for wildlife and plants through suitable verges, helping to decrease the isolation of habitat patches and facilitate their connectivity (Ascensão et al., 2012; Galantinho et al., 2020; Tikka et al., 2001), as highlighted in chapter 3.

However, it is worth mentioning that this research is based on a two-year sampling, which constitute a limitation of the study, as management strategies should be based on more years of monitoring and more information on the persistence of those populations, to better apply protection measures. A comprehensive understanding on where such microreserves persisted was difficult for the present study since Sentinel-2 is a relatively new sensor that has not yet covered long monitoring periods. Another limitation stands on the problem that supporting habitat for threatened species may also promote the abundance of other more common species, which may be likewise controversial, since they may exacerbate vehicle collisions, in specific with mesocarnivores, but also raptors and snakes, as these may be attracted to road verges due to the increased abundance of prey (D'Amico et al., 2015; Meunier et al., 2000; Silva et al., 2019). In this respect, despite the research in this thesis is capable of understand where suitable road verges may coincide with risky area of predator species, it did not have found a comprehensive solution in the matter of abovementioned side-effect. This requires further efforts and challenges in finding a coexistence between the support of biodiversity along liner habitats, and the mortality preventions.

Highlights of Research Goal II

- The spatiotemporal accuracy of EOS remote sensing data is tested to overcome SDMs limitations in dynamic landscapes, which is of special relevance for small and rare species occupying fragmented habitats.
- Sentinel-2 dynamic habitat metrics overall explained species distribution spatially and temporally, along with static traditional products, notably road proximity.
- Performances indicated a high discrimination capacity and low error rates, with species higher favourability for green and heterogenous areas during the wet season.
- The main finding is that integrating EOS products within SDMs ensure invaluable insights in ecological studies, and for identifying particular habitats (e.g., road verges), hence improving the allocation of resources for wildlife conservation and monitoring.

5.3 Optimising roadkill risk models towards a multi-taxa mitigation planning (Research Goal III)

The spatial and temporal extent to which roads negatively affect multiple taxa, in particular mammal and bird populations at the landscape level represent a research priority, given that this knowledge may greatly benefit a better green infrastructure design and more effective mitigation planning (Benítez-López et al., 2010; Jaeger, 2015). The chapter 4 forms the cornerstone of the thesis in this regard, by integrating road ecology, remote sensing and landscape connectivity science within EIA-based modeling tools, developed to represent an essential guidance for increasing mitigation efficiency during the strategic planning phase (*sensu* Rytwinski et al., 2015). This methodology integrated connectivity through multifunctional corridors, which is generalisable for other contexts, hence having the potential to be replicated by conservation planners and practitioners in a costly-efficient way through occurrence data type models (SDMs), still, some cautions should be maintained. In fact, given the findings in chapter 2, we did not taken occurrences as an absolute preferred choice in chapter 4, because an inter-individual heterogeneity in habitat resource selection still could have constituted a potential bias (Duchesne & Courbin, 2010), possibly leading to poor functional connectivity proxies for roadkill predictions, thereby undermining mitigation measures efficiency. More specifically, taking into account the context-dependent conditions of similarity between different data types in predicting mortality, described in chapter 2, we respected such conditions in the following objectives in chapter 4. This involved filtering out scales larger than home range, as well as mobile species most likely prone to show heterogeneity in selecting habitat resources within their biological cycle. Basically, the latter consisted in filtering out species such as apex predators (Dupke et al., 2017) and large herbivores (Duchesne & Courbin, 2010), along with non-resident species, for obvious reasons (e.g. migratory-induced changes in habitat selection). For resident species, the phenological trait was also considered to exclude species presenting a pronounced divergence in habitat selection between hibernation and reproduction sites (e.g., bats, Smeraldo et al., 2018).

Given the support of occurrence data (from the findings in chapter 2), and the support of costly-effective remote sensing data (from the findings in chapter 3) analysed with a free-of-charge cloud computing platform (Google Earth Engine; Gorelick et al., 2017), it was constituted an even-more financially convenient and replicable framework baseline, which facilitated the realisation of the 3 proposed goals in chapter 4. In fact, the long-term monitoring from Landsat data taken landscape alterations into account, which was relevant in the context of the *montado* system here addressed, due to its degradation trend over the last decades imposed by two contrasting processes, land abandonment and land use intensification (e.g. increase in livestock density) (Machado et al., 2020; Godinho et al., 2014). Insofar, landscape dynamism had scarce attention in road ecology (Oddone Aquino, 2021), and in addressing landscape dynamics concurrently with road mortality, it was also highlighted on the necessity of include multiple stressors to reduce the biodiversity loss, as those may act iteratively (Simmons et al., 2021). By these means, the relevant scale of impacts was considered, and road planning units were contextualised within the entire landscape of study area (southern Portugal), which encompassed multifunctional corridors and resilient to inter-annual spatiotemporal variation, both representing key principles for increasing the efficiency of mitigation measures (Clevenger & Huijser, 2011). While this has little research attention, minimising impacts over large temporal scales is considered to benefit wildlife populations across multiple generations, possibly allowing for improvements in demographic exchange and genetic flow (Cosgrove et al., 2018). The EOS data in chapter 4 involved a long monitoring period, in relation to which the availability of remote sensing data was secured by selecting Landsat TM, ETM+ and OLI sensors. This allowed the representation, at the landscape level, of *montado* environmental attributes and relative variations over long periods and at fine-scales, given the optimal compromise between spatiotemporal resolution, the "swath" and temporal coverage of these sensors. Objective 4 addressed in chapter 4, in fact, demonstrated that EOS data are able to accurately describe prime habitat characteristics of the wildlife taxonomic groups addressed in the study, namely non-flying mammals, birds and bats. Several habitat metrics were developed, and each metric was grouped on the basis of biophysical and biochemical characteristic. Findings indicated that species within the same taxon responded preferentially to a particular group of metrics (optical sensor information with spectral and thermal infrared indices, respectively for non-flying mammals and bats; whereas radar sensor information with L-band SAR polarisation indices for birds), which supports the usefulness of continuous and detailed EOS-derived predictors (He et al., 2015; Pettorelli et al., 2005). Indeed, as summarised in chapter 4, the major limitation of categorical products (e.g., CORINE Land Cover maps) resides in their inability to represent particular habitat requirements, which may be critical when considering multiple species. More specifically, Cushman et al., (2010) on this issue casted doubts about the ability of categorical product in the multispecies context by questioning: (1) "if biological communities are multivariate gradients of species composition, with each species responding individualistically to particular combinations of limiting factors, is a categorical patch-based representation appropriate?" and (2) "isn't representing biological communities as categorical patches in a mosaic a de facto ratification of a Clementsian model of community composition at the landscape level?" (for further details see Cushman et al., 2010). The results of the species ecological responses in objective 4, are in agreement with these doubts, as various degrees of environmental tolerance emerged in habitat requirements from the employed habitat metrics, reflecting various degrees of habitat specialisation. This means that remote sensing information was able to accurately capture those multivariate gradients, resulting into a novel ecological aspect in this research area and a relevant step for further analyses. In fact, what may emerge is that the utilisation of a categorical product has the risk of misrepresentation of multivariate gradients, therefore in capturing ecological differentiations between habitat specialist and generalist species, leading to serious consequences across the addressed goals, namely to a possible oversimplification and overestimation in EIAs prediction mortality risk (objective 5), hence in an inadequate spatial prioritisation capacity for mitigation plans (objective 6). Remote sensing in this sense was capable of overcoming these limitations, guaranteeing the possibility to represent unique habitat characteristics across species. Habitat representation, jointly with resistance surfaces, was based on the previously described "data fusion" through a pixel-based procedure which considered species-specific significant landscape attributes and finalised through SDMs (Schulte to Bühne & Pettorelli 2018). The findings in chapter 4 overall indicated an efficient discrimination capacity through different accuracy metrics, supporting the use of freely available cost remote sensing data. It needs to further be emphasised that the thresholding procedure to discriminate habitat from matrix (non-habitat) in resistance surfaces could easily result in a lack of accurate information, as multiple species-specific thresholding procedure is preferable to the generalisation of a single one across species (Moilanen, 2011). Even if it represented secondary aspects in chapter 4, it pursued the necessity for conceptual models of species-specific structural connectivity integrated within species-specific functional connectivity, in the form of "hybrid models" ('*patch-matrix*' and '*continuous*'; for further details see Brudvig et al., 2017), which have been demonstrated of increasing the robustness of connectivity assessments (Brudvig et al., 2017), and overcome theoretical limitations, as briefly anticipated in chapter 2.

Consequently, a main relevance in chapter 4 resides in constructing multispecies corridors from species overlay rather than considering connectivity as a general surrogate, meaning that consideration was given to how the relationship between functional connectivity and road-related mortality varied for each species. As such, this included disentangling species-specific habitat, functional connectivity, and road metrics to gain comprehensive insight into independent effects on the influence of roadkill vulnerability for each species, as aimed for the objective 5 in chapter 4. Because functional connectivity and habitat suitability significantly explained roadkill patterns across species, findings emphasised the importance of disentangling these effects as different ecological processes. In fact, studies in this sense supported to find the most suitable explaining factor that may diverge between habitat and connectivity to increase the efficiency of mitigation measures (Fabrizio et al., 2019), and our findings agreed with this argument. We found in objective 5 that connectivity was a predominant mortality trigger for non-flying mammals, but that habitat was equally important in explaining mortality across bird and bat species, which highlight the necessity of considering those two factors when explaining mortality, which may be useful to lower biases and minimise errors. Results for this objective indicated an excellent discrimination capacity in predicting road-related mortality from the employed accuracy metrics, increasing the robustness of spatial prediction for implementing mitigation structures. Overall, the most important finding here is that an ameliorated efficiency of mitigation plans may rely in structures intercepting both connected habitat woodlands and the neighbouring non-habitat matrix (open areas), corroborating previous research (e.g., Malo et al., 2004). These findings should receive more attention, especially when considering that research in road-related mortality has been predominantly focused on habitats (Gunson et al., 2011; Oddone Aquino, 2021; Pagany, 2020).

Then, research to finalise objective 6 was conducted through previous objectives in chapter 4 to represent the multispecies cumulative mortality risk on road planning units, supporting the implementation of site-specific structures, such as crossing structures and/or fences, for each taxon and for all taxa. Our results here clearly reported a much higher focus in prioritisation, toward road planning units representing high risk of mortality for the maximum number of species attainable. This resulted into a more convenient resource allocation and an ameliorated mitigation planning efficiency. Indeed, a possible optimisation of mitigation structures on the basis of present findings, is that site-specific structures such as underpasses (e.g., culverts), can be adapted to multi-use for species with different degree of habitat specialisation, contrary to what previously described in literature (Clevenger & Huijser, 2011). It has also been verified the condition of reducing mortality for multiple taxa, given the congruence in mortality patterns between birds and non-flying mammals, ensuring cost opportunities in planning structures such as fences (Armsworth, 2014), so in optimised multitaxa mitigation plans. Acceptable variability in predicted mortality risk was found within each taxonomic group, ensuring for flexibility in implementing mitigation structures on road sites (or planning units) beyond best sites (highest mortality richness). However, based on the current results relative to objective 6, caution is needed when a significant mismatch between taxa occurs in terms of mortality risk variability and thus flexibility, meaning that in this condition, best road sites should be preferred. This was the case of bat group as predicted mortality variability diverged significantly when compared to other taxa (birds and non-flying mammals). We advanced the hypotheses on such mismatches likely due to greater variability of bats in terms of mobility and home ranges, compared to other taxonomic groups.

From the metrics employed in accuracy and variation across predictions in chapter 4, the findings reveal that this work is applicable, as well as replicable, for producing more detailed and useful information of sites most prone to multispecies mortality risk, and across different taxa. This represents an unprecedented outcome in EIAs towards prioritising road mitigation structures and increasing their effectiveness, that are able to benefit species with different home-ranges, ecological requirements, and dispersal movements. In fact, their integration within mitigation plans is still mostly neglected (Polak et al., 2019), in part because there is likely no way of collecting movement data for a multitude of species and with different sizes (Salgueiro et al., 2021), in part also due to the difficulty of representing the variety of wildlife ecological and movement requirements (Brennan et al., 2020). Such aspects in fact continue to pose a challenge to the scientific community, and as solution, some initiatives analysed multispecies connectivity as '*species-agnostic*' (Koen et al., 2014), or through umbrella species, inferring functional connectivity for one species as a surrogate for a wider range of species (Krosby et al., 2015). Yet, the shortcomings of these studies lie on a tendency in connectivity oversimplification. In this sense, chapter 4 demonstrated that in the absence of known movements, there may still be an ameliorated focus with respect to planning units, by integrating multifunctional corridors through species-based functional connectivity and habitat models that depend on more affordable data. The solutions proposed are not only circumscribed to wildlife, but also relevant for the maintenance of *montado*, as protection of selected species can contribute for the functioning of its ecosystems (e.g., Lourenço et al., 2021), with no cost to human societies, excluding the collateral deriving from wildlife vehicle collisions. The latter aspect is even more relevant when considering some species that are dangerous for human safety on roads (e.g., wild boar), which emphasises even more practical reasons of reducing wildlife road-related mortality, beyond those intrinsically linked to ethic or conservation. As such, the chapter 2, 3 and 4 have evidenced and supported a mental workflow capable of addressing theoretical and practical gaps in EIAs, which should not be ignored (Bennet, 2017). Such combined works demonstrated that spatially explicit models, and in specific EIA-based modeling tools are useful to cut costs, as well as leveraging opportunity costs at the community level, intended for multiple species and taxa (Fletcher et al., 2016), so increasing the efficiency of conservation and mitigation measures for multiple generations (Cosgrove et al. 2018), optimising at best the financial return throughout more targeted and flexible management options.

Nevertheless, a limitation of the developed research in chapter 4 is that prioritisation of road planning units for mitigation is based solely on the '*conservation value*' (*sensu* Margules & Pressey, 2000), which included biodiversity, environmental conditions (e.g., variability) and risk, but excluded the cost associated with each site for planning mitigation structures, which arguably may vary on the basis of type of road, permissions and local site conditions (e.g., topography, soil, etc.). This is an aspect that solicitates further research efforts and could be potentially solved through pixel-based benefit/cost formulations (e.g., Kujala et al., 2018), which are rapidly being employed in road ecology (Polak et al., 2019), though with scarce attempts in real-world context and no attempts when including the gaps addressed in this research. The post-hoc integration of this work into benefit/cost analysis will guarantee, on site-specific road units, an even more straightforward identification of where to place costly-effective structures to reduce mortality risk.

Highlights of Research Goal III

- Findings from both chapter 2 and 3 paved the way for occurrence and remote sensing-based functional connectivity targeting small and mid-sized resident species for mitigation plans.
- SDMs integrating EOS (Landsat) habitat metrics revealed similar ecological requirements for similar species belonging to the same taxonomic group.
- Roadkill was explained by functional connectivity and habitats, highlighting both connected woodlands and surrounding matrix areas for mitigation measures.
- Highest spatial prioritisation for mitigation resources was attained when including the maximum number of species vulnerable to mortality risk.
- Low variability in mortality risk emerged within and across taxonomic groups, allowing for opportunity costs and flexibility during the mitigation planning phase.
- Our findings overall showed that the present inter-disciplinary and costly-effective framework is capable to optimise roadkill mitigation plans, that can be transversal for species with different size, home range, ecological requirements, and mobility.

5.4 *Future prospective*

Site-specific conservation measures can be ameliorated through costly-efficient plans and strategical optimisations by integrating different disciplines, namely remote sensing and connectivity science, as they may help to solve theoretical and practical gaps still overlooked, such as those presented in this thesis about road ecology. The advantages of considering remote sensing information are increasingly attainable, given the open access policies adopted by agencies and space missions, which is even more convenient when considering data sources nowadays readily accessible at high spatial and temporal resolution, from online repositories storing vast amounts of information (e.g., tens of million images only considering Landsat missions; Wulder et al., 2016). Such large repositories of remote sensing information are organised on cloud platforms, allowing a collection, preparation and manipulation of data that can rapidly be finalised (Gorelick et al., 2017). This facilitates applications including remote sensing, which is becoming a paradigm in ecology (Pettorelli et al., 2014a; Pettorelli et al., 2014b; Schulte to Bühne & Pettorelli 2018). With regard to connectivity, faster computations are also actually possible given the recent software technological advances (Hall et al., 2021; Landau et al., 2021; Leonard et al., 2017). Approaches linking wildlife conservation with fine-grain connectivity, and/or remote sensing, are also encouraged (Marrec et al., 2020; Hu et al., 2015; Zeller et al., 2020). Cross-disciplinary empirical applications in conservation biology are leading to the advent of international journals geared toward specific partnerships (Pettorelli et al., 2017), as well as special issues (Jennings et al., 2021). For road ecology, some authors have also been recently encouraged for bridging disciplines to further develop generalisable principles (D'Amico et al., 2018).

The last two decades have been characterised by a rapid development of societies, by a global road expansion acknowledged as the most pronounced in history (Gross, 2016), and recognised as a proper "infrastructure tsunami" incoming (Laurance, 2018), that will require monitoring Earth from space to track impacts on habitats and ecosystems (Scholes et al., 2012). Likewise, since the last two decades, humanity has entered in the information age (Castells, 1996), characterised by an unprecedented level of automatisation, and increasing capacity in generating, organising and processing data (e.g., machine learning for image classification; Keitt & Abelson, 2021), which promoted progresses in conservation biology through interdisciplinary initiatives. These, in fact, are increasingly mandatory for evidence-based prioritisation solutions, for costly-effective efforts and to enhance the guidance for resource allocation towards wildlife protection (Pressey et al., 2017; Rose et al., 2019).

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Publications included in the thesis

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- Valerio, F., Carvalho, F., Barbosa, A. M., Mira, A., & Santos, S. M. (2019). Accounting for connectivity uncertainties in predicting roadkills: a comparative approach between path selection functions and habitat suitability models. *Environmental management*, ***64***(3), 329-343. https://doi.org/10.1007/s00267-019-01191-6

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