**Universidade de Évora - Instituto de Investigação e Formação Avançada**

Programa de Doutoramento em Biologia

Tese de Doutoramento

# Are roads refuges or barriers for small mammals? Implications for road verge management.

Ana Cristina Pereira da Cruz Galantinho

Orientador(es) | António Mira
Russell Alpizar-Jara

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This thesis was prepared as a compilation of three scientific papers published in international peer-reviewed journals registered in the Web of Science (WoS). The three papers were carried out in collaboration with other authors. However, the author of this thesis clarifies that she led and actively participated in all phases of the work for each paper including conception, data collection, data analysis and manuscript writing, editing, and submitting. In the three chapters corresponding to the published papers, numeration of headlines, tables and figures were adjusted according to the sequence presented in this thesis.

The Jury Committee did not suggest any alterations to this thesis.

# Table of contents

To Rodrigo,

Faith, persistence, courage, friendship, and love help us endure and overcome any adversity.

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# Are road refuges or barriers for small mammals? Implications for road verge management

## Abstract

Roads are often considered barriers to animal movement thereby reducing the persistence of species in their vicinity. Nonetheless, road verges may provide refuges or corridors for small mammals across highly human-modified landscapes. In these landscapes, road verges can be the last remnants of suitable habitats. However, in well-preserved habitats, road verges may show a similar vegetation structure to surrounding areas. In these circumstances, the effects of roads, road verges and roadside management on small-mammal conservation are still not clearly understood. These effects are expected to depend on management practices on road surrounding land. This thesis aims to assess the effects of roads on demographic traits, movements patterns and landscape connectivity for small mammals in a well-preserved agro-silvo pastoral system. Populations of wood mice (*Apodemus sylvaticus*) were studied using capture-mark-recapture methods in a road area and in a similar roadless area. Extended Pollock’s robust design models were used to assess several population parameters. Movement patterns were evaluated by step analysis. Graph theory was used to quantify landscape connectivity. Road and roadside management decreased abundance and body condition, hampered road crossings, changed movement direction, and decreased overall landscape connectivity for wood mice. Road verges provided habitat and pathways, promoting functional connectivity for this species in the road area. Nevertheless, road verges’ role depended on the vegetation management on verges and surrounding areas. The recommendations suggested in this work for roadside vegetation management also account for the potential increase in roadkill and fire risk. The outcomes of this thesis emphasize the importance of properly managing vegetation on road verges and surrounding private land for the conservation of small mammals in well-preserved habitats.

**Keywords:**

*Apodemus sylvaticus*; Landscape functional connectivity; Movement patterns; Population parameters; Road verge vegetation management

# As estradas são refúgios ou barreiras para os pequenos mamíferos? Implicações para a gestão das bermas de estrada

## Resumo

As estradas podem ser barreiras ao movimento dos animais reduzindo a persistência das espécies na sua vizinhança. No entanto, as bermas de estrada podem oferecer refúgios ou corredores para pequenos mamíferos em paisagens altamente modificadas. Nestas paisagens, as bermas podem ser os últimos redutos de habitat favorável. Contudo, em habitats bem-preservados, as bermas de estrada podem ter uma estrutura de vegetação semelhante às áreas circundantes. Assim, os efeitos nos pequenos mamíferos das estradas, das bermas e da sua gestão permanecem por clarificar. Estes efeitos dependerão da gestão na área circundante à estrada. Esta tese pretende avaliar os efeitos das estradas nos parâmetros populacionais, padrões de movimento e conectividade da paisagem para pequenos mamíferos num sistema agro-silvo-pastoril bem-preservado. As populações de rato-do-campo (*Apodemus sylvaticus*) foram estudadas através de captura-marcação-recaptura numa área com estrada e numa área semelhante sem estrada. Vários parâmetros populacionais foram avaliados usando modelos de desenho robusto de Pollock. Os padrões de movimento foram analisados com base em etapas de movimento. A teoria dos grafos serviu de base para a quantificação da conectividade da paisagem. A estrada e a gestão das bermas diminuíram a abundância e a condição corporal, dificultaram os atravessamentos da estrada, alteraram a direcção do movimento, e diminuíram a conectividade para o rato-do-campo. As bermas de estrada proporcionaram habitat e caminhos, promovendo a conectividade funcional da área para esta espécie. No entanto, o papel das bermas de estrada dependeu da gestão da vegetação nas bermas e na área circundante. As recomendações sugeridas neste trabalho para a gestão das bermas de estrada têm em consideração os riscos de atropelamento e de incêndio. Os resultados desta tese realçam a importância de uma gestão adequada da vegetação nas bermas de estrada e propriedades privadas circundantes para a conservação de pequenos mamíferos em habitats bem-preservados.

**Palavras-chave:**

*Apodemus sylvaticus*; Conectividade funcional da paisagem; Padrões de movimento; Parâmetros populacionais; Gestão da vegetação das bermas

# Chapter 1

## General Introduction

# 1. General introduction

## 1.1. Consequences of road expansion on wildlife

Roads are crucial to modern human development. These infrastructures facilitate access to food, education, employment, medical support, law enforcement and administrative services (Meijer et al., 2018). Therefore, roads have become essential in the fight against poverty and the promotion of a brighter society. Roads already cross most landscapes, but the economic and social needs of a fast-growing human population demand a rise on their extension, complexity and use around the world (Forman et al., 2003; Torres et al., 2016; Meijer et al., 2018). Thus far, 21 to 36 million km of roads exist worldwide (Ibish et al., 2016; Meijer et al., 2018). Estimates indicate that there will be an additional 3 to 4.7 million km of roads by 2050 (Meijer et al., 2018). Half of Europe's area already is less than 1.5 km away from a transportation infrastructure (Torres et al., 2016).

The rapid expansion of road networks imposes a challenge to United Nations' Sustainable Development Goals because these infrastructures are among the ten major threats to biodiversity worldwide (Laurance et al., 2014; Ibisch et al., 2016; Maxwell et al., 2016). Moreover, road expansion is set to rise especially on less developed countries which still hold extraordinarily high levels of biodiversity and greatly contribute for ecosystem services (Torres et al., 2016; Meijer et al., 2018). On the other hand, road width, traffic volume and velocity tend to grow in developed countries where road networks are already established, contributing to increase the pressure on ecosystems. For instance, farmland habitats are the dominant land use in Europe, and some support high levels of biodiversity. However, biodiversity on farmland has been declining due to agricultural intensification and to road expansion (Stoate et al., 2009; Torres et al., 2016). Actually, farmland habitats are the most exposed to, and so the most negatively affected by roads (Torres et al., 2016). Indeed, the biodiversity hotspot most affected by urban expansion, including roads, is the Mediterranean Basin (Torres et al., 2016).

The construction and use of roads have many negative ecological effects on terrestrial and aquatic ecosystems: increased pollution, change of waterflows (water runoff and sediment yield), facilitation of human access to previously remote areas (increasing the risk of poaching and wildfire, and accelerating deforestation), promotion of dispersion of invasive species (plants and animals), increased mortality by vehicle collision (roadkill), barrier to animal movement, and increased loss, change, and fragmentation of habitats (Forman and Alexander, 1998; Forman et al., 2003; Laurance et al., 2014) (Figure 1.1). Moreover, these effects may persist for several hundreds of meters beyond the road itself (road-zone effect; Forman and Alexander, 1998). Although roadkill may be the most immediate and visible effect of roads, their greatest ecological impact can be habitat fragmentation and the associated barrier to movement which splits and isolates wildlife populations (Forman and Alexander, 1998). Consequently, roads may reduce gene flow, decrease genetic diversity, and increase extinction risk (Holderegger and Di Giulio, 2010). Additionally, habitat fragmentation onsets the three major mechanisms responsible for the negative impacts of roads on wildlife: decrease in habitat amount and/or quality, increase in non-natural mortality, and decrease in landscape connectivity (see Teixeira et al., 2020) (Figure 1.1). The changes imposed by roads on habitat composition and configuration (structural connectivity) may modify species' behaviour and limit their movement range (functional connectivity) (Kadoya, 2009).

Along almost a century, studies have demonstrated the deleterious effects of roads on many taxa worldwide (e.g., Stoner, 1925; Huey, 1941; Hodson, 1962; Oxley et al., 1974; van der Zande et al., 1980; Forman and Alexander, 1998; Forman et al., 2003; Fahrig and Rytwinski, 2009; Benitez-Lopez et al., 2010; Grilo et al., 2020). The growing proof of the negative effects of roads on populations should already impose the application of mitigation measures in every project of road implementation or maintenance (Fahrig and Rytwinski, 2009). However, some species may benefit from the existence of roads (Fahrig and Rytwinski, 2009). Small mammals may use road verges (road managed area between the edge of the road and the beginning of private land) as refuge and/or movement corridors across less favourable habitats (Getz et al., 1978; Bennett, 1990; Bellamy et al., 2000; Sabino-Marques and Mira, 2011) (Figure 1.1). Road verges may even sustain a greater abundance of some species of small mammals than adjacent areas (Bissonette and Rosa, 2009). A higher abundance of small mammals at road verges may also increase their use by predators and consequently increase the roadkill risk for both groups of species (Ascensão et al., 2012; Martinig and McLaren, 2019; Silva et al., 2019) (Figure 1.1). Concurrently, studies have also documented several of the above referred negative road effects for many small mammal species: roadkill (Carvalho and Mira, 2011), increase on stress indices due to pollution (Marcheselli et al., 2010), barriers or filters to movement (Macpherson et al., 2011), home range rearrangements (McGregor et al., 2008; Carvalho et al., 2018), changes in community richness and diversity (Goosem, 2000), genetic substructuring (Gerlach and Musolf, 2000) and local extinctions (La Haye et al., 2014; Banaszek et al., 2020).

Altogether, the balance between positive and negative effects of roads on small mammals may affect population dynamics, movements and functional connectivity to an extent not yet completely clarified. In highly modified habitats (e.g., intensively grazed or cultivated areas), road verges may increase abundance (Sabino-Marques and Mira, 2011) or level population outbreaks (Redon et al., 2010). In such landscapes, road verges often provide the only source of suitable habitat, and so may act as important refuges or corridors for small mammals (Bennett, 1990; Forman and Alexander, 1998). In contrast, in less modified landscapes, the abundance of several species decreases near roads (Goosem, 2000). In pristine landscapes, roads tend to hinder the movement of endemic and/or specialist species but frequently enable the movement of invasive and/or generalist species (Goosem, 2000; Laurance et al., 2009).

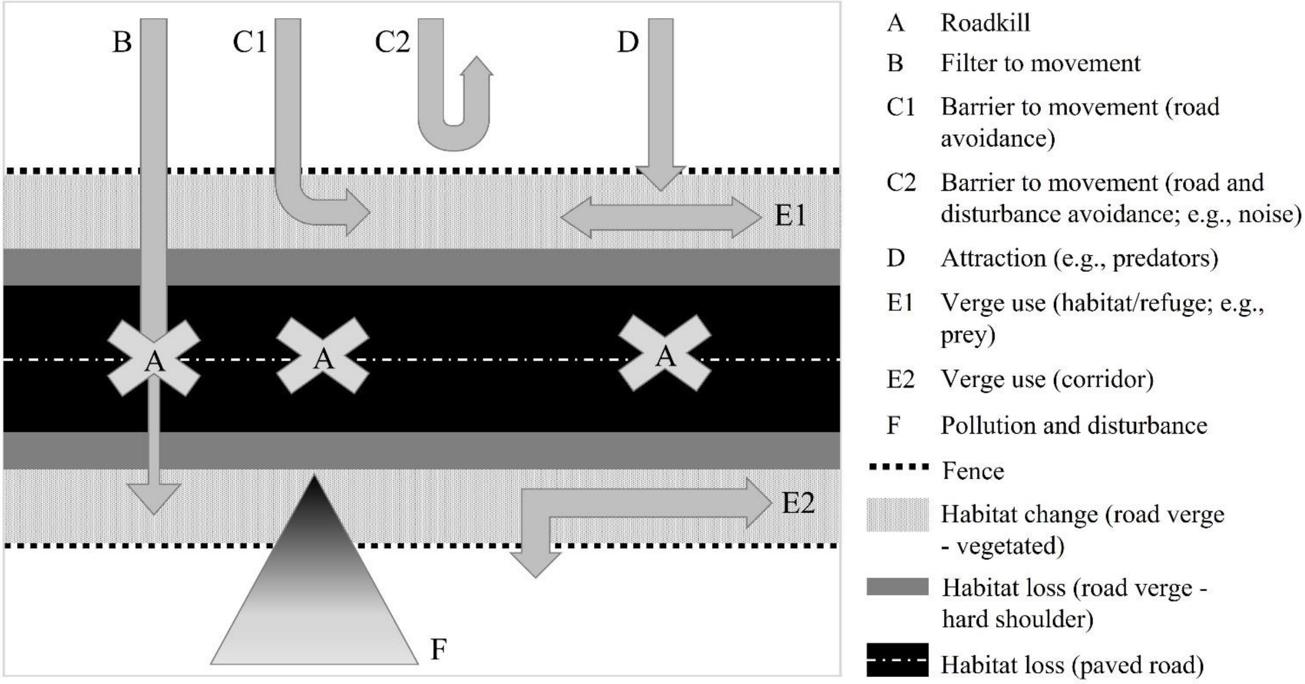

Figure 1.1 – General road structure and main road effects on wildlife (based on Trocmé et al., 2003; van der Ree et al., 2015).

Clarifying road effects on small mammals is particularly important because although these species often have large populations, less connected populations have been affected by genetic substructuring or even genetic drift (Gerlach and Musolf, 2000; Guivier et al., 2011; Visser et al., 2018). In fact, the common hamster (*Cricetus cricetus*), previously considered a pest, has suffered local extinctions and it is currently Critically Endangered (La Hay et al., 2014; Banaszek et al., 2020). One of the main causes for its decline is population isolation imposed by the road network (Banaszek et al., 2020). Isolation or restriction of movements caused by roads can be especially dramatic for low-mobility species like many small mammals (Millward et al., 2020).

Overall, species persistence on fragmented landscapes relies on their ability to survive and to move across a hostile environment to reach adequate resources (Gardiner et al., 2019). Thus, management focused on maintaining landscape functional connectivity is essential for the conservation of biodiversity in human-modified landscapes (Ernst, 2014). Vegetation management at road verges (beyond the primary goal of road safety for vehicles and people) has been mostly directed for the conservation of plants, pollinator insects, birds, and larger mammals (e.g., Grimm and Yahner 1988; Rea, 2003; Jakobsson et al., 2018; Knight et al., 2019). The most common management practices implemented for small-mammal conservation involve enabling safe road crossings using overpasses and underpasses (Meaney et al., 2007; White and Huges, 2019). Nevertheless, vegetation management for small-mammal conservation near roads can be especially important where safe passages are absent. Therefore, this thesis approach is indeed less common for directly minimising road effects on small mammals.

## 1.2. Study areas

The study was conducted in Évora district, Alentejo, Southern Portugal (Figure 1.2). This region holds large extensions of well-preserved *montado* (Figure 1.2). The *montado* is a traditional Mediterranean agro-silvo pastoral system of cork oak (*Quercus suber*) and holm oak (*Quercus ilex rotundifolia*) trees with grass and shrubs (Pinto-Correia et al., 2011). This farmland system is characteristic of the south-western Iberian Peninsula and has a high value for biodiversity conservation (Habitat 6310, Annex I, Habitats Directive, Council Directive 92/43/EEC of 21 May 1992) being a favourable habitat for many threatened species (Pinto-Correia et al., 2011). In Portugal, *montado* systems occupy 1 million ha (approximately one third of the Portuguese woodland area; ICNF, 2019). This region is also part of the Mediterranean Basin Hotspot for Biodiversity (Myers et al., 2000) and includes two Special Areas of Conservation: Monfurado (PTCON0031) and Cabrela (PTCON0033). This landscape is crossed by several national roads and a motorway (A6) which are included in the main terrestrial transportation corridor connecting Lisbon to Madrid. This road network is responsible for high roadkill rates of different vertebrate groups (Carvalho and Mira, 2011; Santos et al., 2011; Medinas et al., 2021), increase in habitat fragmentation, and consequently, decrease in habitat connectivity (Carvalho et al., 2016; 2018). This study was conducted in two areas (road and roadless areas) of well-preserved *montado*, one of which was bisected by a national road (EN257; road area). The roadless area was more than 1 km away from any national paved road.

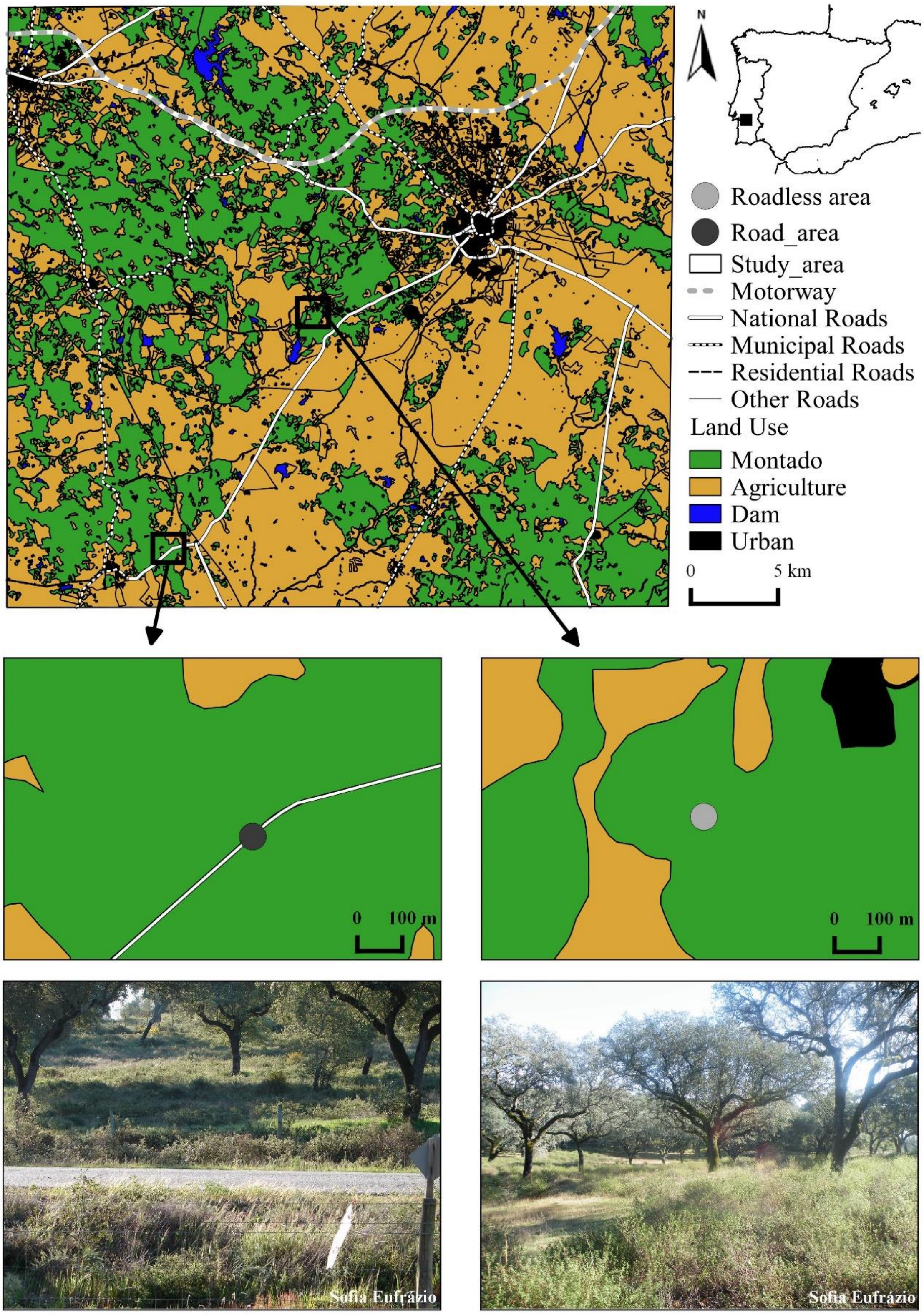

Figure 1.2 – Study areas, road network and main land uses in the study region. Dots indicate the location of the two study areas: road (dark grey) and roadless (light grey) areas. Pictures show the *montado* at each study area.

## 1.3 Study species

Using a species as a model for assessing road and roadside management effects enables to provide information and guidelines for the conservation of similar species on road surroundings. This approach is quite valuable, especially when conservation management cannot afford or wait for studies on every species. In fact, different groups of species may react differently to roads but species with similar body sizes, movement abilities and habitat requirements tend to behave in a similar way in similar habitats (Fahrig and Ritwsiky, 2009; Duffet et al., 2020).

The main challenge of a model species approach (frequently based on more abundant and generalist species) is to adapt information for a specialist species. Specialist species often have specific and so different behaviours from most species. However, finding how significantly a generalist model species is affected by road and roadside management will exacerbate concerns for specialist species in similar environments. Additionally, specialist species are often less abundant and with a conservation status of higher concern than generalist species. Therefore, targeting an abundant generalist species is usually easier and more effective.

This thesis used the wood mouse (*Apodemus sylvaticus*, Linnaeus 1758) as a model for forest-dwelling small mammals (Ascensão et al., 2016) (Figure 1.3). This species is a habitat generalist (Jubete, 2007) but is more abundant in woodland areas with dense understory cover, such as the *montado* (Alcántara and Díaz, 1996; Rosário and Mathias, 2004). Forest-dwelling small mammals may be affected by vegetation gaps caused by road pavement and verge maintenance, as well as by farmland management (Ascensão et al., 2016). In fact, wood mice react swiftly to changes in vegetation cover at fine and broad spatial scales (Tew et al., 1992; Tew and Macdonald, 1993; Tattersall et al., 2001). More specifically, paved lanes may be a partial barrier or filter for wood mice movement (Macpherson et al., 2011). This species is also one of the most road-killed small mammals in Portugal (Carvalho and Mira, 2011). Wood mice are relatively easy to trap and have a low trap related mortality rate (Montgomery, 1980; Gurnell, 1982). Additionally, knowledge about wood mice’s diet, reproduction, population dynamics, movements and habitat use already exist (e.g., Zubaid and Gorman 1991; Montgomery et al., 1997;

Rosalino et al., 2011) allowing for a better isolation and understanding of road and roadside management effects on this species.

Figure 1.3 – Wood mouse released after capture and handling.

The wood mouse (average (± SD) weight at our study sites: 23,4 ± 6,6 g) belongs to the order Rodentia, family Muridae, subfamily Murinae. The species is listed by the IUCN as Least Concern (Schlitter et al., 2021). Wood mice are common in Europe (except Latvia, Estonia, Finland, and most of Russia), also occur in Northern Africa (Schlitter et al., 2021) and have a limited distribution in Asia where they are rare and restricted to a small area near the coast of the Black Sea (Suzuki et al., 2008). The white-footed mouse (*Peromiscus leucopus*) is the ecological equivalent to the wood mouse in America (Marrotte et al., 2014). Wood mice density varies according to different habitats and seasons. In *montado* systems in spring wood mice densities can reach 60 ind/ha (Rosário and Mathias, 2004). Breeding season differs across its distribution range according to climatic conditions being supressed during the most harsh seasons: summer in the Mediterranean region and winter in central and northern Europe (Rosário and Mathias, 2004). Wood mice home ranges and movements vary according with habitat (Corp et al., 1997). In *montado* systems mean home range size (± SD) is 1.12 ± 0.97 ha (Rosalino et al., 2011) but in woodland areas with abundant resources it may be smaller (0.35 ± 0.09 ha) (Corp et al., 1997). This species consumes mainly seeds but can also eat fruits, flowers, leaves, mushrooms, and invertebrates (Zubaid and Gorman, 1991; Rogers and Gorman, 1995). The wood mouse is not considered an agricultural pest, though it may cause damage occasionally (Montgomery, 1999). In fact, the wood mouse is a major consumer and moderate disperser of acorns and may play an important role in tree regeneration (Gómez et al., 2008; Perea et al., 2011). Wood mice are particularly important acorn dispersers in smaller forest patches where long distance dispersers are absent (Moran López et al., 2016). The lack of tree regeneration in *montado* systems with lower shrub cover maybe due to the lower abundance of dispersers that cannot find shelter in shrubs (Pulido and Diaz, 2005). The wood mouse is an important prey of many birds (e.g., tawny owl, *Strix aluco*; Southern and Lowe, 1968) and mammals, some of which are of conservation concern (e.g., european wildcat, *Felis sylvestris*; Sarmento, 1996).

## 1.4. Research main goals and hypothesis

The effects of roads on population dynamics and movements patterns of small mammals, and consequently, on habitat connectivity for these species are still not yet clarified in well-preserved habitats. Understanding such effects is particularly important in well-preserved farmland systems that are responsible for high levels of biodiversity in the European Mediterranean Region. In this context, providing useful information for roadside management to effectively aim biodiversity conservation and so offset the negative effects of roads on wildlife is a paramount goal.

The main goals of this thesis are to assess the effects of roads on demographic traits (chapter 2), movements patterns (chapter 3) and landscape connectivity (chapter 4) for small mammals in a well-preserved agro-silvo pastoral system. In these areas, road verges and road surrounding habitats should offer similar resources. Therefore, the main hypothesis of this thesis is that, on those circumstances, the presence of roads will negatively affect population parameters and compromise movements of forest-dwelling small mammals (e.g., Goosem, 2001; Carvalho and Mira, 2011). However, on some occasions, management practices can occur at road verges or at road surrounding habitats and change vegetation structure (e.g., road verge vegetation cutting; cattle grazing). If management reduces vegetation cover on road surrounding habitats, small mammals will use road verges more frequently (e.g., Ascensão et al., 2012) and, consequently, connectivity along road verges will also increase.

## 1.5 Thesis structure and list of papers

This thesis includes 5 main chapters: the general introduction (chapter 1), three papers published in peer-review international journals (chapters 2, 3 and 4) and the general conclusions (chapter 5).

The general introduction (chapter 1) addresses the main consequences of road expansion on wildlife and the need to clarify road effects on small mammals in well-preserved habitats. This chapter also presents the study area, the model species, and the main goals and hypothesis of this thesis. The thesis' structure and contents are also summarized in this chapter.

Chapter 2 analyses road effects on several wood mice's demographic traits. Abundance, survival, and temporary emigration are assessed using extended Pollock's robust design capture-recapture models. Population turnover, sex ratio, age structure and body condition are also analysed.

Chapter 3 examines road effects on wood mice´s movement patterns. Road crossings, road verge use, and length and direction of movements are assessed using movement step analysis. The effects of management practices (at road verges and surrounding land) on movement patterns are also addressed.

Chapter 4 quantifies the effects of road verges and paved lanes on the fine-scale landscape connectivity for the wood mice using graph theory. The impact on connectivity of vegetation cutting at road verges and of management practices at road surroundings is also assessed.

Chapter 5 summarizes this thesis' main findings and implications for road verge management practices aiming small-mammal conservation. The main limitations of this work are addressed, and future research is suggested. Finally, the overall conclusions of this thesis are presented.

According to this thesis' structure, the three published papers are:

- **Galantinho, A.,** Eufrázio, S., Silva, C., Carvalho, F., Alpizar-Jara, R., Mira, A., 2017. Road effects on demographic traits of small mammal populations. European Journal of Wildlife Research. 63, 22. DOI: https://doi.org/10.1007/s10344-017-1076-7

- **Galantinho, A.**, Santos, S., Eufrázio, S., Silva, C., Carvalho, F., Alpizar-Jara, R., Mira, A., 2022. Effects of roads on small-mammal movements: opportunities and risks of vegetation management on roadsides. Journal of Environmental Management, 115272, DOI: https://doi.org/10.1016/j.jenvman.2022.115272

- **Galantinho, A.,** Herrera, J.M., Eufrázio, S., Silva, C., Carvalho, F., Alpizar-Jara, R., Mira, A., 2020. Road verges provide connectivity for small mammals: A case study with wood mice (*Apodemus sylvaticus*) in an agro-silvo pastoral system. Journal of Environmental Management, 258, 110033. DOI: https://doi.org/10.1016/j.jenvman.2019.110033

## 1.6 References

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# Chapter 2

## Road effects on demographic traits of small mammal populations

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ORIGINAL ARTICLE

**Road effects on demographic traits of small mammal populations**

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# Road effects on demographic traits of small mammal populations

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## Compliance with Ethical Standards

Research involving Animals

# Abstract

Recent studies have highlighted the positive effects of roads verges on the abundance of small mammals. However, most of these studies occurred in intensively grazed or cultivated areas, where verges were the last remnants of suitable habitats, which could mask the true effects of roads on population traits. We analysed the effects of roads on small mammal populations living in a well-preserved Mediterranean forest. We used the wood mouse (*Apodemus sylvaticus*) as a model of forest-dwelling small mammals that probably are amongst the species most affected by road clearings. Our study compared populations in similar habitat areas with and without road influence. We assessed abundance, survival and temporary emigration using extended Pollock's robust design capture-recapture models. Moreover, we analysed population turnover, sex ratio, age structure and body condition. We found that wood mouse abundance and body condition were lower at the road bisected area, whereas the remaining population traits were similar. This suggests that the reduced habitat availability and quality due to the physical presence of the road and verge vegetation clearing are the main drivers of demographic differences in wood mouse populations between areas. Nevertheless, our results also suggest that in high-quality habitats surrounding national roads, wood mice populations present similar dynamics to others living in undisturbed areas, despite the decrease in abundance and body condition. Overall, the often-reported increased small mammal abundance in road surroundings should not be generalized independently of habitat quality, nor to other population traits.

**Keywords:** *Apodemus sylvaticus*; capture-recapture; extended robust design models; population estimation; roadless area; road effects

## 2.1 Introduction

Roads are essential to modern human societies. These infrastructures exist throughout most landscapes, and their extension, complexity and use are set to rise around the world due to growing economic and social demands (Forman et al., 2003). Numerous studies have addressed the impacts of roads on wildlife and pointed out their deleterious effects on many species (Forman et al., 2003; Fahrig and Rytwinski, 2009; Benítez-Lopéz et al., 2010).

Documented negative road effects common to several small mammal species or communities include road kills (Carvalho and Mira, 2011), enhanced metal concentrations in tissues and negative consequences on stress indices (Marcheselli et al., 2010), barriers or filters to movement (Macpherson et al., 2011), home range rearrangements (McGregor et al., 2008) and changes in community structure (species richness and diversity) (Goosem, 2000). Nevertheless, several studies have highlighted positive or neutral effects of roads on small mammal abundance (e.g., Fahrig and Rytwinski, 2009; Ascensão et al., 2012; Bissonette and Rosa, 2009). This is because most species have small home ranges, high reproductive rates and abundance and avoid crossing roads regardless of traffic volume (Fahrig and Rytwinski, 2009). Therefore, small mammals may use road verges as habitats and dispersal routes (Bennett, 1990; Bellamy et al., 2000), undergoing only low levels of road kills (Ruiz-Capillas et al., 2015).

In non-natural habitats, such as intensively grazed or cultivated areas, roads seem either to enhance abundance (Sabino-Marques and Mira, 2011) or to level population outbreaks (Redon et al., 2010), whereas in less modified landscapes, the abundance of some species is lower near roads (Goosem, 2000). Therefore, the effects of roads on small mammal populations may depend on the quality of the surrounding habitats.

However, the effects of roads on small mammal populations are still poorly understood in well-preserved habitats. Most studies on small mammals rely on relative abundance to infer habitat suitability and population responses to roads (Fahrig and Rytwinski, 2009). Nonetheless, abundance is insufficient to truly reveal the effects of roads on wildlife populations (van Horne, 1983). Moreover, the most common measures of abundance (number of individuals per sampling effort and minimum number known alive) tend to be negatively biased because they ignore detection probabilities (Efford, 1992).

Our main goal was to assess the effects of roads on the demography of small mammals in a well-preserved habitat. We used the wood mouse (*Apodemus sylvaticus*) as a model of forest-dwelling small mammal species (Ascensão et al., 2016). Gaps in vegetation cover due to the presence and maintenance of roads would affect mostly forest-dwelling species (Ascensão et al., 2016). The wood mouse is common on road verges and surrounding woodland areas and is a key prey of many mammalian carnivores and birds of prey (Sarmento, 1996; Pezzo and Morimando, 1995). We hypothesize that roads decrease the quality of a well-preserved habitat and will therefore negatively affect wood mouse populations, as suggested for other species (D'Amico et al., 2016; Torres et al., 2016). Previous studies have shown that the wood mouse is one of the most road-killed small mammals in Portugal (Carvalho and Mira, 2011), hence we expect lower abundance and survival near roads. Also, as paved lanes hinder movement (Ford and Fahrig, 2008) and force individuals to disperse through verges (Bennett, 1990), we predict higher population turnover near roads. Moreover, poorer body condition due to traffic-induced stress may occur, as suggested by other studies (Ware et al., 2015). We compared two populations living on a well-preserved Mediterranean woodland region, one of which was bisected by a medium-traffic-intensity national road. We used capture-mark-recapture data to assess several population traits besides abundance, such as survival, recruitment and turnover. The lack of studies such as ours that account for imperfect detection is likely due to the amount of effort needed to collect enough data to estimate these parameters. Our work allows us to isolate the pure effect of roads on several population parameters rather than to assess their combined effect with that of habitat disturbance.

## 2.2 Methods

### 2.2.1 Study area

Our study was conducted in Alentejo, southern Portugal. The climate is Mediterranean with hot, dry summers and mild winters. During the study period, the monthly mean temperature was 17.6 ºC (ranging from 11.0 ºC in April to 22.8 ºC in August) and the monthly mean precipitation was 16.3 mm (ranging from 0.4 mm in August to 42.4 mm in October) (CGE, 2011).

The landscape is dominated by *montado*, a traditional Mediterranean savannah-like forest of stands of cork (*Quercus suber*) and holm (*Quercus rotundifolia*) oaks trees with herbaceous and shrub strata (Pinto-Correia and Mascarenhas, 1999). Several national roads cross the region, and firebreaks (~15 m wide) are opened along both verges every year to decrease the fire risk associated with traffic.

### 2.2.2 Study design

We carefully selected trapping sites, accounting simultaneously for high similarity between areas and optimal habitat for the species.

The study was conducted on two plots of 1.2 ha each, 16 km apart (Figure 2.1). The roadless area (38º31’N, 8º01’W) was more than 1 km away from any national paved road at the University of Évora field station. The road area (38º24’N, 8º06’W) was bisected by the national road EN257, a two-lane paved road with an average traffic volume of approximately 5000 vehicles per day (~600 vehicles per night) (EP, 2005).

The areas were very similar in vegetation structure and composition, soil type slope and, *a priori*, also in predator pressure. The main difference between areas was the presence/absence of road verges and paved lanes. The study sites were sampled simultaneously every four weeks from March to October 2009 (eight trapping sessions) using a square grid of 10 x 10 traps spaced at 12-m intervals. This period includes the most relevant events in the wood mouse annual cycle in the Mediterranean region: the peak of reproduction (March and April); the harsh dry season when reproduction almost ceases (June and July); and the resumption of reproduction after the first autumn rains (September and October) (Rosário and Mathias, 2004).

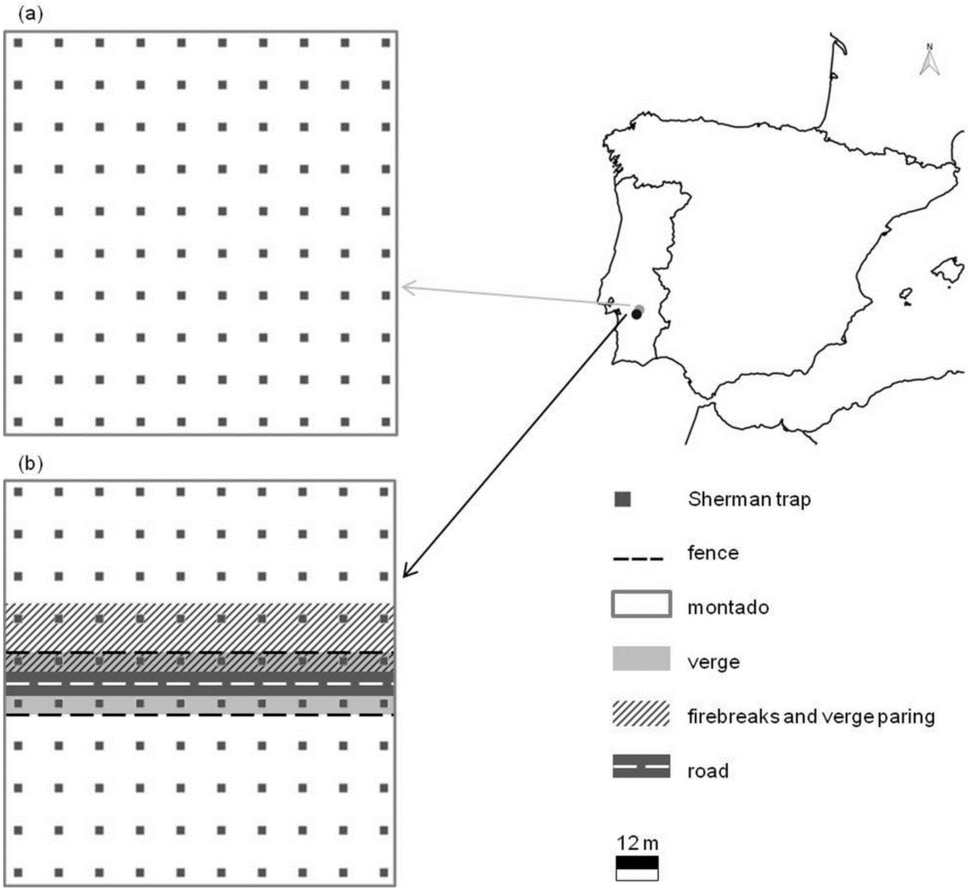

Figure 2.1- Schematic location of Sherman traps on the roadless (a) and road (b) areas. Diagonal lines show the grid section affected by verge paring and firebreaks during 2009

### 2.2.3 Data collection

Wood mice were live trapped at each site and trapping session with Sherman medium-sized live traps (8 x 9 x 23 cm). Traps remained opened on the field for four consecutive nights and were checked every day at sunrise, summing 6400 trap-nights (3200 trap-nights per area). One trap was placed on every nodule of each square grid (Figure 2.1). At the road area, the two central trap lines were placed at each road verge. Road verges were flanked by wired fences (~10 m from the asphalt) permeable to both small mammals and their predators. A mixture of sardines, oil and oat flakes was used as bait and hydrophobic cotton was provided for nesting.

Trapped individuals were sexed, aged, measured, weighted to the nearest 0.5 g (micro-line spring scale Pesola AG, Baar, Switzerland) and released at the place of capture. Males with scrotal testes and females with either perforated vagina, vaginal plug, visible or enlarged nipples or those that were pregnant were considered reproductively active (Gurnell and Flowerdew, 2006).

Each individual was assigned to an age class based on its weight, body length and breeding condition using measurement references for the Iberian Peninsula (Jubete, 2002). Upon first capture, animals were individually marked with passive integrated transponders tags (PIT, TXP148511B, 8.5 mm x 2.12 mm, 134.2 kHz ISO, 0.067g, Biomark, Boise, USA).

At every field session, we collected information on vegetation traits in a 1-m square around each trap to control for changes across time and similarities between areas. We assessed cover (%) and height (cm) for herbaceous and shrub strata and also cover (%) for bare ground, litter, rocks and tree strata. We categorized measurements in 25% classes for cover and in 10-cm classes for height of herbaceous and shrub strata (Ascensão et al., 2012).

### 2.2.4 Data analysis

Capture-recapture data were analysed using extended Pollock's robust design models (Kendall et al., 1997). The eight trapping sessions (months) were defined as primary periods, and the four consecutive trapping nights as the secondary periods within each primary period, totalling 32 trapping occasions. Among primary periods, the population is considered open, allowing for immigration, emigration, births and deaths, and among secondary periods, the population is considered closed to gains and losses (Kendall et al., 1997). Extended Pollock's robust design models estimate abundance (N) and capture probabilities (p) within primary periods and survival probability ($\phi$), temporary emigration (Y") and temporary immigration (1-Y') among primary periods (Kendall et al., 1995; Kendall et al., 1997). All parameters are estimated jointly using a full likelihood approach (Kendall et al., 1995; Kendall et al., 1997). This approach allows for parameter estimation considering them constant or time varying (Kendall et al., 1995).

Analyses were performed using closed captures parameterization for robust design models in the program MARK (White and Burnham, 1999). Several candidate models were proposed to find evidence of time variation in each of the population parameters and to test for temporary emigration at each study site. We estimated population parameters with the corresponding 95% confidence intervals. Estimates with coefficients of variation greater than 50% and/or confidence intervals including zero were considered unacceptable for further analysis (Brandstätter, 1999; White et al., 1982). In the capture-recapture context, coefficients of variation should be less than 20% for reliable scientific studies and up to 50% for management or monitoring studies (White et al., 1982). Parameter estimates were obtained assuming in both areas even flow movement (the probability of leaving the area and re-entering is the same: $Y'' = 1 - Y'$) and this type of movement was tested against the no movement model ($Y'' = 0$; $Y' = 1$) (Sanders and Trost, 2013). Even flow models were plausible because habitat type was similar across and outside of the trapping grids. Analyses were conducted for each area separately to account for the possibility of different parameter estimates and movement models. Dead individuals were excluded from the analysis (Pollock et al., 1990).

Population turnover was accounted for in each area and primary period as the ratio of the sum of recruits and losses to the number of residents (Bertolino et al., 2001). Body condition was evaluated by the scaled mass index (SMI) to account for the scaling relationship between body mass and a linear body measurement as growth occurs (Peig and Green, 2010). For comparison purposes, we only used male body condition to exclude the effect of pregnancies on scaling (Díaz and Alonso, 2003). Age structure (juveniles and adults), sex ratio (males/females), turnover, residents, losses and recruits were compared between the two areas using the Wilcoxon rank sum test (W) with continuity correction (Sokal and Rohlf, 1997). The overall sex ratio was tested for deviations from the balanced sex ratio (1:1) in each area with a chi-square test (Sokal and Rohlf, 1997).

SMI was estimated using the package lmodel2 (Legendre, 2011) for R software, version 2.13.0 (R Core Team, 2011). The effect of roads on body condition was modelled with linear mixed-effects models (nlme package; Pinheiro et al., 2015) considering area (roadless/road) as the fixed effect and individual identity (PIT tag) as the random effect. Also, in the road area, we analyzed the effect of microhabitat (eight variables mentioned above), row (to account simultaneously for distance to the road and firebreaks) and session with linear mixed models, considering individual identity as the random effect. We log transformed body condition to approach normality. Temporal patterns in body condition were checked using the autocorrelation function (nlme package; Pinheiro et al., 2015). The Akaike's Information Criterion was used for selection of robust design models (corrected for small sample sizes, AICc) and linear mixed-effects models (AIC; Burnham and Anderson, 2002). We considered models within two AIC units of the best model to have substantial support, except those with non-informative parameters (Burnham and Anderson, 2002; Arnold, 2010). Effect sizes were considered as the magnitude of the differences found between areas and were significant if their confidence intervals did not overlap zero (Cooch and White, 2013).

Estimates for all parameters (except age structure and sex ratio) are presented with their corresponding 95% confidence intervals. We report the dominant classes (mode) for each microhabitat variable.

## 2.3 Results

### 2.3.1 Abundance, capture probability, survival and temporary emigration

We recorded 494 captures of 119 different wood mice (66 in the roadless area and 53 in the road area) in 6400 trap nights. Trap mortality was extremely low; we found only three dead individuals (one in the road area and two in the roadless area) and excluded them from the data set.

From the 24 robust design candidate models fitted (Supplementary materials 2), only four had reliable estimates for all parameters for each study area (Tables 2.1, 2.2 and 2.3). All models with parameters varying among sessions resulted in estimates with poor precision (coefficient of variation >50% or confidence intervals including zero), except for time-varying abundance models.

Thus, the four models considered for further analysis comprised even flow movement and no movement models, both applied with either all parameters held constant, or with time-varying abundance. In the roadless area, the second best model adds time varying abundance and has delta AIC of 0.88. Since the change in deviance was not enough to compensate for the increase in parameters, we considered time-varying abundance as uninformative and inferred only from the top model. The best plausible models emphasized the presence of temporary emigration in both areas. The more parsimonious models assume the same probability for individuals temporarily leaving and re-entering the area (even flow movement), and all parameters constant along the eight primary sessions. In each area, the top model had coefficients of variation below 10% for all estimates except for temporary emigration, which was, nevertheless, below 31% (Tables 2.2 and 2.3). Probabilities of capture were 0.43 (95%CI: 0.38–0.48) in the road area and 0.46 (95%CI: 0.41–0.50) for the roadless area. Temporary emigration was 0.24 (95%CI: 0.13–0.42) in the road area and 0.21 (95%CI: 0.11–0.36) for the roadless area. Probability of survival was 0.69 in both areas (95%CI: 0.59–0.78 for the road area; 95%CI: 0.60–0.77 for the roadless area). Abundance estimates were significantly lower in the road area (20.79; 95%CI: 20.23–22.67) than in the roadless area (32.69; 95%CI: 32.19–34.45) (effect size = 11.9; 95%CI: 10.46–13.34). More specifically, estimates of abundance were significantly lower in the road area from March to July (Figure 2.2).

Table 2.1- Extended Pollock's robust design model selection for the road and roadless areas. Notation: survival (ϕ), temporary emigration (Y''), temporary immigration (1-Y'), recapture rate (p), type of movement (even flow Y''= 1-Y' and no movement Y'' = 0, Y'=1), abundance (N), constant parameter (.), time-dependent parameter (t), difference in AIC value (ΔAICc), Akaike weights ($w_i$), number of parameters (No. Par.).

| Area | Model | AICc | ΔAICc | $w_i$ | No. Par. | Deviance |
|---|---|---|---|---|---|---|
| Road | even flow; all parameters constant | | | | | |
| | ϕ(.); Y''(.) = 1-Y'(.); p (.); N(.) | 393.58 | 0.00 | 0.77 | 4 | 446.98 |
| | no movement; all parameters constant | | | | | |
| | ϕ(.); Y''= 0 Y'=1; p(.); N(.) | 396.57 | 2.99 | 0.17 | 3 | 452.04 |
| | even flow; time-varying abundance | | | | | |
| | ϕ(.); Y''(.) = 1-Y'(.); p (.); N(t) | 399.82 | 6.24 | 0.03 | 10 | 440.31 |
| | no movement; time-varying abundance | | | | | |
| | ϕ(.); Y''= 0 Y'=1; p(.); N(t) | 401.14 | 7.56 | 0.02 | 9 | 443.83 |
| Roadless | even flow; all parameters constant | | | | | |
| | ϕ(.); Y''(.) = 1-Y'(.); p (.); N(.) | 412.45 | 0.00 | 0.58 | 4 | 610.68 |
| | even flow; time-varying abundance | | | | | |
| | ϕ(.); Y''(.) = 1-Y'(.); p (.); N(t) | 413.34 | 0.89 | 0.37 | 9 | 601.05 |
| | no movement; time-varying abundance | | | | | |
| | ϕ(.); Y''= 0 Y'=1; p(.); N(t) | 418.58 | 6.13 | 0.03 | 8 | 608.42 |
| | no movement; all parameters constant | | | | | |
| | ϕ(.); Y''= 0 Y'=1; p(.); N(.) | 419.92 | 7.46 | 0.01 | 3 | 620.20 |

Table 2.2 - Extended Pollock's robust design model results for the road area. Notation: parameter (Par), estimates (Est), standard errors (SE), confidence intervals (LCI – lower limit and UCI – upper limit), coefficient of variation (cv), survival (ϕ), temporary emigration (Y"), temporary immigration (1-Y'), recapture rate (p), type of movement (even flow Y"= 1-Y' and no movement Y" = 0, Y'=1), abundance (N), constant parameter (.), time-dependent parameter (t). No movement models imply fixed temporary movements and hence variation coefficients are not computed.

| Model | Par | Est | SE | LCI | UCI | cv |
|---|---|---|---|---|---|---|
| **even flow; all constant** | ϕ | 0.694 | 0.048 | 0.594 | 0.779 | 0.069 |
| **ϕ(.); Y"(.) = 1-Y'(.); p (.); N(.)** | Y" | 0.244 | 0.075 | 0.127 | 0.417 | 0.307 |
| | Y' | 0.756 | 0.075 | 0.583 | 0.873 | 0.099 |
| | p | 0.430 | 0.027 | 0.377 | 0.484 | 0.064 |
| | N | 20.786 | 0.542 | 20.232 | 22.670 | 0.026 |
| **no movement; all constant** | ϕ | 0.607 | 0.044 | 0.518 | 0.689 | 0.073 |
| **ϕ(.); Y"= 0; Y'=1; p(.); N(.)** | p | 0.400 | 0.024 | 0.354 | 0.449 | 0.061 |
| | N | 21.156 | 0.612 | 20.436 | 23.065 | 0.029 |
| **even flow; time-varying abundance** | ϕ | 0.694 | 0.048 | 0.592 | 0.780 | 0.070 |
| **ϕ(.); Y"(.) = 1-Y'(.); p (.); N(t)** | Y" | 0.225 | 0.080 | 0.106 | 0.417 | 0.355 |
| | Y' | 0.775 | 0.080 | 0.583 | 0.894 | 0.103 |
| | p | 0.409 | 0.028 | 0.355 | 0.466 | 0.070 |
| | N | 22.256 | 1.883 | 20.542 | 29.384 | 0.085 |
| | N | 31.369 | 2.272 | 29.015 | 39.185 | 0.072 |
| | N | 24.534 | 1.985 | 22.653 | 31.830 | 0.081 |
| | N | 19.976 | 1.779 | 18.437 | 26.943 | 0.089 |
| | N | 15.417 | 1.555 | 14.248 | 22.093 | 0.101 |
| | N | 8.569 | 1.167 | 8.046 | 15.050 | 0.136 |
| | N | 5.134 | 0.929 | 5.003 | 11.406 | 0.181 |
| | N | 1.000 | 1.98E-04 | 1.000 | 1.000 | 0.000 |
| **no movement; time-varying abundance** | ϕ | 0.610 | 0.044 | 0.521 | 0.692 | 0.072 |
| **ϕ(.); Y"= 0 Y'=1; p(.); N(t)** | p | 0.383 | 0.025 | 0.336 | 0.432 | 0.065 |
| | N | 22.880 | 2.096 | 20.802 | 30.336 | 0.092 |
| | N | 32.241 | 2.523 | 29.442 | 40.476 | 0.078 |
| | N | 25.220 | 2.208 | 22.954 | 32.871 | 0.088 |
| | N | 20.539 | 1.980 | 18.658 | 27.804 | 0.096 |
| | N | 15.856 | 1.732 | 14.393 | 22.757 | 0.109 |
| | N | 8.824 | 1.299 | 8.092 | 15.363 | 0.147 |
| | N | 5.298 | 1.031 | 5.013 | 11.873 | 0.195 |
| | N | 1.000 | 1.85E-04 | 1.000 | 1.000 | 0.000 |

Table 2.3 - Extended Pollock’s robust design model results for the roadless area. Notation: parameter (Par), estimates (Est), standard errors (SE), confidence intervals (LCI – lower limit and UCI – upper limit), coefficient of variation (cv), survival (ϕ), temporary emigration (Y"), temporary immigration (1-Y'), recapture rate (p), type of movement (even flow Y"= 1-Y' and no movement Y" = 0, Y'=1), abundance (N), constant parameter (.), time-dependent parameter (t). No movement models imply fixed temporary movements and hence variation coefficients are not computed.

| **Model** | **Par** | **Est** | **SE** | **LCI** | **UCI** | **cv** |
|---|---|---:|---:|---:|---:|---:|
| **even flow; all constant** | ϕ | 0.691 | 0.042 | 0.603 | 0.768 | 0.061 |
| **ϕ(.); Y"(.) = 1-Y'(.); p (.); N(.)** | Y" | 0.211 | 0.063 | 0.114 | 0.358 | 0.296 |
| | Y' | 0.789 | 0.063 | 0.642 | 0.886 | 0.079 |
| | p | 0.456 | 0.023 | 0.411 | 0.502 | 0.051 |
| | N | 32.685 | 0.497 | 32.192 | 34.450 | 0.015 |
| | | | | | | |
| **even flow; time-varying abundance** | ϕ | 0.691 | 0.043 | 0.601 | 0.768 | 0.062 |
| **ϕ(.); Y"(.) = 1-Y'(.); p (.); N(t)** | Y" | 0.195 | 0.065 | 0.097 | 0.351 | 0.332 |
| | Y' | 0.805 | 0.065 | 0.649 | 0.903 | 0.080 |
| | p | 0.434 | 0.024 | 0.387 | 0.481 | 0.055 |
| | N | 35.161 | 2.143 | 32.947 | 42.552 | 0.061 |
| | N | 44.082 | 2.426 | 41.389 | 51.997 | 0.055 |
| | N | 27.354 | 1.874 | 25.596 | 34.295 | 0.069 |
| | N | 24.008 | 1.751 | 22.460 | 30.766 | 0.073 |
| | N | 20.660 | 1.620 | 19.334 | 27.249 | 0.078 |
| | N | 6.131 | 0.913 | 6.003 | 12.296 | 0.149 |
| | N | 4.000 | 0.001 | 4.000 | 4.001 | 0.000 |
| | N | 1.000 | 0.000 | 1.000 | 1.000 | 0.000 |
| | | | | | | |
| **no movement; time-varying abundance** | ϕ | 0.613 | 0.039 | 0.535 | 0.685 | 0.063 |
| **ϕ(.); Y"= 0 Y'=1; p(.); N(t)** | p | 0.409 | 0.022 | 0.367 | 0.452 | 0.053 |
| | N | 35.941 | 2.374 | 33.324 | 43.729 | 0.066 |
| | N | 45.056 | 2.686 | 41.903 | 53.433 | 0.060 |
| | N | 27.964 | 2.077 | 25.859 | 35.231 | 0.074 |
| | N | 24.545 | 1.940 | 22.675 | 31.592 | 0.079 |
| | N | 21.126 | 1.796 | 19.504 | 27.959 | 0.085 |
| | N | 6.284 | 1.007 | 6.012 | 12.732 | 0.160 |
| | N | 4.000 | 0.000 | 4.000 | 4.000 | 0.000 |
| | N | 1.000 | 0.000 | 1.000 | 1.000 | 0.000 |
| | | | | | | |
| **no movement; all constant** | ϕ | 0.610 | 0.039 | 0.533 | 0.683 | 0.063 |
| **ϕ(.); Y"= 0 Y'=1; p(.); N(.)** | p | 0.429 | 0.021 | 0.388 | 0.470 | 0.049 |
| | N | 33.011 | 0.561 | 32.366 | 34.789 | 0.017 |

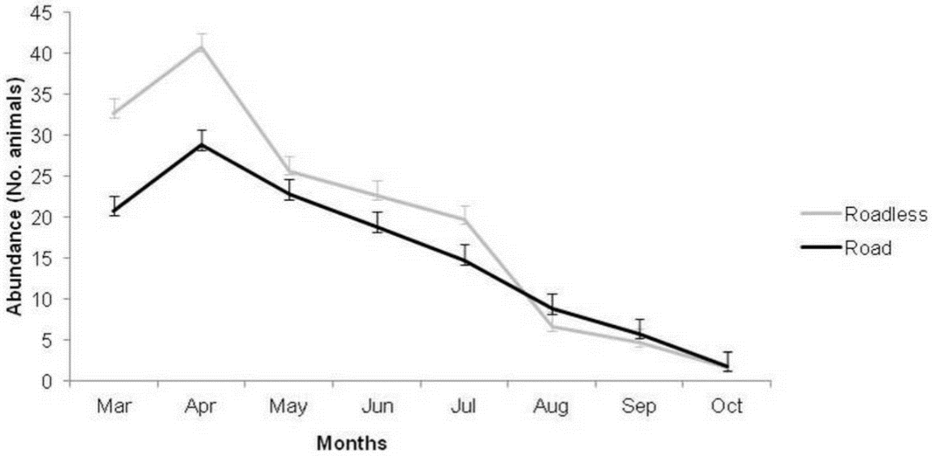

Figure 2.2 - Abundance estimated by extended Pollock's robust design models at the road and roadless areas. Bars represent 95% confidence intervals

### 2.3.2 Turnover, age structure, sex ratio and body condition

Population turnover and proportion of residents were slightly higher at the roadless area (1.59; 95%CI: 0.65–2.53 and 0.46; 95%CI: 0.32–0.60, respectively) than at the road area (1.20; 95%CI: 0.59–1.81 and 0.42; 95%CI: 0.24–0.60, respectively). The reverse situation occurred for the proportion of recruits (0.12; 95%CI: 0.02–0.22 at the roadless area; 0.17; 95%CI: 0.05–0.29 at the road). However, none of these differences were statistically significant (Table 2.4). In both areas, on average, the joint number of losses and recruits outweighed the number of residents (turnover >1).

Table 2.4 - Population turnover and percentages of recruits, losses and residents for the road and roadless areas (means and 95% CI). Differences between areas were assessed with the Wilcoxon rank sum test statistics (W) and associated significance level (*p*).

| | **Road** | **Roadless** | **Wilcoxon Test** |
|---|---|---|---|
| **Turnover** | 1.20 (0.59, 1.81) | 1.59 (0.65, 2.53) | W=21; *p*=0.5286 |
| **Recruits (%)** | 0.17 (0.05, 0.29) | 0.12 (0.02, 0.22) | W=30.5; *p*=0.2404 |
| **Losses (%)** | 0.41 (0.25, 0.57) | 0.42 (0.26, 0.58) | W=27.5; *p*=0.3744 |
| **Residents (%)** | 0.42 (0.24, 0.60) | 0.46 (0.32, 0.60) | W=21; *p*=0.3504 |

The number of juveniles was low at both sites: three juveniles out of 20 individuals in March in the road area; two juveniles out of 32 in March and one out of 40 in April in the roadless area.

The sex ratio was similar between areas along the eight sessions (W = 31.5; *p* = 0.7) and the global values were not significantly different from the balanced sex ratio: 34 (52%) females and 32 (48%) males in the roadless area ($\chi^2$ = 0.0606, df = 1, *p* = 0.81); 29 (57%) females and 22 (43%) males in the road area ($\chi^2$ = 0.9608, df = 1, *p* = 0.33).

Body condition was significantly lower in the road area (22.44 g; 95%CI: 21.63–23.25) than in the roadless area (24.16 g; 95%CI: 23.44–24.87) (*p* = 0.0019) (effect size = 1.72; 95%CI: 0.64–2.80). In the road area, we did not find a considerable effect of row or cover of shrubs and trees on body condition (Table 2.5). Models including each of these three variables were within 2 AIC units from the top model, but did not improve the likelihood considerably. According to the top model, body condition was lower from April to August than in March and decreased with litter cover above 50% and herbaceous and shrub height from 10 to 20 cm. Also, body condition increased with herbaceous cover from 20 to 50% and herbaceous and shrub height above 20 cm (Table 2.6).

Table 2.5 - Selection of models explaining male body condition at the road area (98 observations of 22 males). Notation: Akaike weights ($w_i$), difference in AIC value (ΔAICc), log-likelihood (logLik), degrees of freedom (df), variables accounted by each model (+). We only show models within 2 AIC units from the top model (ΔAIC<2).

| | | **Cover** | | | | **Height** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **(Intercept)** | **Litter** | **Herbaceus** | **Shrub** | **Tree** | **Shrub** | **Herbaceus** | **Session** | **Row** | **df** | **logLik** | **AIC** | **ΔAIC** | **$w_i$** |
| **model 1** | 3.40 | + | + | | | + | + | + | | 28 | 108.71 | -161.40 | 0.00 | 0.29 |
| **model 2** | 3.51 | + | + | | | + | + | + | + | 37 | 117.55 | -161.10 | 0.31 | 0.25 |
| **model 3** | 3.37 | + | + | + | | + | + | + | | 31 | 111.51 | -161.00 | 0.40 | 0.24 |
| **model 4** | 3.36 | + | + | | + | + | + | + | | 31 | 111.44 | -160.90 | 0.55 | 0.22 |

Table 2.6 - Estimates, standard errors (SE) and 95% confidence intervals (LCI – lower limit and UCI – upper limit) for variables in the selected top model explaining male body condition at the road area (98 observations of 22 males). Confidence intervals excluding zero are in italic.

| | **Estimate** | **SE** | **LCI** | **UCI** |
|---|---:|---:|---:|---:|
| **(Intercept)** | *3.394* | *0.056* | *3.281* | *3.507* |
| **Session** | | | | |
| April | *-0.229* | *0.049* | *-0.327* | *-0.130* |
| May | *-0.226* | *0.052* | *-0.330* | *-0.122* |
| June | *-0.257* | *0.053* | *-0.362* | *-0.151* |
| July | *-0.278* | *0.054* | *-0.387* | *-0.170* |
| August | *-0.421* | *0.084* | *-0.589* | *-0.252* |
| September | -0.110 | 0.066 | -0.243 | 0.024 |
| October | -0.074 | 0.172 | -0.432 | 0.284 |
| **Litter cover (%)** | | | | |
| ]25-50] | -0.052 | 0.055 | -0.162 | 0.057 |
| ]50-75] | *-0.128* | *0.045* | *-0.219* | *-0.036* |
| ]75-100] | *-0.167* | *0.031* | *-0.230* | *-0.105* |
| **Herbaceus cover (%)** | | | | |
| ]25-50] | *0.138* | *0.031* | *0.076* | *0.201* |
| ]50-75] | 0.008 | 0.041 | -0.074 | 0.090 |
| ]75-100] | -0.047 | 0.048 | -0.144 | 0.050 |
| **Herbaceus height (cm)** | | | | |
| ]10-20] | *-0.082* | *0.039* | *-0.160* | *-0.005* |
| ]20-30] | *0.108* | *0.043* | *0.021* | *0.195* |
| ]30-40] | 0.003 | 0.051 | -0.099 | 0.105 |
| ]40-50] | *0.158* | *0.061* | *0.035* | *0.280* |
| ]50-60] | -0.069 | 0.089 | -0.248 | 0.110 |
| ]90-100] | *0.256* | *0.096* | *0.063* | *0.450* |
| **Shrub height (cm)** | | | | |
| ]10-20] | *-0.078* | *0.034* | *-0.146* | *-0.010* |
| ]20-30] | *0.192* | *0.043* | *0.106* | *0.278* |
| ]30-40] | 0.042 | 0.039 | -0.036 | 0.120 |
| ]40-50] | 0.054 | 0.056 | -0.058 | 0.166 |
| ]50-60] | -0.042 | 0.065 | -0.173 | 0.088 |
| ]100-110] | *0.337* | *0.094* | *0.148* | *0.526* |

### 2.3.3 Microhabitat structure

Both areas presented the same dominant classes for cover and height for all of the variables measured. The most frequent cover classes were 0–25% for herbaceous, trees, rocks and bare ground; 25%–75% for shrubs and 75%–100% for litter. Herbaceous and shrub height were below 20 and 60 cm in more than 70% and 75% of traps (out of a total of 100 traps per area), respectively.

During our study, firebreaks were only opened on one side of the road (May 2009). The grid lines affected by the firebreaks (20 traps; Figure 2.1) had lower shrub cover and higher herbaceous strata than the corresponding lines of the roadless area, where no firebreaks occurred. Also, the dominant class cover for the herbaceous strata was 0–25% before the firebreaks and 25%–50% afterwards, whereas in the roadless area, herbaceous strata remained in the lowest class (0–25%).

## 2.4 Discussion

We found that a wood mouse population living in an area surrounding a road has similar demographic parameters to another in a roadless area within a comparable habitat. Nevertheless, the road population has a lower abundance and males present on average a lower body condition. Both areas have similar habitat structure, except for the presence/absence of the road, therefore we believe that this infrastructure was the main factor responsible for our findings. These results show that the previously documented positive effects of roads on the abundance of small mammals (Fahrig and Rytwinski, 2009; Ascensão et al., 2012) do not hold true under all circumstances. Thus, the effects of roads seem to depend on the quality of the surrounding habitat, or more precisely, on the quality of road verges within each habitat.

### 2.4.1 Road vicinity effects on small mammal population traits

In similar well-preserved habitats, the road area supported, on average, one third less individuals than the roadless area. Previous positive or neutral effects of roads on the abundance of small mammals highlight verges as refuges in poorer-quality habitat matrices, either intrinsically or due to grazing pressure or agricultural intensification (Fahrig and Rytwinsky, 2009; Ascensão et al., 2012; Ruiz-Capillas et al., 2013). Lower abundance was reported near roads only in a few circumstances and for species prone to using undisturbed habitats (Goosem, 2000; Barrows et al., 2006). The significantly lower abundance at the road area was no longer evident at the end of summer and beginning of autumn when both populations reached their lowest abundances, as previously reported for the Mediterranean dry season (Rosario and Mathias, 2004). Therefore, the road area can maintain the same minimal abundance but is unable to sustain the maximum numbers reached in the roadless area during favourable seasons. Essentially, this may reflect the lower habitat availability at the roadside. The road pavement itself reduces the available habitat by approximately 11% (1/9 inter-row distance). Moreover, at least once a year, nearly one fifth (2/9 inter-row distance) of the sampled road area loses most of its shrub cover due to vegetation clearing on verges and firebreaks reopening along the road. These interventions are enforced by law to prevent fires (Decree-Law 156/2004, 30th June of the Portuguese Ministry of Agriculture, Rural Development and Fisheries) and are applied on verges of every national road in Portugal. Similarly, verges are managed in other countries with documented effects on animal communities (e.g., Meunier et al., 1999). Together, road and vegetation clearing reduce the area of available suitable habitat by approximately one third. Vegetation clearing occurred on only one occasion, but a lower shrub cover and a higher herbaceous cover at those cleared grid lines remained throughout the entire study period. Moreover, although at the occasion of firebreak opening, the global proportion of individuals lost was similar in both study areas (30%), 55% of the losses in the road area corresponded to individuals previously trapped at the lines directly affected by clearing (unpublished data). Lower vegetation cover and height reduce resource availability (shelter and food) and the carrying capacity of the area. Furthermore, vegetation clearing occurs just before the beginning of summer, which is the most critical period, with shortages of food and water, in Mediterranean environments (Rosário and Mathias, 2004). The decrease in resource availability could also explain the poorer body condition in the road area during this season. In fact, body condition increased with taller vegetation and decreased from April to August. Distance from the road (row) may not affect body condition, because wood mice may use more than one row per session. The consistent lower values for body condition near the road, besides reflecting scarcity of resources (Alcántara and Díaz, 1996), may also reveal physiological stress (Tête et al., 2013) induced by traffic (Ware et al., 2015). Other studies found that traffic tended to modify several physiological stress indices (cadmium and plumb kidney/liver ratios and kidney/body weight ratios; Marcheselli et al., 2010) and increase levels of stress hormones (faecal corticosterone metabolites; Navarro-Castillla et al., 2014) in the wood mouse. Additionally, the foraging efficiency of animals may decrease in periods of higher traffic volume (Lowry et al., 2013). Usually, males and females have similar body condition patterns (Rosario and Mathias, 2004), thus a poorer body condition could translate into poorer breeding performance and consequently, lower abundance in the road area. However, we have weak evidence (one juvenile in the roadless area vs zero juveniles at the road in April) that reproduction lasted longer in the roadless area. All juveniles (three in each area) were captured only once. Thus, the increase in abundance from reproduction may have resulted from the individuals born earlier in winter (Rosário and Mathias, 2004).

Contrary to our predictions, the remaining parameters analysed were similar between road and roadless populations. Although reproduction, recruitment and turnover in wood mice are known to depend on abundance (Gurnell, 1978; Montgomery, 1989a, b), these parameters represent proportions (e.g., percentage of residents) and rates (i.e., sex ratio) that are comparable as long as populations have similar structures, independently of their sizes.

Survival probabilities are also rates between the number of marked individuals presently found alive and the total number of previously marked individuals (Pollock et al., 1990). Even so, similar survival probabilities were unexpected because road kills should represent an additional source of mortality. However, during our study period, we never found any road-killed wood mice despite the fact that five individuals crossed the road (unpublished data). Therefore, road kills may not have a significant influence on population survival nor threaten the long-term persistence of an abundant and widespread small mammal, as suggested by Ruiz-Capillas et al. (2015). On the other hand, we found two road-killed wood mice predators: *Martes foina* (Serafini and Lovari, 1993) and *Buteo buteo* (occasional predator; Mañosa and Cordero, 1992; Zuberogoitia et al., 2006). This may suggest that in the road area, lower mortality by predation could compensate for a higher mortality by road kills (predation release hypothesis; Fahrig and Rytwinski, 2009). However, the effects of predation release near roads are still not proven (Planillo and Malo, 2013; Downing et al., 2015), and we lack sufficient data to test it.

Temporary emigration results showed that residents entered and exited road and roadless areas at the same rate, meaning that animals’ movements may not be significantly disturbed by traffic as suggested by Ford and Fahrig (2008) for other small mammal species. The similarities between the two areas in terms of temporary emigration, turnover and related traits suggested that the road surroundings were not acting as dispersal routes, as initially expected. Indeed, verges may be favoured dispersal corridors for small mammals only when roads cross habitats highly modified by humans (Getz et al., 1978), or in natural or semi-natural habitats, if they offer additional conditions, such as food or shelter (Brock and Kelt, 2004).

### 2.4.2 Potential limitations and strengths

Precise estimations in capture-recapture studies demand large data sets, frequently preventing the desired replication due to budget constraints (Bailey et al., 2004). Therefore, many capture-recapture studies use one or two areas (e.g., Rosario and Mathias, 2004; Wang and Getz, 2007; Borges and Marini, 2010; Silva et al., 2011). Model complexity increases data demands, and extended Pollock’s robust design is amongst the most complex models available for estimating population parameters (Cooch and White, 2013). This method is particularly important in the presence of temporary emigration and the lack of perfect detectability (Kendall et al., 1997), as we found in our study.

Inferences in capture-recapture studies are often model-based (likelihood-based approach) rather than design-based (Burnham et al., 1987; Bailey et al., 2004; Borchers et al., 2002). Model-based inference is independent of how we choose sampling units and rather relies on model assumptions (e.g., unknown detection probability and other assumptions related to the parametric model structure) (Borchers et al., 2002).

We acknowledge that model-based inference is necessary because the detection probability is unknown, but it could eventually be combined with design-based methods to test model assumptions. However, this would require sampling as many areas as possible over the range of the survey region (Borchers et al., 2002). Nonetheless, our model-based estimates could be used to calibrate parameters in a similar habitat (Pollock et al., 2002; Bailey et al., 2004).

We used a high number of traps and temporal replication (in each area) to compare, for the first time, several traits of small mammal populations living in similar road and roadless areas. Our analysis did not show evidence of capture probabilities being affected by a possible behavioral response to trap bait. However, a possible violation of this assumption, due to a trap-happy scenario, would overestimate encounter probability and underestimate abundance (Pollock, 1982). Although our temporal replication encompassed three seasons, it was conducted in a dry year. Different climatic conditions could affect our estimates. Rosário and Mathias (2004) found higher abundance and better body condition in a wood mouse population during a wetter year in the same roadless habitat and region. However, climate would affect both road and roadless areas and consequently, our conclusions for comparison purposes would not be compromised.

### 2.4.3 Implications for road verge management

Our analysis suggested that common management actions to prevent fire risk associated with roads shape the population dynamics of a common and abundant small mammal, lowering the carrying capacity at a roadside area significantly. Part of this arises from the double intervention (verge vegetation clearing and firebreak opening) in contiguous areas at one side of the road. Probably, this management strategy will have an even greater effect when implemented on both sides of the road as prescribed by law.

On roads crossing areas highly modified by human activity, verge management could hamper the populations of threatened small mammals by destroying their last refuges (e.g., *Microtus cabrerae*; Pita et al., 2006 and *Niviventer cameroni*; Musser and Ruedas, 2008). On the other hand, in intensive agricultural areas, verge management would contribute to the control of small rodent outbreaks that compromise agriculture yield (e.g., *Microtus arvalis*; Redon et al., 2010).

To minimize fire risk and simultaneously maintain the availability of habitat for threatened small mammals, we suggest a maximum width of 10 m for the vegetation clearing strip (currently, this is the minimum width allowed by law). This maximum width should include verge paring and firebreaks. Most small mammal species depend on vegetation cover (Garratt et al., 2012) and would benefit from our recommendation without any additional cost. This would be important because the current habitat loss and fragmentation may compromise even abundant and widespread species. In fact, a former small mammal pest (*Cricetus cricetus*) is now extinct in parts of its distribution range (La Hay et al., 2014). Moreover, small mammals are key prey for many predators, including threatened species (Delibes-Mateos et al., 2011). Thus, any management action promoting small mammal abundance should help in the conservation of its predators (Delibes-Mateos et al., 2011).

## 2.5 Conclusions

In well preserved habitats, a low-traffic road may negatively affect small mammals, even those with high reproductive ability like the wood mouse. We showed that abundance and body condition were lower in the road area, whereas survival and turnover were similar in both areas. We do not have evidence that road kills negatively affect survival, nor that verges positively affect turnover, as initially predicted. Thus, our results stress the need to test for more than one parameter before generalizing population trends.

Despite the study limitations, our conclusions could reasonably be extended to small mammal species that depend on vegetation cover and avoid crossing gaps such as those induced by roads (paved lanes and vegetation clearing on its surroundings) (Oxley et al., 1974, Macpherson et al., 2011).

Less available space to settle in due to road pavement and vegetation clearing associated with the presence of roads seems to drive the differences observed in wood mouse populations living in road and roadless areas. Additionally, physiological stress, presumably induced by traffic, might contribute to our findings (Ware et al., 2015), although we have not gathered data to test this.

Small mammals could be resilient to roads and verge management locally.

Nevertheless, populations might be affected if management further restricts resource availability across the road network, as roads are one of the most widespread infrastructures across all modern landscapes. Moreover, smaller populations with poorer body condition at the roadside may hardly recover after critical periods. Thus, road-dominated environments may hamper the persistence of other endangered small mammal species.

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## Ethical Approval

All applicable international, national, and/or institutional guidelines for the
care and use of animals were followed. All the procedures followed the guidelines approved by the Portuguese Institute for Nature and Forest Conservation (ICNF - Instituto de Conservação da Natureza e das Florestas) and the American Society of Mammalogists for the use of wild mammals in research (Sikes et al. 2011).

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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# Supplementary materials 2

Table 2.S - Extended Pollock’s robust design models for the road and roadless areas. Notation: survival (ϕ), temporary emigration (Y''), temporary immigration (1-Y'), recapture rate (p), type of movement (even flow Y''= 1-Y'; no movement Y'' = 0, Y'=1), abundance (N), constant parameter (.), time-dependent parameters (t). Models with variation coefficients below 50% for all estimates in both areas are highlighted in grey.

| Type of movement | Model |
|---|---|
| **Even flow** | ϕ(.) Y''(.) = 1-Y'(.) p(.) N(.) |
| | ϕ(.) Y''(.) = 1-Y'(.) p(.) N(t) |
| | ϕ(.) Y''(.) = 1-Y'(.) p(t) N(.) |
| | ϕ(.) Y''(.) = 1-Y'(.) p(t) N(t) |
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| | ϕ(.) Y''(t) = 1-Y'(t) p(.) N(t) |
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| | ϕ(t) Y''(t) = 1-Y'(t) p(.) N(t) |
| | ϕ(t) Y''(t) = 1-Y'(t) p(t) N(.) |
| | ϕ(t) Y''(t) = 1-Y'(t) p(t) N(t) |
| **No movement** | ϕ(.) Y''= 0 Y'=1 p(.) N(.) |
| | ϕ(.) Y''= 0 Y'=1 p(.) N(t) |
| | ϕ(.) Y''= 0 Y'=1 p(t) N(.) |
| | ϕ(.) Y''= 0 Y'=1 p(t) N(t) |
| | ϕ(t) Y''= 0 Y'=1 p(.) N(.) |
| | ϕ(t) Y''= 0 Y'=1 p(.) N(t) |
| | ϕ(t) Y''= 0 Y'=1 p(t) N(.) |
| | ϕ(t) Y''= 0 Y'=1 p(t) N(t) |

# Chapter 3

## Effects of roads on small-mammal movements: opportunities and risks of vegetation management on roadsides

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# Effects of roads on small-mammal movements: opportunities and risks of vegetation management on roadsides.

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# Abstract

Roads can block animal movement and reduce persistence of species living in road surroundings. Movement restrictions on local populations may even increase extinction risk of abundant small mammals. However, road verges (road managed area between the edge of the road and the beginning of private land) may provide refuge and corridors for small mammals when properly managed. Information on the effects of roads and roadside management on small-mammal movement is still scarce for low traffic roads (< 20000 vehicles per day) crossing well-preserved habitats. We aimed to fill this gap by comparing fine-scale movement patterns of wood mice (*Apodemus sylvaticus*) in a road and in a similar roadless area without management. Both areas consisted of a well-preserved Mediterranean agro-silvo pastoral system. We studied several movement patterns: road crossings, verge use, length, and direction of movement. Additionally, we assessed how roadside management, animals' sex and residency status, season and microhabitat affect movement at the road area. At the roadless area, we defined a virtual road and verges at equivalent locations to the road area for comparison purposes. We gathered capture-mark-recapture data for two years to characterize movement patterns. Wood mice tended to avoid the road by crossing it less often and moving away from it more frequently than from equivalent locations in the roadless area. Wood mice used road verges more frequently than virtual verges and moved more often parallel to the road than to the virtual road. Road crossings were more frequent after firebreak openings (strips of mowed land) in surrounding areas and near taller shrubs. Also, males used road verges more often than females. Differences on several movement patterns between areas and their trends within the road area can be explained mainly by the presence of the road and roadside vegetation management (e.g., firebreaks openings). We suggest roadside vegetation management practices (e.g., avoid land mowing; maintain vegetation strips) to promote the role of verges as refuges and/or corridors for small mammals.

**Keywords:** *Apodemus sylvaticus*; Roadless area; Road barrier effect; Road verges; *Montado*; Movement step analysis

## 3.1. Introduction

Roads are among the ten major threats to biodiversity worldwide (Maxwell et al., 2016) and road networks are expanding rapidly (Laurance et al., 2014). The road network in Europe is already substantial with half of its area being less than 1.5 km away from a transportation infrastructure (Torres et al., 2016). Therefore, mitigation of road effects on biodiversity will become essential tools to fulfil United Nations' Sustainable Development Goals (Ibisch et al., 2016).

Among many harmful effects, roads contribute to habitat loss and fragmentation, which are main causes for species decline and extinction (Forman et al., 2003; Fahrig and Rytwinski, 2009; Benítez-López et al., 2010). Species persistence on fragmented habitats depends on their ability to move (through a hostile environment) and reach suitable patches to access food, shelter, and mates (Gardiner et al., 2019). Roads become barriers to movement when animals avoid roads and their surroundings or are road-killed (Fey et al., 2015; Carvalho et al., 2018). In fact, roads may affect wildlife populations some distance (>100 m) beyond their paved lanes (road-effect zone; Forman and Deblinger, 2000). The barrier effect of roads can be their greatest ecological impact because it may split and isolate populations (Forman and Alexander, 1998) and consequently reduce gene flow, decrease genetic diversity, and increase extinction risk (Holderegger and Di Giulio, 2010). Isolation induced by roads is particularly important for species with low mobility like most small mammals (Millward et al., 2020). Although small mammals often have large effective populations sizes (and so are less likely affected by genetic drift; Gaines et al., 1997), more isolated populations have been affected by genetic drift (e.g., Cape mole-rat, *Georychus capensis,* Visser et al., 2018; bank vole, *Myodes glareolus*, Guivier et al., 2011). In fact, a 25-year-old motorway (with an average of 28680 vehicles per day) has already significantly reduced movement, decreased gene flow and increased genetic substructuring of a population of bank voles (Gerlach and Musolf, 2000). This shows the importance of assuring safe road crossings even for abundant and common species, as the bank vole.

Small-mammal road crossings also depend on the species, sex, season (Garland and Bradley, 1984; Bakowski and Kozakiewicz, 1988; Goosem 2001; Gomez et al., 2011) and on the type of road pavement and width (Brock and Kelt, 2004; Grilo et al., 2016; Ji et al., 2017). Sex and residency status may affect movement patterns near roads particularly because in the breeding season resident male home ranges expand and they become more aggressive to transient males (e.g., wood mice, *Apodemus sylvaticus*; Gurnell, 1978; Wolton, 1985).

Although road pavement can block animal movement, its verges can be movement corridors across unsuitable habitat (Bennett, 1990), and promote connectivity even in suitable habitat (Galantinho et al., 2020). Nevertheless, the importance of road verges for small-mammal movements also depends on management practices on verges and surrounding areas (Galantinho et al., 2020; Ouédraogo et al., 2020). Those practices are related to road maintenance and safety (e.g., vegetation cutting, firebreak openings) or to agro-pastoral activities (e.g., ploughing, grazing). Management involves frequently the reduction of ground cover vegetation, which decreases habitat quality and consequently influences small-mammal movements (Gardiner et al., 2019).

On the road immediate surroundings, the effects of management on small-mammal movements have already been studied (Brock and Kelt, 2004; Chen and Koprowski et al., 2016a, 2016b; Ascensão et al., 2017), but still need to be clarified to properly adapt road mitigation measures (Ouédraogo et al., 2020). The road coupled with roadside vegetation management can further restrict small-mammal movement, isolate populations and compromise their long-term viability. Such studies may be especially relevant in small and low traffic roads crossing well-preserved habitat where roadkill may be particularly dramatic (van Langevelde et al., 2009) and animals have a lower body condition than in similar roadless areas (Galantinho et al., 2017). Low traffic roads represent 52% of the European road network (16.1% less than 5000 vehicles per day; 35.9% from 5000 to 20000 vehicles per day) (CEDR 2020).

In this study, our main goal was to understand the effect of roads on small-mammal movement patterns. We used the wood mouse (*Apodemus sylvaticus*) as a model for forest-dwelling small mammals because it often uses road surroundings and is sensitive to changes in ground vegetation cover (Tattersall et al., 2001, Ascensão et al., 2016). We compared wood mouse fine-scale movements in two similar areas in a well-preserved Mediterranean woodland. The main difference between areas was the presence of a road, road verges and management at the road area and their absence at the roadless area. We used capture-mark-recapture data to analyse movement steps (Calenge et al., 2009; McClintic et al., 2014; Edelhoff et al., 2016). Specifically, we investigated whether road crossing rates, verge use, length, and direction of movement differed between road and roadless areas. We expected a reduction in movements across the road and an increase in verge use at the road area when compared with equivalent lines in a roadless area (Bennett, 1990; Galantinho et al., 2020). Additionally, we examined whether animals´ sex and residency status, season, microhabitat characteristics, and management practices influenced movement patterns on the road area. Non-residents and male wood mice during the breeding season tend to move larger distances and to be less selective on habitat use (Gurnell, 1978; Wolton, 1985; Rosalino et al., 2011). Thus, we hypothesized that during the breeding season males and non-resident animals would cross the road and use the verge more often than females and resident animals, respectively. We anticipated that road crossing rates and verge use would increase when management practices reduced ground vegetation cover on road surroundings beyond verges (Galantinho et al., 2020; Ouédraogo et al., 2020). Animals would move away from the road when such management practices occurred in the verge (Gardiner et al., 2019). Understanding how roads and management on their surroundings affect animal movement would allow us to tailor guidelines for selective vegetation management, particularly on road verges. Differentiating vegetation management adjacent to and away from the road pavement, will enhance the role of road verges as small-mammal refuges and corridors, while simultaneously reducing roadkill risk.

## 3.2. Methods

### 3.2.1 Study area

This study was conducted in Alentejo, Southern Portugal (Figure 3.1) where the climate is Mediterranean with mild winters and hot, dry summers. Mean annual total rainfall is 609.4 mm and mean annual daily temperature is 15.9 °C (Évora 1971–2000; IPMA, 2019). The landscape is dominated by *montado,* a traditional Mediterranean savannah-like agro-silvo pastoral system of cork oak (*Quercus suber*) and holm oak (*Quercus ilex rotundifolia*) trees with grass and shrubs (Pinto-Correia et al., 2011). In spring, vegetation cutting and/or firebreak openings are implemented in verges of paved roads to reduce fire ignition risk and improve traffic safety (Decree-Law 156/2004, 30th June of the Portuguese Ministry of Agriculture, Rural Development and Fisheries). Firebreaks are strips of mowed land (often 10 to 15 meters wide) in adjacent farmland, but may also include the verge itself, depending on landowners and road operators. Adjacent farmland does not always belong to the same owner on both sides of the road. Therefore, decisions on firebreaks including or not road verges may differ between both sides of the road. There is an additional reduction in ground vegetation cover in road surrounding areas due to agro-silvo-pastoral practices, such as land ploughing and cattle grazing. Cork stripping also compromises vegetation structure despite occurring less often. This management practice removes the outer layers of cork from the tree trunk and wider branches usually every 9 years (Costa et al 2004).

### 3.2.2 Study species

The wood mouse frequently uses road verges (Galantinho et al., 2017, 2020) and is often road-killed (Carvalho and Mira, 2011) in the study region. This species is an important prey of many birds (e.g., tawny owl, *Strix aluco*; Southern and Lowe, 1968) and mammals, some of which are of conservation concern (e.g., European wildcat, *Felis sylvestris*; Sarmento, 1996). The wood mouse is common in Europe but is more abundant in dense understory cover in woodland areas, such as the *montado* (Alcántara and Díaz, 1996; Rosário and Mathias, 2004). Forest-dwelling small mammals may be affected by vegetation gaps caused by road pavement and verge maintenance, as well as by farmland management (Ascensão et al., 2016). In fact, wood mice react swiftly to changes in vegetation cover at fine and broad spatial scales (Tew et al., 1992; Tew and Macdonald, 1993; Tattersall et al., 2001). More specifically, paved lanes may be a partial barrier or filter for wood mice movement (Macpherson et al., 2011). Wood mice home ranges and movements vary according to habitat (Corp et al., 1997). In *montado* systems mean home range size (± SD) is 11240 ± 9250 $m^2$ (Rosalino et al., 2011) but in woodland areas with abundant resources home ranges may be smaller (e.g., 3457 ± 862 $m^2$, Corp et al., 1997). Shrubs and trees are among the most important microhabitat features for wood mice movement in *montado* systems (Rosalino et al., 2011). Presence and abundance of wood mice (Boitani et al., 1985; Tew et al., 2000), and trap efficiency (Cusak et al., 2015) also depend on microhabitat characteristics.

### 3.2.3 Study design

To study the effects of roads on wood mouse movements, we chose two 12000 m$^2$ areas in a landscape dominated by well-preserved *montado* woodland. The road area (38º24’N, 8º06’W) was bisected by the national road EN257 (average traffic volume of approximately 5000 vehicles per day and 600 vehicles per night; E.P., 2005). The roadless area (38º31’N, 8º01’W; University of Évora field station) was 16 km apart from the road area and more than 1 km away from any national paved road (more than 400 m from a regional low traffic road).

The two areas were carefully selected based on habitat similarity and suitability for wood mice. Wood mouse abundance was lower at the road area than at the roadless area, but density may be similar when excluding the area occupied by the road pavement and roadside management (Galantinho et al., 2017). Vegetation structure and composition and slope were similar on both areas (Supplementary materials 3.A). The key differences between areas were the presence at the road area (and absence at the roadless area) of paved lanes, road verges, and roadside management. Unburied wired fences bordered road verges and were permeable to small mammals and to non-volant predators (e.g., small carnivores). Although birds of prey may use fence posts as artificial perches (Malan and Crowe, 1997), we believe that fences would not have a major role in the differences in movement patterns because both areas have trees that are frequently used as perches.

### 3.2.4 Data collection

Wood mice were sampled at the road and roadless areas simultaneously, from March 2009 to March 2011. We conducted a trapping session consisting of four consecutive nights per area every four weeks, totalling 26 trapping sessions and 20800 trap-nights. Each trap was set on the first day and checked once a day on the following days at sunrise. At each area, we used medium size Sherman traps (8 x 9 x 23 cm) arranged in a square live-trapping grid (10 x 10 traps) (Figure 3.1). Distance between consecutive trap rows was 12 m to match the approximate width of the paved lanes and ensure equal spacing between rows. At the road area, the two central trap rows were established along road verges. At the roadless area, the two central trap rows were defined as “virtual verges” delimiting a “virtual road” for comparison purposes (*i.e.*, the “virtual road” was the space between the two central trap rows). Trap position was marked permanently for an accurate relocation on subsequent sessions. We provided a mixture of sardines, oil and oat flakes as bait and hydrophobic cotton as nest material. We registered the sex, age, length, and weight of every trapped wood mouse. Animals were individually marked with passive integrated transponders tags (PIT, TXP148511B, 8.5 mm x 2.12 mm, 134.2 kHz ISO, 0.067g, Biomark, Boise, USA) and released at capture location immediately after handling. These procedures were reviewed by the animal care committee of the Portuguese Institute for Nature and Forest Conservation (ICNF - Instituto de Conservação da Natureza e das Florestas). All these procedures followed the guidelines from ICNF and the Directive 2010/63/EU for animal experiments. Altogether, we captured 159 different wood mice (on 540 occasions) at the road area and 353 different wood mice (on 1637 occasions) at the roadless area. We also sampled three microhabitat characteristics - shrub height, shrub cover and tree cover - in a 1 $m^2$ around each trap on every session to assess differences within and between areas over time.

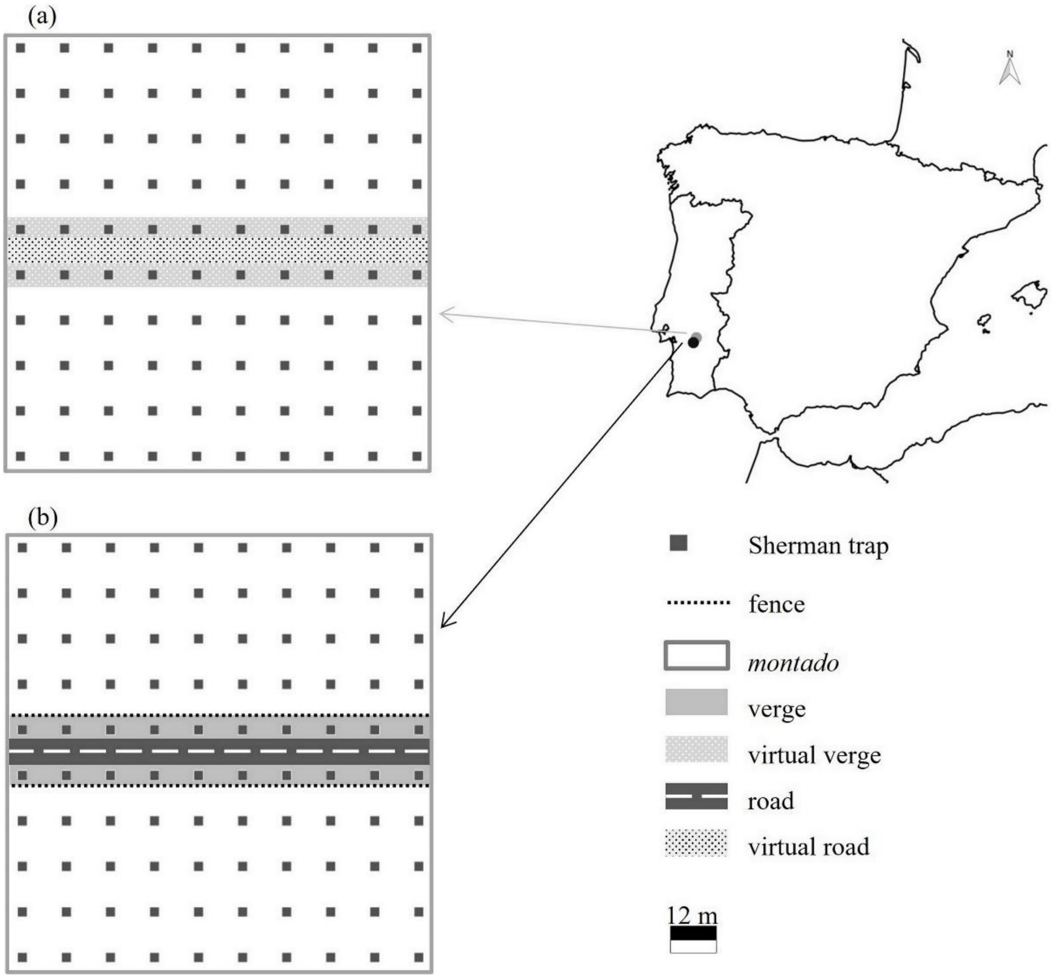

Figure 3.3 - Schematic arrangement of traps in a square grid (10 x 10 traps; 12 m trap spacing) at the roadless (a) and road (b) areas.

### 3.2.5 Data analysis

#### *3.2.5.1 Response and explanatory variables*

We used capture-recapture data collected at our trapping grids to analyse wood mice movement. Trapping data were converted into movement steps using the function “as.ltraj” from “adehabitatLT” package version 0.3.25 (Calenge, 2006; Calenge et al., 2009) in R version 3.0.2 (R Core Team, 2013). We considered a movement step as the straight-line distance between the trapping locations of two consecutive captures of the same animal (during the same trapping session). A sequence of at least three captures (two steps) of the same animal in a trapping session was considered a movement burst. We then used two movement metrics - step length and step absolute angle - to derive four of the five studied response variables: “verge use” (verge/virtual verge use; *i.e.,* whenever a step started, ended or crossed a verge), “parallel” (parallel movement to the road/virtual road), “move away” (movement away or towards the road/virtual road) and “length” (length of movement step). “Road (or virtual road) crossings” was our fifth response variable. However, due to the low number of crossings within each session at the road area, we also accounted for crossings that occurred between sessions. Thus, we considered a “crossing” whenever an animal was captured in the side of the road (or virtual road) opposite to the side where the animal was last captured, even if both captures occurred in different sessions. Each response variable was modelled separately. Altogether, we used three binary response variables (presence/absence) - “crossings”, “verge use” and “parallel” – and two continuous response variables - “length” (m) and “move away” (sine of the steps’ absolute angle in relation to the road/virtual road). “Move away” ranged from -1 (moving towards: in a 270º angle in the north side of the grids; in a 90º angle in the south side of the grids) to +1 (moving away: in a 90º angle in the North side of the grids; in a 270º angle in the South side of the grids) (Figure 3.2). At the road area, crossing behaviour was analysed for 86 animals (50 females and 36 males) with 224 movements (length > 0m). The analyses for the remaining movement patterns (“verge use”, “move away”, “parallel”, “length”) included 47 animals (25 females and 22 males) with 73 movement steps (length > 0m) at the road area. At the roadless area, crossing behaviour (of the virtual road) was analysed for 209 animals (100 females and 109 males) with 952 movements (length > 0m). The analyses for the remaining movement patterns (“verge use”, “move away”, “parallel”, “length”) included 123 animals (65 females and 58 males) with 385 movement steps (length > 0m) at the roadless area.

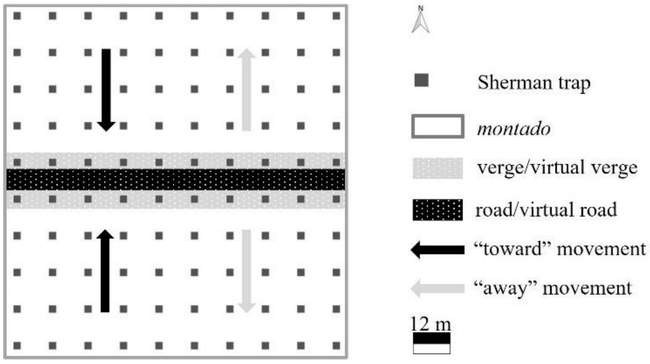

Figure 3.2 – Schematic example of “toward” and “away” movement on both sides of the road/virtual road.

We used as explanatory variables: “area type” (road/roadless), two variables referring to animals’ condition (“residency status” and “sex”), three microhabitat characteristics (“shrub cover”, “tree cover” and “shrub height”), and “season”. Wood mice were considered residents if trapped in more than one session (Garland and Bradley, 1984). Each microhabitat value assigned for each movement was the mean between the value at the source and at the destination traps. The mean values were then classified into four classes to reduce measurement errors. We classified shrub and tree cover in four cover classes (class 1: 0-25%; class 2: 25-50%; class 3: 50-75%; class 4: 75-100%) and shrub maximum height (cm) in four height classes (each class represents 25% of the observations, *i.e.,* quartiles; Altman and Bland, 1994). At the road area, five management practices occurred: “verge vegetation cutting”, “firebreak openings”, “cattle grazing”, “ploughing” and “cork stripping”. We did not observe movement bursts during or immediately after “ploughing” and “verge vegetation cutting”, therefore, we only used “firebreak openings”, “cattle grazing” and “cork stripping” as explanatory variables. We considered that a step occurred at a managed section of the road area whenever it started, ended, or crossed that section during or immediately after (subsequent session) management practices.

We also used “initial distance” to the road as an explanatory variable for the analysis of “move away” and “parallel” because steps that begin on the outermost lines cannot move away from the centre any further. Additionally, “length” was an explanatory variable when analysing “verge use” because longer steps should be more likely to reach the verges.

#### *3.2.5.2 Differences in wood mouse movement patterns at the road and roadless areas*

We used a first set of models to assess if movement patterns were different between the road and the roadless areas. For this set of models, we considered 1176 movements when analysing “crossings” and 458 steps when analysing the remaining response variables. We modelled each of the five response variables as function of “area type” and of each of the three microhabitat characteristics (“shrub cover”, “shrub height” and “tree cover”), using different model structures (see details on section 2.5.4). The three microhabitat variables accounted for differences in movement characteristics between the road and roadless areas that could result from differences in vegetation between the two areas rather than from the presence of the road itself.

Metric and temporal traits of movement may be distinctive of each individual wood mouse (Benhamou, 1990). Therefore, movement patterns may be similar for the same animal (and different from the overall population) or within the same trap session or season. Random effects allow us to accommodate such idiosyncrasies in our analysis. Thus, we used two random effects - “animal” and “season” – for all response variables. We also used “burst” as a random effect for all variables except for “crossings”. “Crossings” include movements within and between sessions while bursts only account for movements within sessions.

#### *3.2.5.3 Drivers of wood mouse movements at roadsides*

We used a second set of models to specifically assess how road and management practices affect movements at the road area. This second model set exclusively used data collected at the road area and included only the response variables that were significantly different between the road and the roadless areas (i.e., variables for which “area type” had a significant effect), in the first model set (section 2.5.2). For this set of models, we considered 224 movements when analysing “crossings” and 73 steps when analysing the remaining response variables.

The selected response variables were modelled using as explanatory variables: three management variables (“firebreak openings”, “cattle grazing” and “cork stripping”), two animal’s condition variables (“residency status” and “sex”), three microhabitat variables ("shrub cover", "shrub height" and "tree cover") and "season". Only microhabitat variables significant in the first set of models (section 2.5.2) were considered as explanatory variables for the second set of models. Additionally, we used as explanatory variables "initial distance" to the road (to analyse "move away" and "parallel") and "length" (to analyse "verge use"). We also considered two random effects ("animal" and "burst") for each response variable (but only "animal" for "crossings").

### *3.2.5.4 Model building and selection*

On both model sets (sections 2.5.2 and 2.5.3), the significance of every explanatory variable for each response variable was preliminarily assessed individually with generalized linear models (GLM) (Hosmer and Lemeshow, 2000). Only explanatory variables with 95% confidence intervals excluding zero were considered to have a strong support for an effect and were retained for further analysis. Then, in a multivariate analysis (i.e., response variable vs all relevant explanatory variables), we checked for collinearity using the generalized variation inflation factor (GVIF) and discarded variables with GVIF > 3 (Zuur et al., 2009). The candidate model set for each response variable included all possible combinations of non-collinear variables. We used Akaike's Information Criterion corrected for small samples (AICc) to rank models for each response variable (Burnham and Anderson, 2002). When necessary, we used generalized least squares estimation (GLS) to incorporate variance structures in the models to account for heterogeneity and spatiotemporal autocorrelation (Zuur et al., 2009).

For each response variable, we only included a random effect (generalized linear mixed models; GLMM) if it improved the models (lower AICc). We accounted for the best model and proceeded with the analysis whenever the best model explained considerably more variation in a response variable than the null model ($\Delta$AICc > 4 between best model and null model; Burnham and Anderson, 2002). Model averaging was used to estimate parameters and associated unconditional standard errors (SE) for the subset of competing models at $\Delta$AICc < 4 from the best model (Burnham and Anderson, 2002). We assessed the relative importance of each variable ($w+$) by the sum of Akaike weights ($w$) of all models of each set where the variable was included (i.e., the relative importance of the variable increases with its weight; Burnham and Anderson, 2002). Additionally, a variable was considered to have low support if the 95% confidence interval for its estimate included zero.

The autocorrelation in model residuals was assessed by Moran’s I for spatial patterns and by the autocorrelation function for temporal patterns (ACF). Random effects and variance structures (for heterogeneity and temporal and spatial autocorrelation) are only mentioned in the results sections (3.1 and 3.2) when their use originated models with a lower AICc.

All statistical analyses were conducted in R version 3.0.2 (R Core Team, 2013). GLMM and GLS were implemented using function “lme” and “gls” from package “nlme” version 3.1.-113 (Pinheiro et al., 2013), respectively. Function “glm” from package “stats” version 4.0.1 (R Core Team, 2017) was used to implement GLM. We used function “vif” from package “car” version 3.0-8 to assess collinearity (Fox and Weisberg, 2011); package “spdep” version 1.1-3 to check for spatial autocorrelation (Bivand et al., 2018); package “nlme” version 3.1.-113 to check for temporal autocorrelation (Pinheiro et al., 2013) and package “MuMIn” version 1.43.17 to implement model averaging (Barton, 2014).

Variables and random effects are summarized in Table 3.1. Data collection, data processing and model building are summarized in Supplementary materials 3.B. Detailed results for model building, selection and validation are provided as Supplementary materials 3.C.

Table 3.1 – Response variables, random effects and explanatory variables used in the analysis of (1) differences in wood mouse movement patterns at the road and roadless areas and of (2) drivers of wood mouse movements at roadsides.

| **Variable** | **Definition** | **Type** | **Units** |
|---|---|---|---|
| **Response variables** | | | |
| Length (1) | distance in a straight line between beginning and end of step | continuous | (m) |
| Move away [a] (1,2) | sine of steps’ absolute angle in relation to road/virtual road | continuous | [-1, 1] |
| Parallel (1,2) | step was/was not (1/0) parallel to road/virtual road | binary | (1/0) |
| Crossings [b] (1,2) | movement did/did not cross (1/0) road/virtual road | binary | (1/0) |
| Verge use (1,2) | step did/did not (1/0) start, end or cross verge/virtual verge | binary | (1/0) |
| **Random effects** | | | |
| Animal (1,2) | animals' identification | nominal | na |
| Burst (1,2) | bursts' identification | nominal | na |
| Season [c] (1) | Spring, Summer, Autumn, Winter | nominal | na |
| **Explanatory variables** | | | |
| ***Area Type* (1)** | | | |
| Area | road/roadless (1/0) | binary | (1/0) |
| ***Animal's condition* (2)** | | | |
| Residency status | resident captured in >1 sessions/ transient captured in 1 session (1/0) | binary | (1/0) |
| Sex | male/female (1/0) | binary | (1/0) |
| ***Movement* (2)** | | | |
| Initial distance [d] | distance in a straight line between beginning of step and road | ordinal | (m) |
| Length [e] | distance in a straight line between beginning and end of step | continuous | (m) |
| ***Microhabitat* [f] (1,2)** | | | |
| Shrub cover | | ordinal | (%) |
| | shrub cover at beginning and end of step (class 1) | | [0-25] |
| | shrub cover at beginning and end of step (class 2) | | ]25-50] |
| | shrub cover at beginning and end of step (class 3) | | ]50-75] |
| | shrub cover at beginning and end of step (class 4) | | ]75-100] |
| Tree cover | | ordinal | (%) |
| | tree cover at beginning and end of step (class 1) | | [0-25] |
| | tree cover at beginning and end of step (class 2) | | ]25-50] |
| | tree cover at beginning and end of step (class 3) | | ]50-75] |
| | tree cover at beginning and end of step (class 4) | | ]75-100] |
| Shrub height | | ordinal | (cm) |
| | shrub maximum height at beginning and end of step (class 1) | | [1.00-116.00] |
| | shrub maximum height at beginning and end of step (class 2) | | ]116.00 – 208.50] |
| | shrub maximum height at beginning and end of step (class 3) | | ]208.50-297.25] |
| | shrub maximum height at beginning and end of step (class 4) | | ]297.25-412.00] |
| ***Management practices* (2)** | | | |
| Cattle grazing | step did/did not (1/0) start, end or cross cattle grazing | binary | (1/0) |
| Firebreak openings | step did/did not (1/0) start, end or cross firebreaks | binary | (1/0) |
| Cork stripping | step did/did not (1/0) start, end or cross cork stripping | binary | (1/0) |

Each variable was either used (1) in the first set of models, (2) in the second set of models or (1,2) in both sets of models. [a] “Move away” ranged from -1 (moving towards: in a 270° angle in the north side of the grids; in a 90° angle in the south side of the grids) to +1 (moving away: in a 90° angle in the North side of the grids; in a 270° angle in the South side of the grids). [b] Road and virtual road “crossings” accounted for all crossing movements, *i.e.*, not only those that occurred within (steps) but also between trapping sessions. [c] In the second set of models (2) “season” was used as fixed effect. [d] “Initial distance” to the road was used as explanatory variable for “move away” and “parallel” models. [e] Step “length” was used as explanatory variable for “verge use” models. [f] Shrub and tree cover were averaged and classified in four classes and shrub maximum height was averaged and classified in quartiles to reduce possible measurement errors.

## 3.3. Results

### 3.3.1 Differences in wood mouse movement patterns at the road and roadless areas

Wood mice crossed the road (5.8% of all movements) less often than the virtual road at the roadless area (12.8% of all movements), and mostly at locations with high tree cover (50-75%) (Figure 3.3.a; Table 3.2). At the road area, 52% of wood mouse steps included the verges (82% of which were entirely within the verge) while at the roadless area, 30% of the steps included the virtual verges (28% of which were entirely within the virtual verges). Wood mice used road verges more than virtual verges and mostly where shrub cover was high (50-75%) (Figure 3.3.b; Table 3.3).

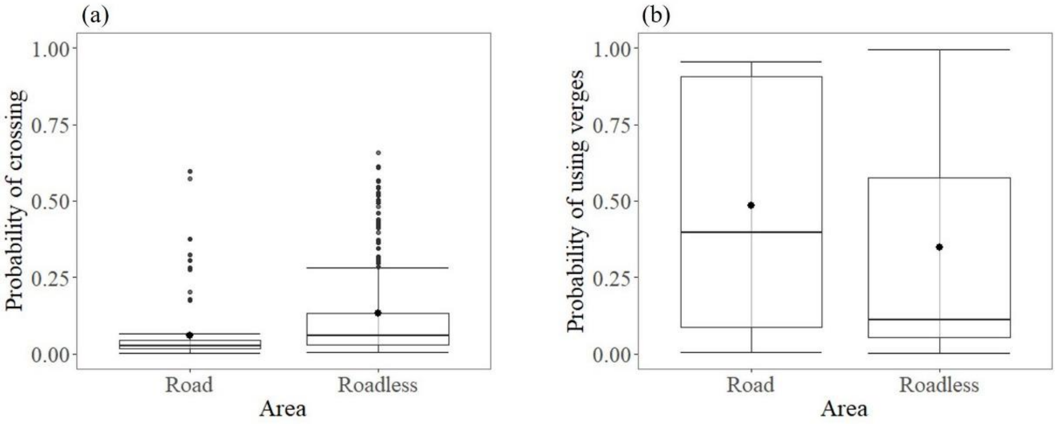

Figure 3.3 - Boxplots represent the empirical distribution of the: (a) the probability of crossing the road and the virtual road (at the roadless area) and (b) the probability of using verges and virtual verges (at the roadless area). Probabilities were calculated using the averaged model with data from road and roadless areas (results section 3.1). On each boxplot the bigger dot is the mean, and the darker horizontal line is the median. The length of the box represents the interquartile range (i.e. difference between 75th and 25th percentiles). The dots outside the whiskers are outliers (a).

Table 3.2 - Averaged model (GLMM) of the estimated effects of “area type” (road or roadless) and “tree cover” on road and virtual road “crossings”. Model averaging accounted for “animal” as random effect and temporal autocorrelation. Model averaging is based on the confidence set of models at ΔAICc < 4 from the best model. We show standard errors (SE), 95% confidence intervals (CI) and relative importance ($w_+$) of each variable involved. Estimates whose 95% CI excluded zero are in bold. The reference categories are “road” for “area type” and “class 1” for “tree cover”.

| | Estimate | SE | CI | w+ |
|---|---|---|---|---|
| (Intercept) | 0.05 | 0.03 | (-0.01, 0.12) | |
| **Area type** | | | | **1.00** |
| **roadless** | **0.08** | **0.03** | **(0.02, 0.15)** | |
| **Tree cover** | | | | **0.63** |
| class 2 | 0.01 | 0.02 | (-0.03, 0.04) | |
| **class 3** | **0.08** | **0.03** | **(0.02, 0.14)** | |
| class 4 | 0.04 | 0.04 | (-0.03, 0.12) | |

Table 3.3 - Averaged model (GLMM) of the estimated effects of “area type” (road or roadless) and “shrub cover” on “verge use”. Model averaging accounted for “animal” as random effect and temporal autocorrelation. The reference categories are “road” for “area type” and “class 1” for “shrub cover”. See Table 3.2 for other details.

| | Estimate | SE | CI | w+ |
|---|---|---|---|---|
| (Intercept) | 0.31 | 0.12 | (0.08, 0.54) | |
| **Area type** | | | | **0.77** |
| **roadless** | **-0.18** | **0.09** | **(-0.35, -0.01)** | |
| **Shrub cover** | | | | **1.00** |
| class 2 | 0.14 | 0.09 | (-0.04, 0.31) | |
| **class 3** | **0.21** | **0.09** | **(0.03, 0.39)** | |
| class 4 | 0.10 | 0.09 | (-0.09, 0.28) | |

Wood mice moved mostly towards the virtual road at the roadless area (42.4% of steps towards and 27.3% away), but frequently away from the road at the centre of the road area (17.8% of steps towards and 24.7% away) (Figure 3.4; Table 3.4). “Parallel” movement to the road or virtual road occurred in 57.5% and 30.4% of movement steps at the road and roadless areas, respectively (Figure 3.4). Wood mice moved more frequently “parallel” to the road than to the virtual road, mostly in areas with taller shrubs (Table 3.5).

Movement “length” was on average 20.8 ± 11.8 m at the road area (range: 12 – 64.6 m) and 19.3 ± 10 m at the roadless area (range: 12 – 80.5 m). Movement “length” was not significantly different between the road and the roadless areas (length estimate for roadless area = -1.4; CI (-4.0, 1.2)). Therefore, movement “length” was excluded from any further analysis.

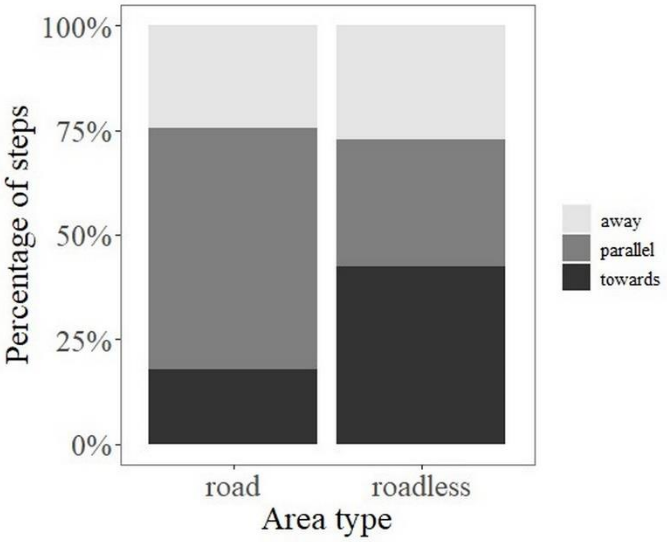

Figure 3.4 – Percentage of steps moving “away”, “parallel” and “towards” the road (at the road area) and the virtual road (at the roadless area). Percentages are based on 73 steps at the road area and 385 steps at the roadless area.

Table 3.4 – Model (GLS) describing the estimated effects of "area type" (road or roadless) on "move away" (from the road or virtual road) accounting for temporal autocorrelation. The reference category for "area type" is "road". See Table 3.2 for other details.

| | Estimate | SE | CI |
|---|---|---|---|
| (Intercept) | 0.06 | 0.07 | (-0.07, 0.19) |
| **Area type** | | | |
| **roadless** | **-0.19** | **0.07** | **(-0.32, -0.05)** |

Table 3.5 - Averaged model (GLMM) of the estimated effects of "area type" (road or roadless), "shrub height", and "tree cover" on "parallel" movement (to the road or to the virtual road) accounting for "burst" as a random effect. The reference categories are "road" for "area type" and "class 1" for the remaining variables. Exponent notation means multiplication by: a=$10^{-1}$. See Table 3.2 for other details.

| | Estimate | SE | CI | w+ |
|---|---|---|---|---|
| (Intercept) | 0.55 | 0.07 | (0.43, 0.68) | |
| **Area** | | | | **1.00** |
| **roadless** | **-0.29** | **0.07** | **(-0.42, -0.15)** | |
| **Shrub height** | | | | **1.00** |
| class 2 | 0.09 | 0.07 | (-0.04, 0.22) | |
| class 3 | 0.21 | 0.12 | (-0.04[a], 0.42) | |
| **class 4** | **0.34** | **0.12** | **(0.11, 0.57)** | |
| Tree cover | | | | 0.31 |
| class 2 | -0.03 | 0.05 | (-0.13, 0.07) | |
| class 3 | 0.08 | 0.08 | (-0.07, 0.23) | |
| class 4 | 0.13 | 0.09 | (-0.06, 0.31) | |

### 3.3.2 Drivers of wood mouse movements at roadsides

Road crossings were more likely during or immediately after “firebreak openings” and at locations with taller shrubs (Table 3.6; Figure 3.5.a and 3.5.b). “Sex” had a strong effect on “verge use” by wood mice (Table 3.7; Figure 3.5.c): males (64.5% of movements) used the verge more frequently than females (42.9% of movements). The effect of “cattle grazing” on “verge use” had low support (95% CI for estimates included zero; Table 3.7).

“Move away” was not explained by any of the descriptive variables (95% CI for estimates included zero) at the road area. The best model explaining “parallel” movement to the road was not better than the null model (ΔAICc < 4; Table 3.C.S6, Supplementary materials 3.C).

Table 3.6 - Averaged model (GLMM) of the estimated effects of “firebreak openings”, “shrub height” and “distance to the road” on road crossings. Model averaging accounted for “animal” as random effect and for temporal correlation. The reference categories are “absence” for “firebreak openings” and “class 1” for “shrub height”. Exponent notation means multiplication by: $b=10^{-2}$; $c=10^{-3}$; $d=10^{-4}$. See Table 3.2 for other details.

| | Estimate | SE | CI | w+ |
|---|---|---|---|---|
| (Intercept) | 0.04 | 0.02 | ($-0.04^b$, 0.09) | |
| **Firebreak openings** | **0.17** | **0.06** | **(0.05, 0.29)** | **1.00** |
| **Shrub height** | | | | **1.00** |
| class 2 | 0.08 | 0.05 | (-0.03, 0.18) | |
| **class 3** | **0.52** | **0.11** | **(0.30, 0.74)** | |
| Distance to road | $-0.39^d$ | $0.09^b$ | ($-1.80^c$, $1.72^c$) | 0.25 |

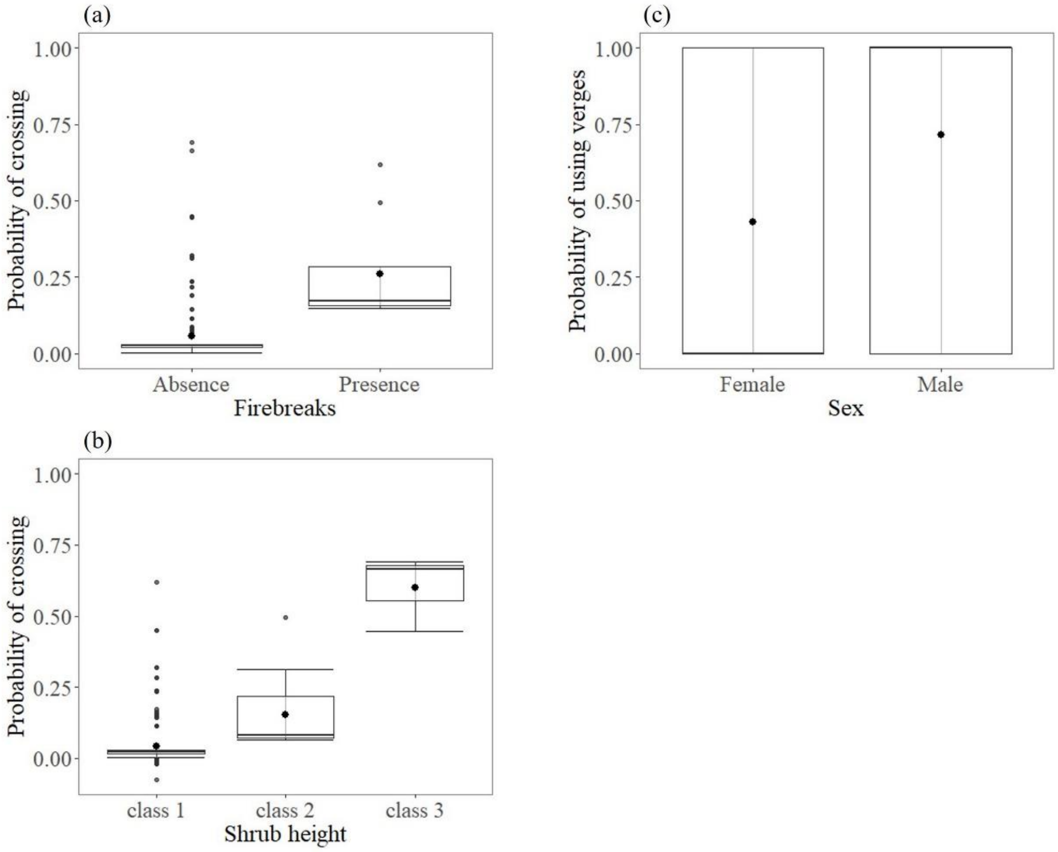

Figure 3.5 - Boxplots represent the empirical distribution of the: (a) probability of crossing the road in the absence and in the presence of firebreaks; (b) probability of crossing the road in the three classes of shrub height; (c) probability of using verges for females and males. Probabilities are based on the averaged model using only road data (results section 3.2). On each boxplot the bigger dot is the mean and the darker horizontal line is the median. The length of the box represents the interquartile range (i.e., difference between 75th and 25th percentiles). The dots outside the whiskers are outliers (a, b).

Table 3.7 - Averaged model (GLMM) of the estimated effects of “cattle grazing” and “sex” on “verge use” at the road area. Model averaging accounted for “burst” as random effect. The reference categories are “absence” for “cattle grazing” and “female” for “sex”. See Table 3.2 for other details.

| | Estimate | SE | CI | w+ |
|---|---|---|---|---|
| (Intercept) | 0.46 | 0.09 | (0.29, 0.63) | |
| Cattle grazing | -0.31 | 0.17 | (-0.66, 0.04) | 0.66 |
| **Sex** | | | | **0.90** |
| **Male** | **0.27** | **0.11** | **(0.04, 0.50)** | |

# 3.4. Discussion

Movement patterns of wood mice living in a road-bisected habitat were clearly different from those observed in a similar roadless area. Wood mice crossed the road less often and moved away from it more frequently than from the virtual road at the roadless area. Simultaneously, wood mice used more road verges than virtual verges and moved more frequently parallel to the road than to the virtual road. These findings support our hypothesis that roads affect wood mouse movement patterns. At the road area, we also found that firebreak openings and higher shrubs promote road crossings and that males tend to use more road verges than females. Our contribution relies on assessing the influence of roads and roadside management on small-mammal movement patterns by contrast with a similar reference area. Our findings support our recommendations for roadside management for small-mammal conservation.

### 3.4.1 Wood mice movement patterns at road and roadless areas

Differences in movement patterns between road and roadless areas can be explained mainly by the presence of the road and associated vegetation management. The percentage of wood mice crossing the road (5.8% of movements corresponding to 5% of captured animals) matches the value obtained from genetic data for this species (5%; Ascensão et al., 2016). These crossing rates may prevent genetic structuring of wood mice populations living near roads (Ascensão et al., 2016). Several small-mammal species avoid crossing roads (e.g., Garland and Bradley, 1984; Brehme et al., 2013; Ascensão et al., 2017), including the white-footed mouse (*Peromiscus leucopus*), which is the ecological equivalent to the wood mouse in America (Marrotte et al., 2014). Road avoidance has been mainly associated with the lack of vegetation cover on road lanes that may increase predation risk (Marrotte et al., 2014; Ascensão et al., 2017). Concurrently, higher vegetation cover at roadsides can attract small mammals and induce an ecological trap effect by also attracting their predators to an area of high roadkill risk (Martinig and McLaren 2019; Silva et al., 2019). Wood mice move more frequently under dense vegetation cover (Montgomery, 1985; Rosalino et al., 2011) and adjust foraging movements and activity to decrease predation risk (Díaz et al., 2005). In our study, road crossings occurred more frequently after implementing firebreaks and where taller shrubs existed at verges. Wood mice also moved more parallel to the road (than to the virtual road), which suggests that they avoid the road surface (e.g., Jaeger et al., 2005) and/or they are looking for a safer passage to cross (D'Amico et al., 2015; Bakker and Van Vuren, 2004). Indeed, wood mice roadkills are more frequent where less culverts occur (Ascensão and Mira 2006). Firebreak openings abruptly remove most of the shrub and herbaceous layers (on verges and adjacent area) forcing animals to seek shelter in patches of taller vegetation. Only one animal crossed the road into a firebreak (between shrubs of similar height). This animal had crossed twice between verges, which suggests that both verges were part of its home range. Home range location (site fidelity) is one of the main factors influencing road crossings by small mammals (Grilo et al., 2018). Animals whose home ranges include road verges could probably continue to use them if vegetation is not fully removed by firebreaks. During our study, firebreaks never occupied both verges simultaneously even when conducted on both sides of the road (i.e., at one side of the road firebreaks were conducted at the private land and at the road verge, while at the other side of the road firebreaks were only conducted at the private land).

Lack of vegetation cover might not be the only factor preventing road crossings because wood mice moved away from the road independently of their distance to the road. Actually, wood mice movements away and parallel to the road could reveal conspecific avoidance since road verges were more used than virtual verges, especially by males. Overall, higher verge use was expected because road verges are particularly good connectivity providers for wood mouse foraging movements when compared with surrounding areas (Galantinho et al., 2020). Typically, wood mice males have larger home ranges that overlap with several females (Wolton, 1985). Additionally, dominant males often patrol their territories (Brown, 1969) and may perceive linear structures as limits of their home ranges such as other small mammals (Underhill and Angold, 2000). In *montado* systems, females move mainly in cork oak woodland with understory, while males do not show any preference (Rosalino et al., 2011). Therefore, females may perceive road verges as more exposed to predation because they are bordering an unvegetated area (Gardiner et al., 2019) thus avoiding them more than males. Female wood mice also have higher physiological stress near roads than males (Navarro-Castilla et al., 2014). Traffic disturbance may also play a role in promoting movement away from the road (Brehem et al. 2013). However, traffic did not have a strong effect on road crossings (Ford and Fahrig, 2008; McGregor et al., 2008; Ascensão et al., 2016) nor on the direction of movement in relation to roads of several small-mammal species (Grilo et al., 2018). Traffic intensity only marginally increased movement away from roads of water voles (*Arvicola sapidus*) (Grilo et al., 2018).

We were also unable to detect strong effects of cattle grazing and cork stripping on wood mouse movement patterns. Although cattle grazing decreases vegetation availability and cork stripping changes its structure, both practices do not strongly affect functional connectivity of the area for wood mice (Galantinho et al., 2020). Cork stripping occurs only during ~2h per tree and every 9 years (Costa et al., 2004). Cattle density was low and never entirely removed shrubs and grass in our study area. Nevertheless, wood mouse movement patterns in areas under high intensity grazing near roads can be different from those found by us (Johnston and Anthony, 2008).

We did not find a strong effect of season on movement patterns. Similarly, seasonality did not affect road crossing rates by the montane akodont (*Akodon montensis*) (Ascensão et al., 2017) despite that males move longer distances than females in the breeding season, such as male wood mice (Wolton, 1985; Gomez et al., 2011). Indeed, roads are a permanent and lasting disturbance (Coffin, 2007; Chen and Koprowski, 2016a) due to the infrastructure itself and the consistency of traffic and thus their impacts on small-mammal movements may not change seasonally.

### 3.4.2. Roadside vegetation management recommendations for small-mammal conservation

The wood mouse was our model species and insights from our results can be applied and are likely to be helpful to other species. We showed that higher shrub cover and height favours the use of verges and road crossings by wood mice. In fact, in open habitats, road verges may be the only refuge and corridor for small mammals (Pita et al., 2006; Sabino-Marques and Mira, 2011). Thus, vegetation management on roadsides should meet conservation goals for these species by providing habitat refuges and/or corridors while minimizing roadkill risks. Patches of well-preserved habitat at road verges and/or surrounding areas need to be strategically considered, particularly in high road density areas.

Management should maintain a strip of verge vegetation when roadkill risk is low (e.g., low traffic roads). This continuous strip of vegetation should be at least 1m wide with 50% shrub cover including taller (more than 3m) shrubs in the outer side of the verge (near the fence). In high traffic roads where roadkill risk is high for vulnerable species, we recommend cutting vegetation at both road verges simultaneously to avoid small-mammal crossings. Land ploughing should be avoided because it removes vegetation and destroys underground nests, forcing small mammals to move and so increasing roadkill hazard. However, systematically suppressing road crossings in an expanding road network may negatively affect even currently abundant small-mammal species. Although the common hamster (*Cricetus cricetus*) was considered a pest (in mid-20th century), it is now extinct in parts of its former range (La Haye et al., 2014) and is overall Critically Endangered (CR) (Banaszek et al., 2020). Road verges provide favourable habitat for this species, but construction and improvement of road networks has increasingly reduced and isolated populations, leading to local extinctions (Banaszek et al., 2020).

Roadside vegetation management must also consider fire risk near roads, especially in Mediterranean regions. In Portugal, 50% of wildfires occur at less than 22m of roads (Parente et al., 2018). In high fire risk landscapes, we suggest maintaining small natural vegetation plots at regular intervals instead of a continuous strip of tall vegetation near the fence. These plots would provide temporary refuges and act as steeping stones for small-mammal movements. However, the minimum size and the maximum distance between plots must still be carefully assessed.

Habitat at road verges will be especially important to maintain small mammals during disturbances on surrounding areas. Ideally, the management on road verges and on surrounding private land should be agreed with landowners to avoid vegetation cutting or removal simultaneously on both areas. Firebreak openings on private land should be temporally asynchronous with verge vegetation cutting to avoid a sudden reduction of habitat availability over a wider area. When formal agreements are not possible, we recommend that firebreak openings on private land and verge vegetation cutting should altogether occupy the minimum clearing width prescribed by law (10 m).

Ensuring safe road crossings is paramount for the viability of populations not only of currently abundant species but especially of rare and endangered ones. Therefore, the increased roadkill risk of maintaining vegetation (unharmed or partially cut) at road verges should be compensated by providing safe passages (e.g., adapting existent culverts or building new ones) and vegetation corridors to guide small mammals to those locations (D’Amico et al., 2015; McDonald and Saint Claire, 2004). In fact, the most common management practices implemented for small mammals involve enhancing safe road crossings by using overpasses and culverts (ledges for small mammals in underpasses, Meaney et al., 2007; arboreal bridge for *Muscardinus avelanarius*, White and Huges, 2019) but few refer to vegetation management (e.g., underpass associated to vegetated highway median, Martinig and McLaren 2019). Nevertheless, vegetation management for small-mammal conservation near roads can be especially important where safe passages are absent.

Additionally, enhancing vegetation at road verges may increase their use by ungulates and small mammals and their predators (carnivores and birds of prey) (Ascensão et al., 2012, Martinig and McLaren 2019, Silva et al., 2019). Therefore, increasing vegetation cover at road verges may increase roadkill risk for larger species. Consequently, management must always consider how the suggested practices affect other species, especially where roadkill risk for endangered species is high. More specifically, on roadkill hotsposts, our recommendations should only be applied when coupled with higher fences with smaller mesh size that would prevent most larger species from moving into the road (e.g., Grilo et al., 2009). Promoting vegetation on verges may decrease drivers’ visibility and increase the risk of colliding with a large animal. This risk may specially increase in high-speed unfenced roads. Under those circumstances, vegetation should be cleared on the 3m strip adjacent to the road pavement.

### 3.4.3. Strengths, potential limitations, and future research

Our trapping effort enabled us to assess movement without using translocation that is the most common method in such studies. We included temporal and spatial autocorrelation in our models to account for the potential limitation of pseudoreplication and enable valid inferences (Davies and Gray, 2015). Additionally, we also accounted for changes in vegetation structure typical of well-preserved *montado* systems. Although species behaviour towards roads depends on their microhabitat preferences and movement ability, and road width and substrate (Ji et al., 2017; Grilo et al., 2018), we provide scientific evidence that can be adapted for management at other roadside areas. Even so, although we accounted for limitations of pseudoreplication, our recommendations should be considered with caution because we only used two study areas in a specific landscape (*montado*).

Although telemetry is one of the best methods to analyse movement, capture-mark-recapture (CMR) enables tracking more animals, during a longer time with a smaller budget (Gomez et al., 2011). Still, capture-mark-capture prevented us from tracking all possible movements during a trapping session. Particularly, telemetry could provide additional information on the effects of residency status and immediate impact of verge vegetation cutting and ploughing on movement patterns. Nevertheless, we do not present movement measures but instead we use movement patterns to compare areas sampled simultaneously with the same method. Therefore, we believe that our results and inferences are not compromised for comparative purposes. Above all, CMR and telemetry have consistent results and are successfully used in studies of small-mammal movements (Clark et al., 2001; Gomez et al., 2011; Ramsay et al., 2019).

Although our study proposes verge management measures as a tool to small-mammal conservation, some aspects deserve further investigation. For example, assessing the minimum amount of verge vegetation to minimize movement disturbance may be particularly important to ensure that specialist small mammals find and use culverts efficiently across roads (D’Amico et al., 2015).

## 3.5 Conclusions

The presence of a road and associated vegetation management influences several wood mouse movement patterns (conditioning road crossings and movement direction) even in a well-preserved and favourable habitat for the species. Verges with shrubs are good habitats and connectivity providers for wood mice (see also Galantinho et al., 2020) but mandatory regular vegetation cutting to prevent fires may hamper their contribution for biodiversity conservation. Road verges’ role for conservation is further compromised when landowners open firebreaks adjacent to the road, displacing small mammals and so increasing roadkill risk. Road operators and landowners agreeing on when and where vegetation cutting on verges and firebreak openings should occur (or even if any of these actions is needed) would increase road verges' value for conservation of small fauna. Maintaining strips of undisturbed vegetation on road verges is an essential tool to optimize their role as refuges and corridors, contributing to offset the widely recognised negative impacts of roads on biodiversity. Nevertheless, management practices must consider and prevent the potential ecological trap effect of enhancing habitat on road verges particularly on areas of high roadkill risk.

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# Supplementary materials 3.A

## Summarized description of vegetation structure, soil type and slop of road and roadless areas

Road and roadless areas were embedded in a well-preserved *montado* landscape. The *montado*, is a traditional Mediterranean savannah-like agro-silvo pastoral system of cork oak (*Quercus suber*) and holm oak (*Quercus ilex rotundifolia*) trees with grass and shrubs (Pinto-Correia et al., 2011). At both areas, cork oak trees were more frequent and occurred at 43% and 46% of the trap points at the road and roadless area, respectively. Salvia cistus (*Cistus salvifolius*) was the most common shrub and occurred at 86% and 90% of the trap points at the road and roadless area, respectively. Most shrub cover was up to 50 cm high (at 98% and 92% of the trap points at the road and roadless area, respectively). However, management practices implemented during our study at the road area decreased shrub cover and height. Soil type is cambisol at the road area and luvisol at the roadless areas (Agência Portuguesa do Ambiente, I.P., 1982). On both areas, average slop is less than 11% (10 % at the roadless area and 5% at the road area; ASF DAAC 2015).

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# Supplementary materials 3.B

## Summary of data collection, data processing and model building procedures

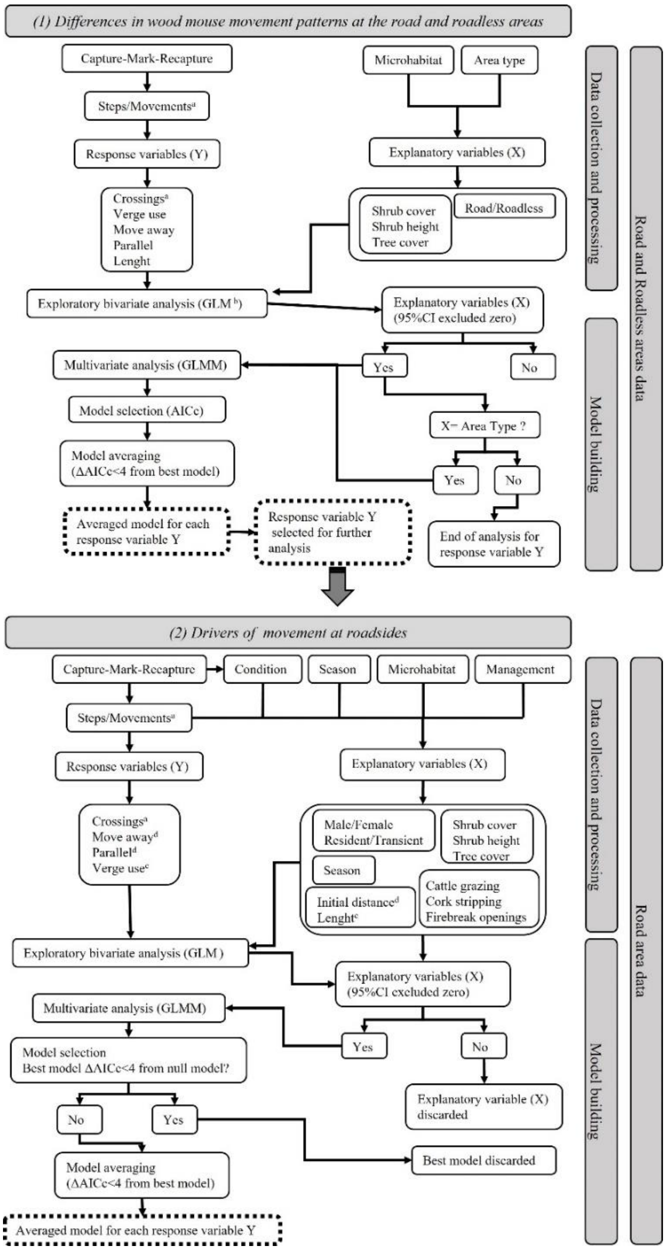

Figure SM3.B1 – Data collection, data processing and model building used in the analysis of (1) road effects on wood mice's movements and (2) drivers of wood mice's movements at roadsides. [a] Road and virtual road "crossings" accounted for all crossing movements, *i.e.*, not only those that occurred within (steps) but also between trapping sessions. [b] GLS was used for "move away" models to account for a temporal autocorrelation structure. [c]Step "length" was used as explanatory variable for "verge use" models. [d] "Initial distance" to the road was used as explanatory variable for "move away" and "parallel" models. Grey boxes and dashed boxes represent the overall procedures and the main outcomes of the analysis, respectively.

# Supplementary materials 3.C

## Details of model selection, model validation, temporal autocorrelation and spatial autocorrelation

Model selection tables include model building details whenever models accounted for random effects, heterogeneity or autocorrelation. We do not show model selection tables whenever none or only one explanatory variable was considered (results section 3.1 for “move away” and for “length”; results section 3.2 for “move away”). Model validation is not presented whenever model selection tables show that the best model found is not better than the null model (ΔAICc<4; results section section 3.2 for “parallel”). Model validation was assessed for the full model that yielded the averaged model (Burnham and Anderson 2002).

## 3.C.1. Model selection

### 3.C.1.1 Differences in wood mouse movement patterns at the road and roadless areas

### *3.C.1.1.1 Crossings*

Table 3.C.S1 - Model selection (GLMM) for road/virtual road “crossings” at the road or at the roadless area. Models relate the presence/absence of “crossings” to “area”, “shrub height” and “tree cover” accounting for “animal” as random effect and temporal correlation. Models are ranked by AICc. We present AICc differences to the best model (delta) and Akaike weights ($w_i$) based on the entire set of models. We also show values for intercept (Int), degrees of freedom (df) and log-likelihood (loglik) for each model. Models included in model averaging (confidence set of models at ΔAICc < 4 from the best model) are highlighted in bold.

| Model | (Int) | Area | Shrub height | Tree cover | df | logLik | AICc | Delta | Wi |
|---|---|---|---|---|---|---|---|---|---|
| **6** | **0.04368** | **+** | | **+** | **8** | **-199.485** | **415.1** | **0.00** | **0.524** |
| **2** | **0.06376** | **+** | | | **5** | **-203.070** | **416.2** | **1.10** | **0.303** |
| 5 | 0.10980 | | | + | 7 | -202.674 | 419.4 | 4.35 | 0.060 |
| 1 | 0.12150 | | | | 4 | -205.707 | 419.4 | 4.35 | 0.059 |
| 8 | 0.04315 | + | + | + | 11 | -199.321 | 420.9 | 5.78 | 0.029 |
| 4 | 0.06281 | + | + | | 8 | -202.884 | 421.9 | 6.80 | 0.018 |
| 7 | 0.10650 | | + | + | 10 | -202.405 | 425.0 | 9.91 | 0.004 |
| 3 | 0.11860 | | + | | 7 | -205.455 | 425.0 | 9.91 | 0.004 |

### *3.C.1.1.2 Road verges and virtual verges*

Table 3.C.S2 – Model selection (GLMM) for “verge/virtual verge use” during a movement step at the road or at the roadless area. Models relate “area”, “shrub cover”, “shrub height” and “tree cover” to the presence/absence of “verge/virtual verge use”. Models account for “animal” as random effect and for temporal autocorrelation. Models are ranked by AICc. We present AICc differences to the best model (delta) and Akaike weights ($w_i$) based on the entire set of models. We also show values for intercept (Int), degrees of freedom (df) and log-likelihood (loglik) for each model. Models included in model averaging (confidence set of models at ΔAICc < 4 from the best model) are highlighted in bold.

| Model | (Int) | Area | Shrub cover | Shrub height | Tree cover | Df | logLik | AICc | Delta | wi |
|---|---|---|---|---|---|---|---|---|---|---|
| **4** | **0.3392** | **+** | **+** | | | **9** | **-152.585** | **323.6** | **0.00** | **0.645** |
| **3** | **0.2102** | | **+** | | | **8** | **-154.836** | **326.0** | **2.42** | **0.192** |
| 8 | 0.3359 | + | + | + | | 12 | -151.833 | 328.4 | 4.79 | 0.059 |
| 12 | 0.3776 | + | + | | + | 12 | -152.145 | 329.0 | 5.42 | 0.043 |
| 7 | 0.2060 | | + | + | | 11 | -154.109 | 330.8 | 7.24 | 0.017 |
| 2 | 0.4996 | + | | | | 6 | -159.371 | 330.9 | 7.36 | 0.016 |
| 11 | 0.2411 | | + | | + | 11 | -154.461 | 331.5 | 7.94 | 0.012 |
| 16 | 0.3846 | + | + | + | + | 15 | -151.092 | 333.3 | 9.70 | 0.005 |
| 1 | 0.3575 | | | | | 5 | -161.611 | 333.4 | 9.78 | 0.005 |
| 6 | 0.5006 | + | | + | | 9 | -158.632 | 335.7 | 12.09 | 0.002 |
| 15 | 0.2470 | | + | + | + | 14 | -153.433 | 335.8 | 12.24 | 0.001 |
| 10 | 0.5250 | + | | | + | 9 | -158.853 | 336.1 | 12.53 | 0.001 |
| 5 | 0.3621 | | | + | | 8 | -160.816 | 338.0 | 14.38 | 0.000 |
| 9 | 0.3700 | | | | + | 8 | -161.340 | 339.0 | 15.43 | 0.000 |
| 14 | 0.5341 | + | | + | + | 12 | -157.886 | 340.5 | 16.90 | 0.000 |
| 13 | 0.3826 | | | + | + | 11 | -160.334 | 343.3 | 19.69 | 0.000 |

### *3.C.1.1.3 Parallel movement*

Table 3.C.S3 - Model selection (GLMM) for “parallel” movement at the road and roadless areas. Models relate “area”, “shrub height” and “tree cover” to the presence/absence of “parallel” movement. All models account for “burst” as random effect. Models are ranked by AICc. We present AICc differences to the best model (delta) and Akaike weights ($w_i$) based on the entire set of models. We also show values for intercept (Int), degrees of freedom (df) and log-likelihood (loglik) for each model. Models included in model averaging (confidence set of models at ΔAICc < 4 from the best model) are highlighted in bold.

| Model | (Int) | Area | Shrub height | Tree cover | df | logLik | AICc | Delta | wi |
|---|---|---|---|---|---|---|---|---|---|
| **4** | **0.5614** | **+** | **+** | | **7** | **-275,841** | **565.9** | **0.00** | **0.648** |
| **8** | **0.5387** | **+** | **+** | **+** | **10** | **-273,533** | **567.6** | **1.63** | **0.287** |
| 2 | 0.5717 | + | | | 4 | -281,891 | 571.9 | 5.94 | 0.033 |
| 6 | 0.5706 | + | | + | 7 | -278,896 | 572.0 | 6.11 | 0.031 |
| 7 | 0.3197 | | + | + | 9 | -281,237 | 580.9 | 14.94 | 0.000 |
| 5 | 0.3681 | | | + | 6 | -284,734 | 581.7 | 15.73 | 0.000 |
| 3 | 0.3251 | | + | | 6 | -285,116 | 582.4 | 16.49 | 0.000 |
| 1 | 0.3552 | | | | 3 | -289,03 | 584.1 | 18.18 | 0.000 |

## 3.C.1.2 Drivers of wood mouse movements at roadsides

### *3.C.1.2.1 Road crossings*

Table 3.C.S4 - Model selection (GLMM) for road “crossings”. Models relate the presence/absence of “crossings” to “distance to road”, “firebreaks”, “shrub height” and “tree cover” accounting for “animal” as random effect and temporal correlation. Models are ranked by AICc. We present AICc differences to the best model (delta) and Akaike weights ($w_i$) based on the entire set of models. We also show values for intercept (Int), degrees of freedom (df) and log-likelihood (loglik) for each model. Models included in model averaging (confidence set of models at ΔAICc < 4 from the best model) are highlighted in bold.

| Model | (Int) | Distance to road | Firebreaks | Shrub height | Tree cover | df | logLik | AICc | delta | wi |
|---|---|---|---|---|---|---|---|---|---|---|
| **7** | **0.04181** | | **+** | **+** | | **7** | **43.432** | **-72.3** | **0.00** | **0.639** |
| **8** | **0.04264** | **-3.848e-05** | **+** | **+** | | **8** | **43.433** | **-70.2** | **2.15** | **0.218** |
| 15 | 0.04253 | | + | + | + | 10 | 44.253 | -67.5 | 4.87 | 0.056 |
| 5 | 0.04791 | | | + | | 6 | 39.731 | -67.1 | 5.27 | 0.046 |
| 16 | 0.04359 | -4.919e-05 | + | + | + | 11 | 44.255 | -65.3 | 7.08 | 0.019 |
| 6 | 0.05091 | -1.388e-04 | | + | | 7 | 39.742 | -65.0 | 7.38 | 0.016 |
| 13 | 0.05196 | | | + | + | 9 | 40.670 | -62.5 | 9.85 | 0.005 |
| 14 | 0.05527 | -1.548e-04 | | + | + | 10 | 40.684 | -60.3 | 12.01 | 0.002 |
| 3 | 0.05678 | | + | | | 5 | 32.980 | -55.7 | 16.66 | 0.000 |
| 4 | 0.06808 | -5.479e-04 | + | | | 6 | 33.148 | -53.9 | 18.43 | 0.000 |
| 11 | 0.04676 | | + | | + | 8 | 34.293 | -51.9 | 20.43 | 0.000 |
| 1 | 0.06388 | | | | | 4 | 29.446 | -50.7 | 21.64 | 0.000 |
| 12 | 0.05829 | -5.484e-04 | + | | + | 9 | 34.466 | -50.1 | 22.25 | 0.000 |
| 2 | 0.07781 | -6.716e-04 | | | | 5 | 29.691 | -49.1 | 23.24 | 0.000 |
| 9 | 0.05629 | | | | + | 7 | 30.839 | -47.2 | 25.18 | 0.000 |
| 10 | 0.07053 | -6.780e-04 | | | + | 8 | 31.094 | -45.5 | 26.83 | 0.000 |

### *3.C.1.2.2 Road verges*

Table 3.C.S1 - Model selection (GLMM) for road "verge use" during movement. Models relate "cattle" and "sex" to the presence/absence of road "verge use". All models account for "burst" as random effect. Models are ranked by AICc. We present AICc differences to the best model (delta) and Akaike weights ($w_i$) based on the entire set of models. We also show values for intercept (Int), degrees of freedom (df) and log-likelihood (loglik) for each model. Models included in model averaging (confidence set of models at $\Delta$AICc < 4 from the best model) are highlighted in bold.

| Model | (Int) | Cattle | Male | df | logLik | AICc | Delta | wi |
|---|---|---|---|---|---|---|---|---|
| **4** | **0,4571** | + | + | **5** | **920,664** | **-1830,4** | **0** | **0,526** |
| **3** | **0,4243** |  | + | **4** | **918,999** | **-1829,4** | **1,02** | **0,316** |
| **2** | **0,5706** | + |  | **4** | **917,743** | **-1826,9** | **3,54** | **0,09** |
| 1 | 0,5366 |  |  | 3 | 916,345 | -1826,3 | 4,09 | 0,068 |

### *3.C.1.2.3 Parallel movement*

Table 3.C.S6 - Model selection (GLMM) for “parallel” movement to the road. Models relate “distance” to the road, “cattle”, “season”, “shrub height” and “tree cover” to the presence/absence of “parallel” movement. All models accounted for “burst” as random effect. Models are ranked by AICc. We present AICc differences to the best model (delta) and Akaike weights ($w_i$) based on the entire set of models. We also show values for intercept (Int), degrees of freedom (df) and log-likelihood (loglik) for each model. The null model is highlighted in bold.

| Model | (Int) | Distance to road | Cattle | Season | Shrub height | Tree cover | df | logLik | AICc | delta | wi |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 0.7378 | -0.007880 | | | | | 4 | -45.701 | 100.0 | 0.00 | 0.200 |
| 4 | 0.7463 | -0.006783 | + | | | | 5 | -44.775 | 100.4 | 0.46 | 0.159 |
| 18 | 0.9105 | -0.008633 | | | | + | 7 | -43.072 | 101.9 | 1.88 | 0.078 |
| 3 | 0.6155 | | + | | | | 4 | -46.668 | 101.9 | 1.93 | 0.076 |
| 10 | 0.7590 | -0.008437 | | | + | | 6 | -44.438 | 102.1 | 2.16 | 0.068 |
| 20 | 0.9092 | -0.007315 | + | | | + | 8 | -41.983 | 102.2 | 2.23 | 0.066 |
| 12 | 0.7691 | -0.007364 | + | | + | | 7 | -43.440 | 102.6 | 2.61 | 0.054 |
| **1** | **0.5706** | | | | | | **3** | **-48.317** | **103.0** | **2.99** | **0.045** |
| 6 | 0.8087 | -0.007952 | | + | | | 6 | -45.013 | 103.3 | 3.31 | 0.038 |
| 19 | 0.7688 | | + | | | + | 7 | -43.969 | 103.7 | 3.67 | 0.032 |
| 8 | 0.8185 | -0.006980 | + | + | | | 7 | -44.185 | 104.1 | 4.10 | 0.026 |
| 11 | 0.6201 | | + | | + | | 6 | -45.709 | 104.7 | 4.70 | 0.019 |
| 26 | 0.9219 | -0.009126 | | | + | + | 9 | -41.975 | 104.8 | 4.82 | 0.018 |
| 22 | 1.0020 | -0.008803 | | + | | + | 9 | -42.186 | 105.2 | 5.24 | 0.015 |
| 28 | 0.9232 | -0.007939 | + | | + | + | 10 | -40.853 | 105.3 | 5.26 | 0.014 |
| 17 | 0.7256 | | | | | + | 6 | -46.005 | 105.3 | 5.29 | 0.014 |
| 7 | 0.6760 | | + | + | | | 6 | -46.253 | 105.8 | 5.79 | 0.011 |
| 9 | 0.5724 | | | | + | | 5 | -47.468 | 105.8 | 5.84 | 0.011 |
| 24 | 1.0020 | -0.007630 | + | + | | + | 10 | -41.172 | 105.9 | 5.90 | 0.010 |
| 14 | 0.8153 | -0.008355 | | + | + | | 8 | -43.994 | 106.2 | 6.25 | 0.009 |
| 5 | 0.6346 | | | + | | | 5 | -47.753 | 106.4 | 6.41 | 0.008 |
| 16 | 0.8272 | -0.007383 | + | + | + | | 9 | -43.041 | 106.9 | 6.95 | 0.006 |
| 27 | 0.7679 | | + | | + | + | 9 | -43.284 | 107.4 | 7.44 | 0.005 |
| 23 | 0.8448 | | + | + | | + | 9 | -43.386 | 107.6 | 7.64 | 0.004 |
| 21 | 0.8070 | | | + | | + | 8 | -45.278 | 108.8 | 8.82 | 0.002 |
| 30 | 1.0070 | -0.009185 | | + | + | + | 11 | -41.281 | 108.9 | 8.90 | 0.002 |
| 25 | 0.7235 | | | | + | + | 8 | -45.348 | 108.9 | 8.96 | 0.002 |
| 15 | 0.6738 | | + | + | + | | 8 | -45.398 | 109.0 | 9.06 | 0.002 |
| 32 | 1.0090 | -0.008119 | + | + | + | + | 12 | -40.194 | 109.6 | 9.60 | 0.002 |
| 13 | 0.6298 | | | + | + | | 7 | -47.044 | 109.8 | 9.82 | 0.001 |
| 31 | 0.8394 | | + | + | + | + | 11 | -42.824 | 112.0 | 11.99 | 0.000 |
| 29 | 0.8014 | | | + | + | + | 10 | -44.770 | 113.1 | 13.10 | 0.000 |

## 3.C.2. Model validation

### 3.C.2.1 Differences in wood mouse movement patterns at the road and roadless areas

#### *3.C.2.1.1. Crossings*

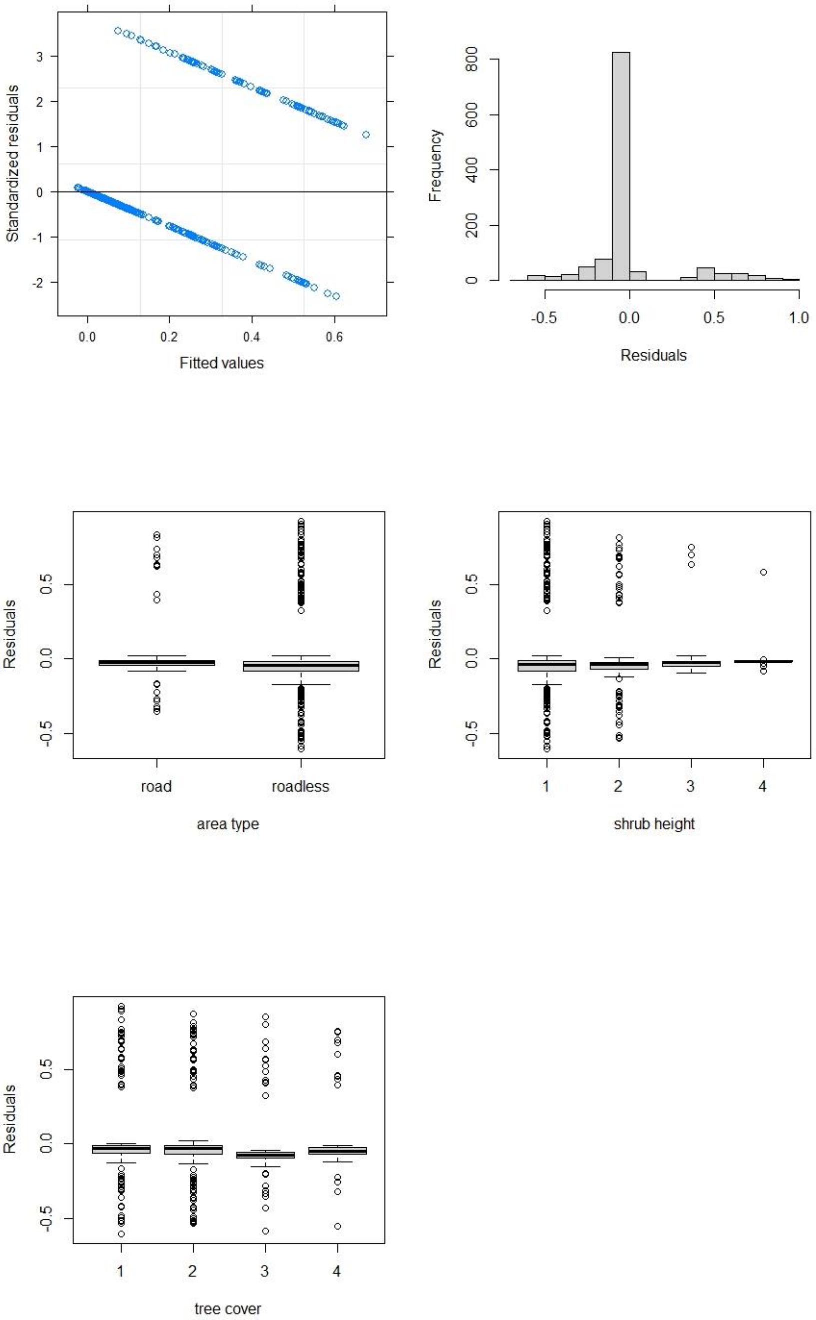

Figure 3.C.S1 – Validation graphs for the model (GLMM) explaining road/virtual road “crossings”. The model relates the presence/absence of “crossings“ to “area” (road or roadless), “shrub height” and “tree cover” and accounts for “animal” as random effect and for temporal autocorrelation. Model validation accounts for the full model that yielded the averaged model.

### 3.C.2.1.2 Verge use

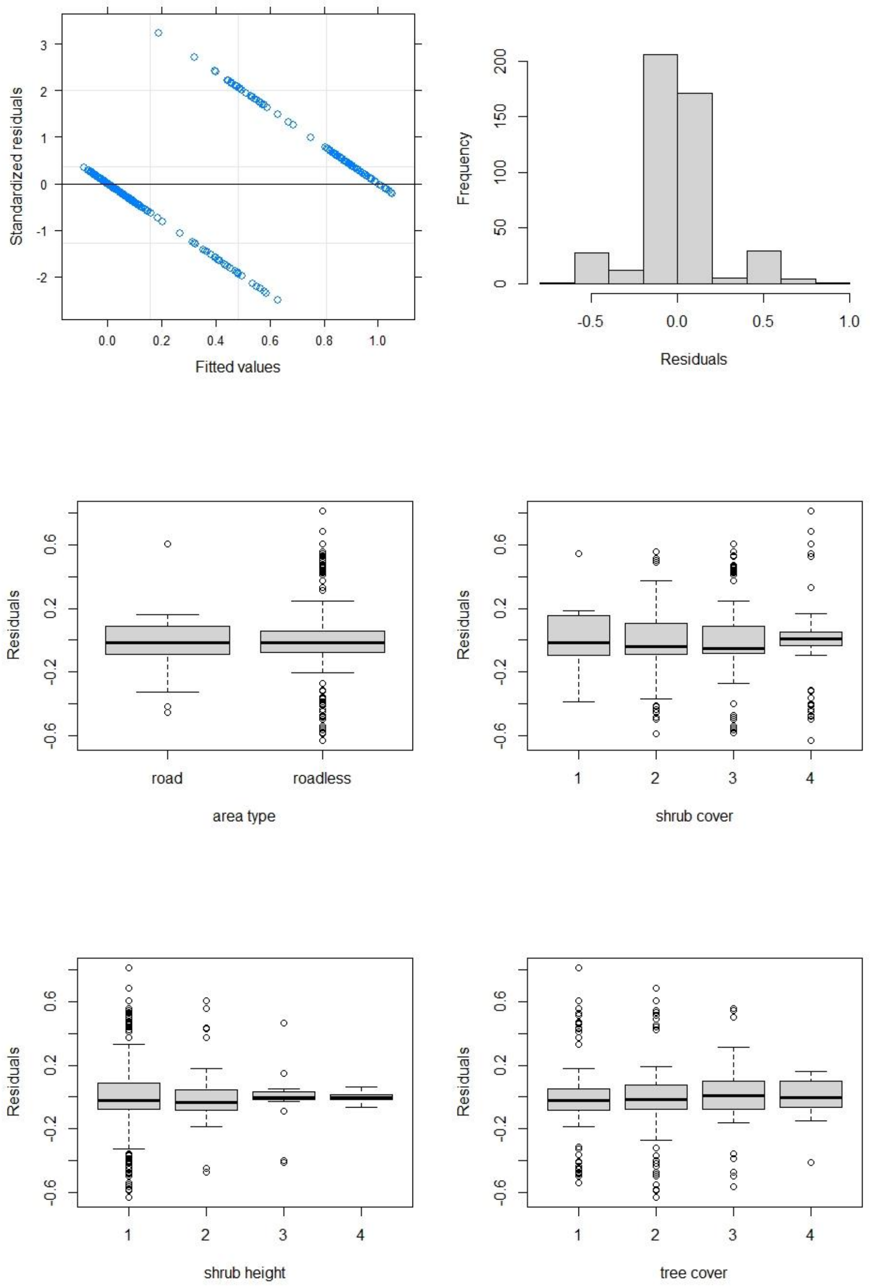

Figure 3.C.S2 – Validation graphs for the model (GLMM) explaining “verge/virtual verge use” at the road and roadless areas. The model relates “area”, “shrub cover”, “shrub height” and “tree cover” to the presence/absence of “verge/virtual verge use” and accounts for “animal” as random effect and for temporal autocorrelation. The model validated accounts for all variables included in the full model. The averaged model only accounted for “area” and “shrub cover” and included “animal” as random effect and temporal autocorrelation.

### 3.C.2.1.3 Move away

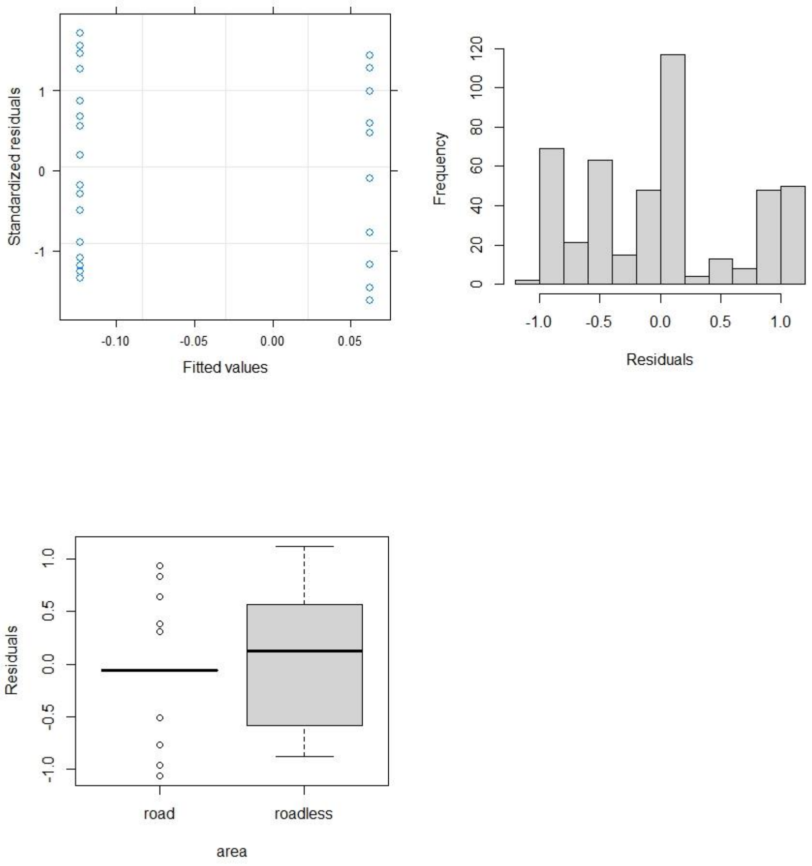

Figure 3.C.S3 – Validation graphs for the model (GLS) explaining “move away” at the road and roadless areas. The model relates “move away” with “area” (road or roadless) accounting for temporal autocorrelation.

### *3.C.2.1.4 Parallel movement*

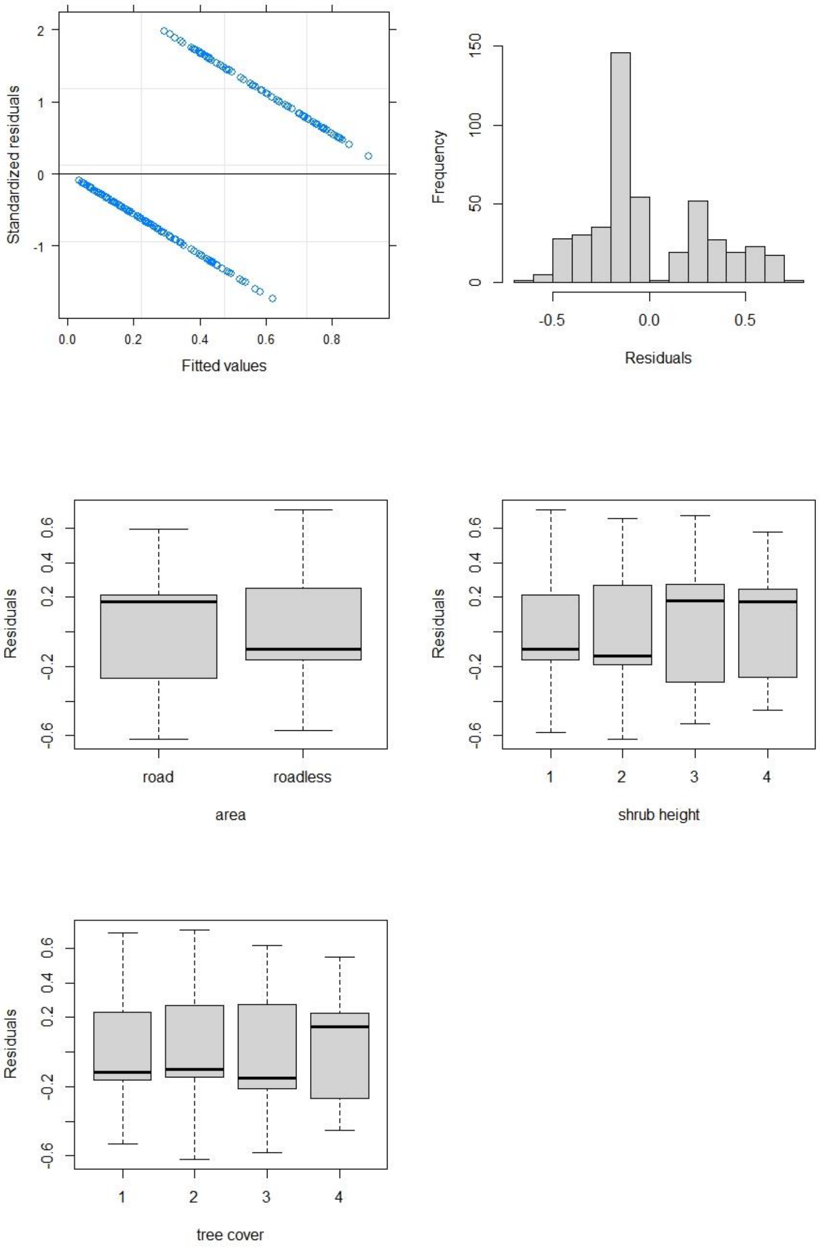

Figure 3.C.S4 – Validation graphs for the model (GLMM) explaining “parallel” movement at the road and roadless areas. The model relates the presence/absence of “parallel“ movement with “area” (road or roadless), “tree cover”, “shrub height” using “burst” as random effect. The model validated accounts for all variables included in the averaged model.

## 3.C.2.2 Drivers of wood mouse movements at roadsides

### *3.C.2.2.1 Road crossings*

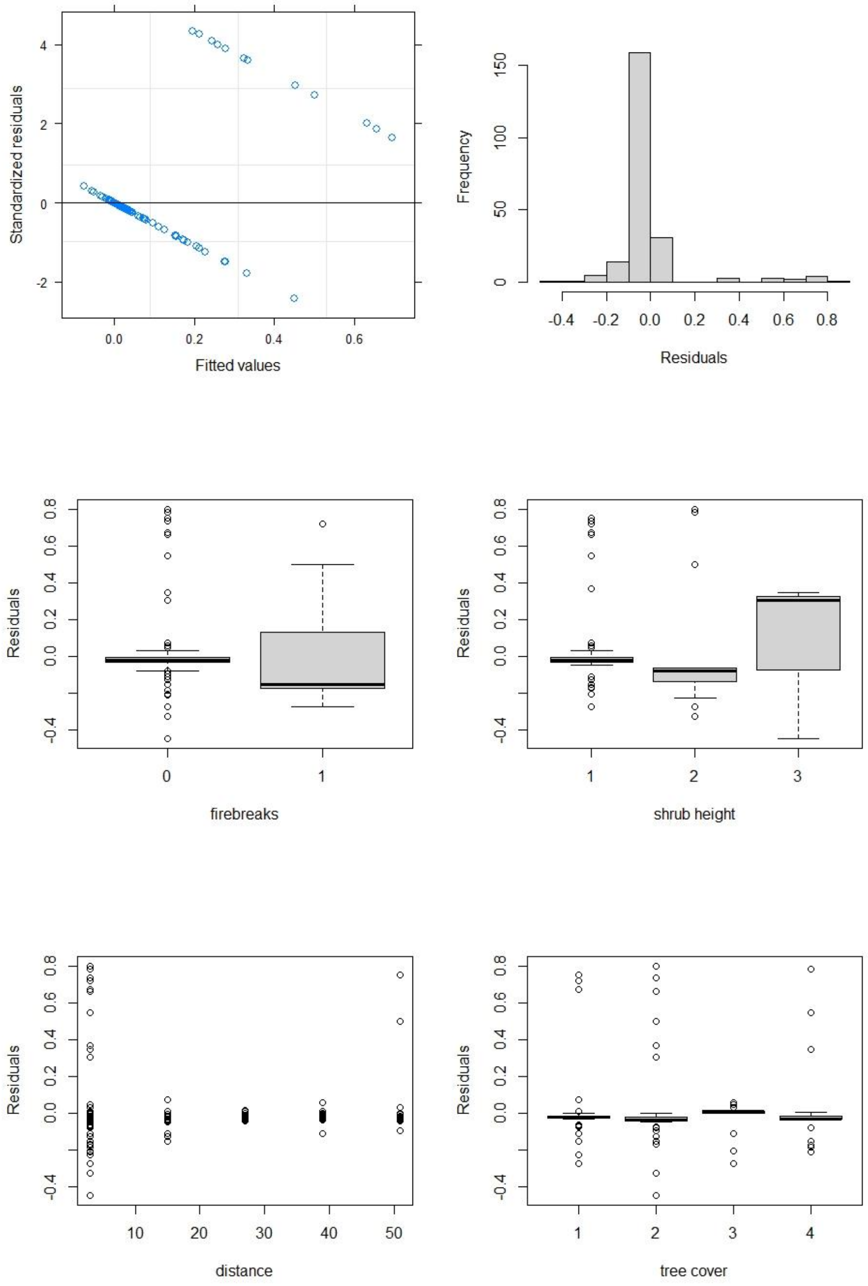

Figure 3.C.S5 – Validation graphs for the model (GLMM) explaining road “crossings”. The model relate the presence/absence of “crossings” to “distance to road”, “firebreaks”, “shrub height” and “tree cover” accounting for “animal” as random effect and temporal correlation. The model validated accounts for all variables included in the averaged model.

### 3.C.2.2.2 Road verges

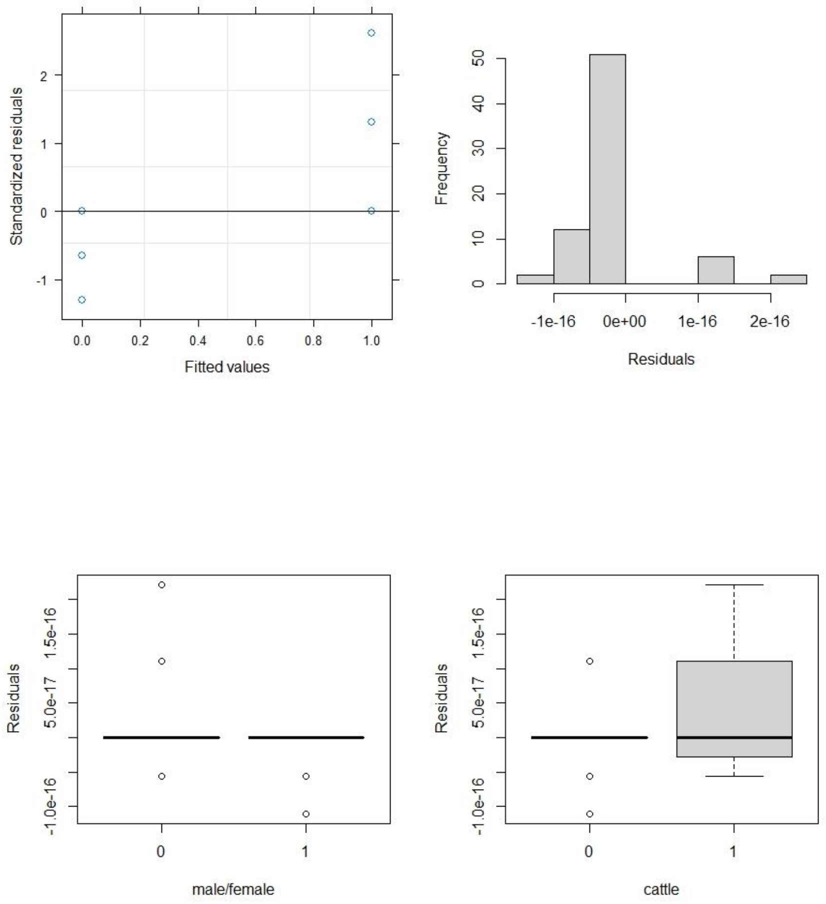

Figure 3.C.S6 - Validation graphs for the model (GLMM) explaining road "verge use". The model relates the presence/absence of "verge use" with "male/female" and "cattle" using "burst" as random effect. The model validated accounts for all variables included in the averaged model.

## 3.C.3. Temporal Autocorrelation

### 3.C.3.1 Differences in wood mouse movement patterns at the road and roadless areas

#### *3.C.3.1.1 Crossings*

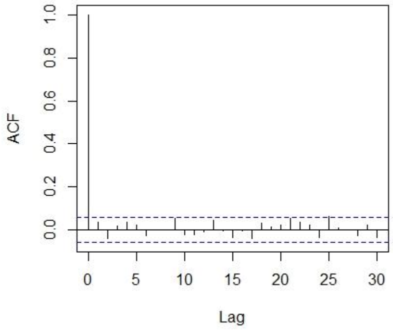

Figure 3.C.S7 – Autocorrelation function (ACF) plot for the residuals of the model (GLMM) explaining road/virtual road "crossings". The model relates the presence/absence of "crossings" to "area" (road or roadless), "shrub height" and "tree cover" and accounts for "animal" as random effect and for temporal autocorrelation. This model is the full model that yielded the averaged model. Autocorrelation on lag 25 is below 0.1.

#### *3.C.3.1.2 Road verges and virtual verges*

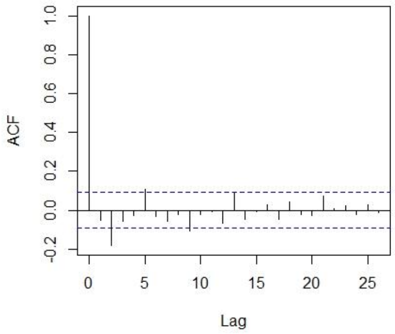

Figure 3.C.S8 – Autocorrelation function (ACF) plot for the residuals of the model (GLMM) explaining "verge/virtual verge use" at the road and roadless areas. The model relates "area", "shrub cover", "shrub height" and "tree cover" to the presence/absence of "verge/virtual verge use" and accounts for "animal" as random effect and for temporal autocorrelation. This model accounts for all variables included in the full model. The averaged model only accounted for "area" and "shrub cover" and included "animal" as random effect and temporal autocorrelation. Autocorrelation on lags 2, 5, 9 and 13 is below 0.5.

### 3.C.3.1.3 Move away

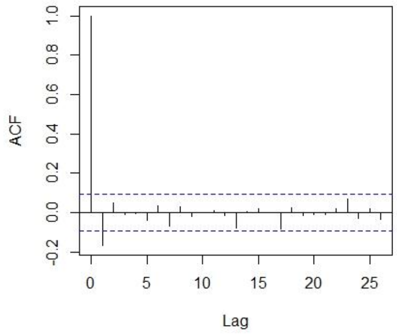

Figure 3.C.S9 – Autocorrelation function (ACF) plot for the residuals of the model (GLS) explaining "“move away” at the road and roadless areas. The model relates “move away” with “area” (road or roadless) accounting for temporal autocorrelation. Autocorrelation on lags 1 is below 0.5.

### 3.C.3.1.4 Parallel movement

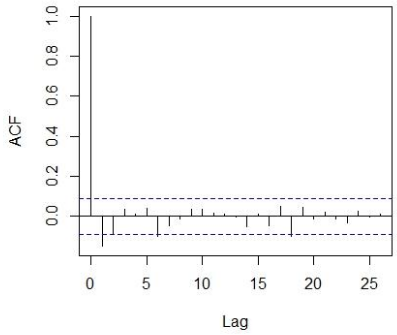

Figure 3.C.S10 - Autocorrelation function (ACF) plot for the residuals of the model (GLMM) explaining “parallel” movement at the road and roadless areas. The model relates the presence/absence of “parallel“ movement with “area” (road or roadless), “tree cover”, “shrub height” using “burst” as random effect. The model validated accounts for all variables included in the averaged model. Autocorrelation on lags 1, 6 and 18 is below 0.5.

## 3.C.3.2 Drivers of wood mouse movements at roadsides

### *3.C.3.2.1 Road crossings*

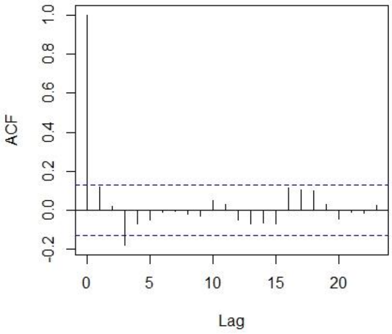

Figure 3.C.S11 - Autocorrelation function (ACF) plot for the residuals of the model (GLMM) explaining road “crossings”. The model relates the presence/absence of “crossings” to “distance to road”, “firebreaks”, “shrub height” and “tree cover” accounting for “animal” as random effect and temporal correlation. The model validated accounts for all variables included in the averaged model. Autocorrelation on lag 3 is below 0.5.

### *3.C.3.2.2 Road verges*

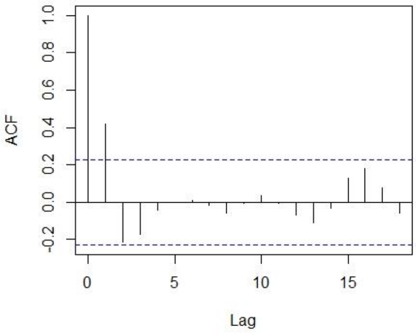

Figure 3.C.S12- Autocorrelation function (ACF) plot for the residuals of the model (GLMM) explaining "verge use”. The model relates the presence/absence of "verge use” with “male/female”, “cattle” and accounting for “burst” as a random effect. This model accounts for the variables (and random effect) included in the averaged model. Autocorrelation on lag 1 is below 0.5.

## 3.C.4. Spatial Autocorrelation

### 3.C.4.1 Differences in wood mouse movement patterns at the road and roadless areas

Table 3.C.S7 – Moran' I statistics (I), standard deviate (Z) and p-value of models (GLS and GLMM) explaining movement patters. Models assessed are the final model (for "move away") and the full models including the variables used in the averaged models (for the remaining movement patterns).

| Response variable | I | Z | p-value |
|---|---|---|---|
| Crossings | -2.009375e-03 | -0.94656 | 0.8281 |
| Road verges and virtual verges | -6.544509e-03 | -1.3842 | 0.9168 |
| Move away | -7.042455e-03 | -1.5367 | 0.9378 |
| Parallel movement | -5.013050e-03 | -0.89472 | 0.8145 |

### 3.C.4.2 Drivers of wood mouse movements at roadsides

Table 3.C.S8 – Moran' I statistics (I), standard deviate (Z) and p-value of models (GLMM) explaining "parallel" movement and "verge use" at the road area. Models assessed include the variables used in the averaged models.

| Response variable | I | Z | p-value |
|---|---|---|---|
| Road crossings | -3.872100e-03 | 0.71398 | 0.2376 |
| Road verges | -1.758801e-02 | -1.5841 | 0.9434 |

## 3.C.5. References

Burnham, K.P., Anderson, D.R., 2002. Model selection and multi-model inference: a practical information—theoretic approach, 2nd ed. Springer-Verlag, New York.

# Chapter 4

## Road verges provide connectivity for small mammals: a case study with wood mice (*Apodemus sylvaticus*) in an agro-silvo pastoral system

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Research article

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# Road verges provide connectivity for small mammals: a case study with wood mice (*Apodemus sylvaticus*) in an agro-silvo pastoral system

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# Abstract

Roads disrupt landscape connectivity for many terrestrial mammals. These infrastructures can be barriers to movement thereby threatening population persistence. Nonetheless, small mammals may use road verges as habitat or corridor, thus increasing migration across intensively managed landscapes. However, in well-preserved habitats where road verges show a similar vegetation structure to surrounding areas, their role is still unknown. Road verges would have an important role as fine-scale connectivity providers for small mammals in a well-preserved habitat depending on land management on road surroundings. We aimed to quantify the effects of road verges and paved lanes on the fine-scale landscape connectivity for the wood mouse (*Apodemus sylvaticus*) in a well-preserved Mediterranean woodland. Additionally, we assessed the impact on connectivity of vegetation cutting on verges and of management in surrounding areas (i.e. firebreaks, grazing, ploughing and cork stripping). We quantified connectivity using graph theory based on two years of capture-recapture data. We compared a set of connectivity metrics (derived from the probability of connectivity index) in a road area and in a virtual roadless scenario. We found that the presence of the road reduced overall fine-scale landscape connectivity, acting as a partial barrier for wood mice movement. However, verges had a key role in promoting movement on road surroundings. Vegetation cutting on verges, and land ploughing in the surrounding landscape were the only management activities compromising connectivity. Our study supports the already known role of road verges as habitat corridors for small mammals. However, it goes beyond existing knowledge by quantifying the connectivity enhancement provide by road verges and demonstrating that this role is highly relevant even in well-preserved landscapes. Therefore, our findings emphasize the critical role of road verges and suggest important management options to enhance landscape connectivity for small mammals.

**Keywords:** Ecological corridors; Fine-scale landscape functional connectivity; *Montado*; Probability of connectivity index; Roadside management;

## 4.1. Introduction

Roads are essential to human development worldwide. Nonetheless, these infrastructures are responsible not only for direct mortality, but also for habitat loss and fragmentation which are two major drivers of extinction for many species (Forman et al. 2003). Paved lanes may also fragment animal populations because they can be a barrier (or filter) to movement for many species (Forman et al. 2003). Smaller populations isolated in smaller habitat patches can be negatively affected by genetic drift and unlikely recover from catastrophic stochastic events, eventually leading to local extinctions (Forman et al. 2003). In pristine environments, roads open the way for invasive and/or generalist species and hamper the movement of endemic and/or specialist species (Goosem 2000; Laurance et al. 2009). However, in highly modified landscapes, road verges may be the only source of habitat, which due to their large extension and linear form may act as important corridors for small fauna (Bennett 1990; Forman and Alexander 1998). Landscape connectivity may be defined as "the degree to which the landscape facilitates or impedes movement among resource patches" (Taylor, 1993). However, landscape connectivity involves structural and functional concepts (Kadoya, 2009). Structural connectivity describes the composition and configuration of habitat patches and their physical relations in a landscape, while functional connectivity results from the species-specific interaction with those structural attributes. Therefore, the distinction between structural and functional concepts of landscape connectivity is pivotal, especially if we are aiming to develop species-based conservation management (Kadoya, 2009). Appropriate management to ensure landscape functional connectivity may overcome the negative effects that habitat loss and fragmentation have on populations' persistence (Rudnick et al. 2012). In fact, maintaining landscape functional connectivity is widely considered a key strategy for the conservation of biodiversity in human-modified landscapes (Ernst, 2014).

Conserving functional connectivity is particularly valuable within organisms' home ranges, as key behavioural processes such as breeding, resting and foraging are performed at this spatial scale (Betts et al., 2014; Carvalho et al., 2016, 2018). Thus, even fine-scale landscape modifications can have deleterious impacts on the population persistence of species with small home ranges (Borthagaray et al., 2014; Saura et al., 2014). Nevertheless, small-sized mammals, such as rodents, can use the vegetation patches that often occur at road verges, which is particularly important where roads cross areas highly modified by humans (Pita et al. 2006; Sabino-Marques and Mira 2011; Ascensão et al., 2012). Yet, the role of road verges and of roadside management in providing landscape connectivity, have been rarely addressed at the foraging scale (i.e. the scale at which an individual uses the landscape; Cumming et al., 2017), particularly in well-preserved habitats (but see Encarnação and Becker 2015; Redon et al., 2015).

In these systems, the low impact management allows for road verges to have similar vegetation structure to the surrounding areas and for both to provide adequate resources for small mammals. The low impact management (e.g., low intensity agriculture; low intensity grazing; shrub layer control) in well-preserved systems involves the conservation of semi-natural vegetation and natural or structural elements (e.g., hedgerows; stone walls) (Paracchini et al. 2008). Moreover, the combined effects that management on road verges and on surrounding landscape have on fine-scale connectivity are still unknown and must be studied to successfully plan species conservation in road areas.

In this study, we quantified and compared fine-scale functional connectivity provided by road verges and by the surrounding landscape over a two-year period. Moreover, we evaluated the impact of regular management actions performed on verges and surrounding areas on fine-scale functional connectivity. Our work was performed within a well-preserved Mediterranean woodland, the Portuguese *montado* (Pinto-Correia et al. 2011). We used the wood mouse *(Apodemus sylvaticus Linnaeus 1758)* as a model for forest-dwelling small mammals (Ascensão et al. 2016). Evaluating fine-scale functional connectivity on a well-preserved system is relevant because the importance of road verges as connectivity providers may depend on the species (Goosem 2000) and also on the structural properties of their surrounding landscape (Arnold and Weeldenburg, 1990). Specifically, we assessed the impact of road verges and management (vegetation cutting, firebreak opening, grazing, ploughing and cork stripping) on functional connectivity using the probability of connectivity index *and* its derived metrics *(*Saura and Pascual-Hortal, 2007*;* Saura and Rubio, 2010). This index accounts for both species-specific habitat availability and movement ability to assess functional connectivity (Saura and Pascual-Hortal, 2007). It is sensitive to habitat changes and identifies the most relevant elements for connectivity (Saura and Pascual-Hortal, 2007). The probability of connectivity index has been used at regional scale but also at fine-scale (e.g., Rodríguez-

Pérez et al. 2014). Overall, we hypothesize that road verges will provide high fine-scale functional connectivity for small mammals because many individuals avoid crossing road pavement (e.g., McGregor et al., 2008). Therefore, fine-scale functional connectivity would decrease across the road but would increase along road verges and across the surrounding farmland. We also expect that the importance of verges for connectivity will decrease during and immediately after vegetation cutting along them but will be reinforced when land management (e.g., ploughing, firebreak opening or grazing) occurs in the surrounding landscape. In fact, previous studies have shown that population connectivity is directly related to habitat quality (Beier et al., 2008), and that land management (e.g., agricultural management) increases the use of unaffected edges by small mammals (Ouin et al., 2000; Wilson et al., 2014). Our conclusions can be applied across other forest/woodland landscapes to most forest-dwelling small mammals which avoid crossing vegetation gaps such as paved lanes or ploughed areas.

## 4.2. Materials and methods

### 4.2.1 Study area

This study was carried out in Alentejo, southern Portugal (38° 24 ′N, 8° 06 ′ W). The climate is Mediterranean with hot and dry summers and mild winters. Mean temperature averages 15.8 °C (9.3–23.3 °C) and mean rainfall averages 50.8 mm (6.6–102.7 mm) (Évora 1971–2000; IPMA, 2014).

The landscape is dominated by *montado*, a traditional agro-silvo pastoral system in the Mediterranean, corresponding to a savannah-like forest of cork oak (*Quercus suber*) and holm oak (*Quercus ilex rotundifolia*) trees with grass and shrub strata (Pinto-Correia et al., 2011). Several national roads dissect the landscape. In spring, vegetation on verges is cut, and firebreaks are opened along national roads according to management guidelines created to prevent fire ignition associated with traffic (Decree-Law 156/2004, 30th June of the Portuguese Ministry of Agriculture, Rural Development and Fisheries). Firebreaks are approximately 15 meters wide and may encompass both the verges and the surrounding farmland.

### 4.2.2 Studied species

The wood mouse (*A. sylvaticus*) was our study model for forest-dwelling small mammals (Ascensão et al. 2016). Forest-dwelling small mammals are probably the more affected by vegetation clearances induced by roads and farmland management in the Mediterranean (Ascensão et al. 2016). The wood mouse is widespread and common across Europe but reaches higher abundance and body condition in woodland areas with dense understory cover, including the *montado* (Alcántara and Díaz, 1996; Rosário and Mathias, 2004). This species responds quickly to changes in landscape structure both at fine (e.g., microhabitat; Tew et al., 1992) and broad spatial scales (e.g., farm-scale alterations; Tew and Macdonald, 1993; Tattersall et al., 2001). Despite the known vagility of this species, in *montado* systems individuals' mean home range size (± standard deviation) is 1.124 ± 0.965 ha and their movements occur mainly in areas with understory vegetation (Rosalino et al. 2011). The wood mouse is a major acorn consumer and disperser (Perea et al., 2011) and an important prey of many mammals and birds, some of which are of conservation concern (Pezzo and Morimando, 1995; Sarmento, 1996). Paved roads may be a partial barrier for the wood mouse (Macpherson et al. 2011) which is one of the most road-killed small mammals in Portugal (Carvalho and Mira, 2011).

### 4.2.3 Study design

To study the effects of road verges and roadside management on habitat connectivity for wood mice, we choose a 1.2 ha plot of well-preserved *montado* bisected by a national road (EN257; average traffic volume of approximately 5000 vehicles per day and 600 vehicles per night; EP, 2005). Road verges were flanked by wired fences, not buried and permeable to small mammals and to non-volant predators (e.g., small and medium size carnivores). Agro-silvo pastoral management differed between the two sides of the road due to different land ownerships. Therefore, for analysis purposes, we divided the study area in four sections – verge North, verge South, surrounding farmland North and surrounding farmland South - using the road and the fences as physical divisions (Figure 4.1).

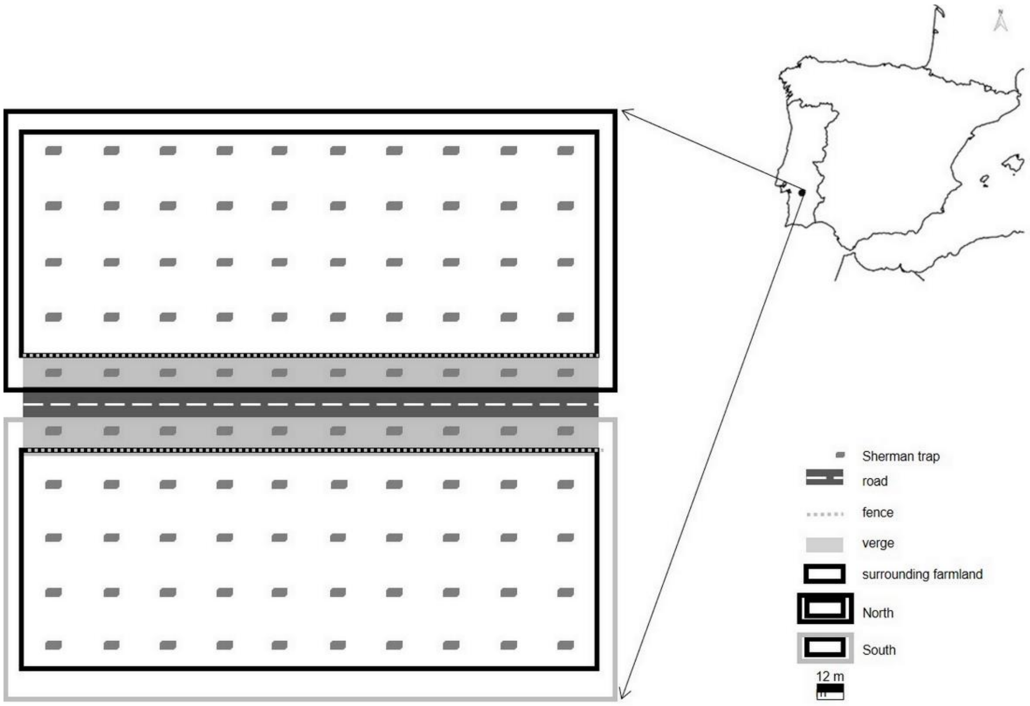

Figure 4.1 - Schematic arrangement of traps in a square grid (10 x 10 traps) at the road area. The area was sub-divided into verge and surrounding farmland both at the North and South patches.

To assess the road effects on connectivity metrics, we built a virtual roadless scenario and compared it to the road area. This scenario consisted in a virtual trapping area of the same size and with the same trap spacing as the road area but with homogenous habitat, where both paved lanes and management were absent. Therefore, connectivity metrics at the virtual roadless scenario were only influenced by distance, assuming that movement was only ruled by the species ability to move, without any interference from landscape features (see details on sections 2.4, 2.5.1 and 2.5.2). Thus, this scenario may act as a null model providing expected values for the importance of each virtual trap site and route for fine-scale functional connectivity.

### 4.2.4 Data collection

Wood mice were sampled every four weeks, from March 2009 to March 2011, with a square live-trapping grid (10 x 10) of medium size Sherman traps (8 x 9 x 23 cm) (Figure 4.1). Traps were spaced at 12 m intervals from each other to match the approximate width of the paved lanes and ensure equal spacing between all rows of traps. Trap points were permanently marked to ensure an accurate relocation. Verges corresponded to the two central trapping lines at both scenarios (road and virtual roadless). We conducted 26 trapping sessions of four consecutive nights, totaling 10400 trap-nights. Each trap was placed on the first day and checked on the subsequent days at sunrise.

We used hydrophobic cotton as nest material and a mixture of sardines, oil and oat flakes as bait. Trapped wood mice were sexed, aged, measured, weighed and released at capture location. Animals were individually marked with passive integrated transponders tags (PIT, TXP148511B, 8.5 mm x 2.12 mm, 134.2 kHz ISO, 0.067g, Biomark, Boise, USA). All the procedures followed the guidelines approved by the Portuguese Institute for Nature and Forest Conservation (ICNF - Instituto de Conservação da Natureza e das Florestas) and the Directive 2010/63/EU for animal experiments.

Microhabitat structure influences wood mice presence, abundance, movements (Boitani et al., 1985; Tew et al., 2000) and trap efficiency (Cusak et al., 2015). Therefore, we sampled microhabitat structure to parameterize connectivity metrics (section 2.5.1). We characterized microhabitat in a 1 square meter centered on each trap in each trapping session. We visually classified cover of grasses, shrubs, trees, litter, rocks and bare ground. To minimize possible measurement errors, we classified cover of each vegetation strata in four classes (class 1: 0-25%; class 2: 25-50%; class 3: 50-75%; class 4: 75-100%). Accordingly, maximum height of each vegetation strata was measured (cm) and categorized based on quartiles.

### 4.2.5 Graph parameterization and connectivity analysis

We considered that individual traps were interconnected by the movement of wood mice during their foraging activity. We used graph theory (Dale and Fortin, 2010) to quantify functional landscape connectivity for wood mice. Graph theory perceives the landscape as a network of nodes (traps and their immediate surroundings in our study) interconnected by links. Links symbolize the relationship between nodes. According to this approach, the routes for wood mice movements would be the links between traps (i.e. nodes). Links and nodes can be hampered by road pavement and land management or facilitated by vegetation growing at verges, thereby changing functional connectivity for wood mice.

The movement of individuals and the frequency of use of links and nodes has already been assessed for other species using capture-recapture data (Pereira et al., 2011). Also, this sampling method has been used to study wood mice activity (Wilson et al. 2014) and has shown comparable results to telemetry data for other species of small mammals (Lira and Fernandez 2009).

#### *4.2.5.1 Node attribute*

To evaluate microhabitat suitability at each node, we modelled the relationship between capture probability (response variable) at each trap and vegetation traits using generalized linear models or, generalized least squares estimation when accounting for heterogeneity was necessary (Supplementary materials 4.A). We assumed that vegetation traits at nodes with a higher probability of capture were more suitable areas for mice. Indeed, small mammals are trapped more frequently at locations with better habitat conditions (Cusak et al., 2015). The capture probability was accounted as the “capture index” which corresponds to the ratio between the number of animals captured and the number of nights each trap was operable, i.e. set and able to capture animals (adapted from Torre et al., 2010). We first built global models using pooled data from all sessions for both captures and vegetation traits. Then, we modelled and estimated the capture probability of each trap according to its vegetation traits in every session. In the virtual roadless scenario, all traps were characterized by the same microhabitat suitability (averaged value of the real road area) derived from the pooled model.

#### *4.2.5.2 Link weight*

Links were weighted by the probability of direct movement between traps using a negative exponential function of the median distance travelled (Saura and Rubio, 2010) by wood mice.

The median distance travelled in two consecutive trapping nights was 12 m (Supplementary materials 4.B). Additionally, for the road area, the probability of movement for each link was weighted by its proportion of use (i.e. the ratio between the number of links used and the total number of links studied). This proportion of use aims to account for the possibility that the road deterred movement (McGregor et al., 2008), but road verges provided corridors for small mammals (Encarnação and Becker, 2015). In the virtual roadless scenario, links were only characterized by the probability of direct movement.

### 4.2.5.3 Fine-scale landscape connectivity

Node and link parameterizations were used to assess fine-scale landscape functional connectivity for wood mice by calculating, for the road and the virtual roadless scenarios, the numerator of the probability of connectivity index ($PC_{num}$; Saura and Pascual-Hortal, 2007; Eq. 1):

$$PC_{num} = \sum_{i=1}^{n} \sum_{j=1}^{n} a_i a_j p_{ij}^{*} \qquad (1)$$

where $n$ is the number of nodes, $a_i$ is the attribute of node $i$ and $p^{*}_{ij}$ is the maximum probability of movement between nodes i and j (hereafter, $PC_{num}$ is referred as PC). This metric relates to the probability that two points in a landscape can be reached from each other considering habitat availability and movement ability of the species (Saura and Pascual-Hortal, 2007). All connectivity metrics were obtained with Conefor Sensinode 2.6 software (Saura and Torné, 2009).

### 4.2.5.4 Node and link importance

We ranked the relative importance of each node and link for connectivity by evaluating the relative decrease (%) in PC after its removal (dPC) (Saura and Rubio, 2010; Eq. 2):

$$dPC_k = 100 \times \frac{PC - PC_{remove,k}}{PC} \qquad (2)$$

where $k$ can be either a node (dPC node) or a link (dPC link). This procedure allowed us to find the better functionally connected traps (nodes) and routes (links) that potentially eased the flow of wood mice.

To assess the role of road verges in maintaining connectivity, the difference in overall connectivity (dPC) was divided into three components (Saura and Rubio, 2010; Eq. 3):

$$dPC_k = dPC\ intra_k + dPC\ flux_k + dPC\ connector_k \qquad (3)$$

Nodes contribute to connectivity through those three components while links only contribute through dPC connector (Saura and Rubio, 2010). dPC intra reflects node quality (e.g., microhabitat suitability) exclusively, while dPC flux depends not only on node quality but also on its position within the landscape network. dPC flux shows how well a node is connected by accounting for the number and extension of connections to all other nodes as beginning or end of the connection. dPC connector stresses the importance of a node or link as a stepping stone or irreplaceable connecting element for maintaining overall connectivity.

#### *4.2.5.5 Road effect on wood mice flow*

We tested the road effect on wood mice flow (i.e. functional connectivity) by comparing PC and the importance of each trap (dPC node) and median distance between traps (dPC link) for the overall connectivity between the road and the virtual roadless scenarios. We analyzed links corresponding to the median distance (12 m; 180 links) because it best represents the central tendency of distances' distribution given the asymmetry of the data (Sokal and Rohlf, 1995). Differences in dPC for nodes and links between the two scenarios were assessed with the Wilcoxon test for: (i) the two central lines (20 nodes and 18 links); (ii) the surrounding farmland (80 nodes, 132 links); (iii) the 20 links between the surrounding farmland and the central lines; and (iv) the 10 links crossing the central lines of the trapping grids (see Figure 4.1 for further details). Also, we represented spatially dPC for each node in both scenarios using five quantiles in the categorized option in QGIS 2.4.0 - Chugiak (Quantum GIS Development Team, 2014).

#### *4.2.5.6 Road verge and management effects on wood mice flow*

The effects of verges and of five management actions (vegetation cutting, firebreak opening, ploughing, cattle grazing and cork stripping) on the functional connectivity for wood mice were tested using two sets of generalized linear models with generalized least squares estimation (one for nodes and one for links) (package nlme 3.1.-113; Pinheiro et al., 2013). Dependent variables were dPC values on each of the 26 sessions either for 100 nodes (dPC node) or for 180 links (dPC link). Explanatory variables were location and management actions. Location for nodes determined if they belonged to the verge or to the surrounding farmland. The location for links was defined considering the location of the nodes linked: within surrounding farmland; within verge; between surrounding farmland and verge; between verges (Figure 4.1). We categorized each of the five management actions depending on their time and location. For each node we considered for each management action either: (1) absence in the session; (2) occurrence at the node; (3) absence at the node but occurrence elsewhere. In the models for dPC link, we used the same categories except occurrence at the link that was further divided into two classes: occurrence at one node of the link; occurrence at both nodes linked. Firebreaks were the only management conducted at the verge and at the surrounding farmland. The remaining management only occurred either at the verge (vegetation cutting) or at the surrounding farmland (ploughing, cattle grazing and cork stripping). Cattle grazing occurred on 9 sessions and firebreaks on 2 sessions. Ploughing, cork stripping and vegetation cutting only occurred once during our study period.

We followed Zuur et al. (2009) for model building and validation and Burnham and Anderson (2002) for model selection. Collinearity between explanatory variables was checked using the variation inflation factor (VIF) (variables with VIF > 3 were excluded; R package AED; Zuur, 2010). Our two candidate model sets (for dPC node and for dPC link) included all possible combinations of the non-collinear variables and variance structures to incorporate heterogeneity and autocorrelation when appropriate (Zuur et al., 2009). On each set, models were ranked using AIC. We used the model averaging procedure to estimate parameters and associated unconditional standard errors (SE) for the 95% confidence subset of models (sum of Akaike weights ≥ 0.95; package MuMIn; Barton, 2014). The relative importance of each variable ($w+$) was assessed by the sum of Akaike weights ($w$) of all models of each set where the variable was included. We checked for autocorrelation in model residuals using the autocorrelation function for temporal patterns (ACF; package nlme; Pinheiro et al., 2013) and bubble plot for spatial patterns (packages *sp*, Pebesma and Bivand 2005 and *gstat*, Pebesma, 2004). All statistical analysis was performed on R version 3.0.2 (R Core Team, 2013).

## 4.3. Results

We captured 159 wood mice, from which 77 were recaptured in consecutive nights and used in the analysis. The 77 animals were recaptured on average twice (2.37 ± 1.89; mean ± standard deviation). Consecutive distances travelled ranged from 0 m to 64.6 m (median distance = 12 m; Supplementary materials 4.B). We registered five road crossings by four animals (5.19% of the animals captured in consecutive nights).

### 4.3.1 Road effect on functional connectivity

As suggested by PC index, overall connectivity was on average seven times lower at the road (80.02 ± 4.32) than at the virtual roadless scenario (592.44 ± 33.31) (Figure 4.2a).

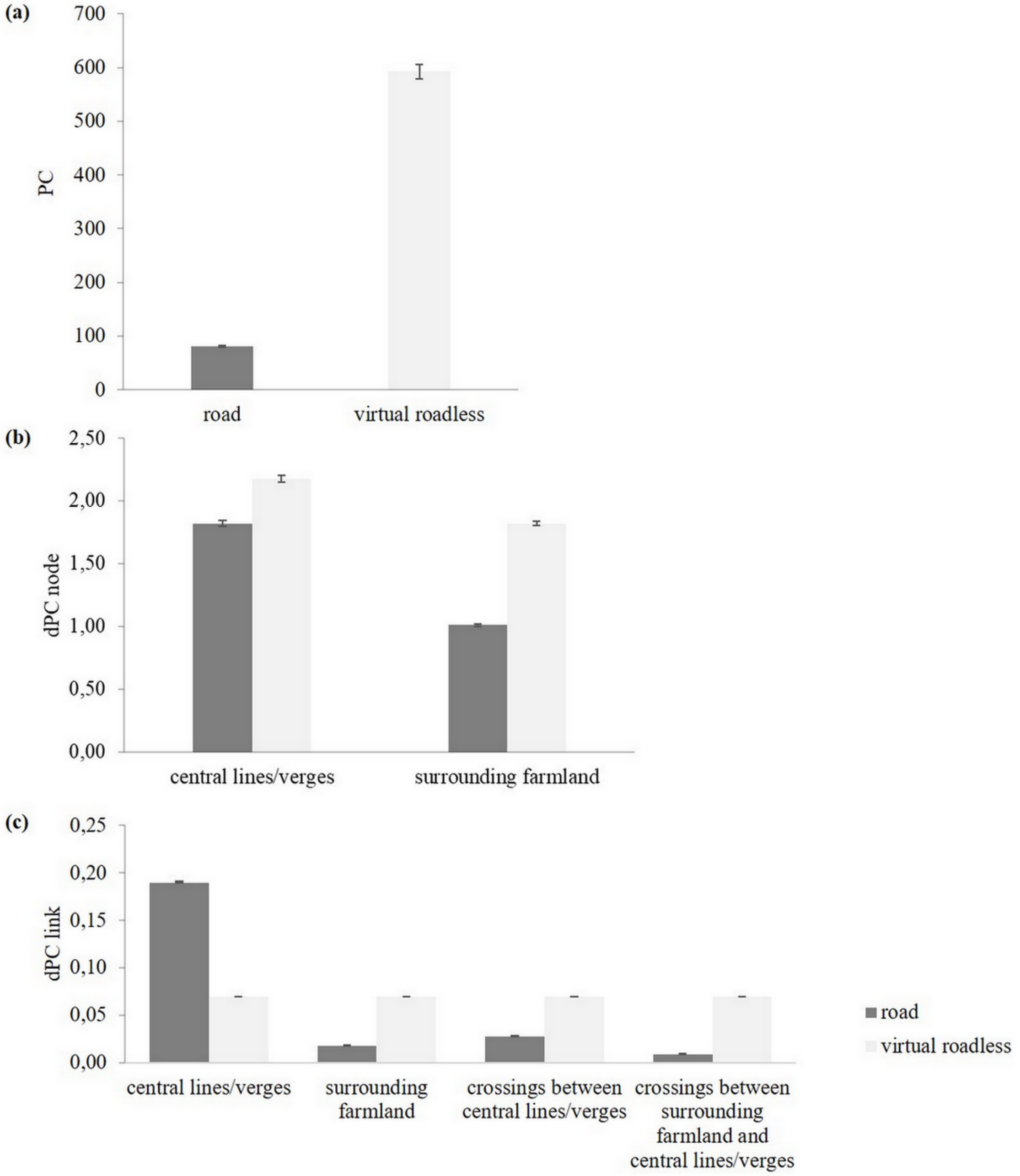

Figure 4.2 - Overall connectivity index (PC; a), relative importance of traps (dPC node; b) and links (dPC link; c) for connectivity at the road and virtual roadless scenario. We show dPC for nodes at central lines or verges (20 nodes) and at the surrounding farmland (80 nodes). We show dPC for links at the central lines or verges (18 links), crossing central lines or verges (10 links), between the surrounding farmland and the central lines (20 links) and at the surrounding farmland (132 links). Values are averaged for dPC considering 26 sessions. Bars represent 95% confidence intervals.

The importance of nodes (dPC node; Figure 4.2b) and links between them (dPC link; Fig 4.2c) was mainly lower at the road than at the virtual roadless scenario (dPC node for central lines and surrounding farmland, $p < 0.0001$; dPC link for crossing central lines, surrounding farmland and between central lines and surrounding farmland, $p < 0.0001$).

Links at verges were the only elements with a greater importance for connectivity at the road than their equivalents at the central lines of the virtual roadless scenario (dPC link; central lines/verges, $p < 0.0001$; Figure 4.2c). Nodes with higher dPC were mostly concentrated along verges at the road but occupied the core of the virtual roadless scenario (Figure 4.3). At the road area, node importance decreased sharply from the verges into the surrounding farmland (Figure 4.3a). However, at the virtual roadless scenario, the importance for connectivity decreased progressively in all directions without a sharp contrast between central lines and surrounding areas (Figure 4.3b).

Scenarios also differed in the contribution of individual nodes (mean dPC ± standard deviation) to the overall wood mice flow. The flux ability was always higher at the virtual roadless scenario ($p < 0.0001$) and the microhabitat suitability was always higher at the road area ($p < 0.0001$). At the virtual roadless scenario, nodes contributed mostly to connectivity by producing or receiving movement fluxes (at central lines: % dPC intra = 5.23 ± 0.96 and % dPC flux = 94.77 ± 0.96; at surrounding farmland, % dPC intra = 6.43 ± 1.63 and % dPC flux = 93.57 ± 1.63). At the road area, microhabitat suitability was as relevant as its flux ability at verges (% dPC intra = 55.13 ± 5.45; % dPC flux = 44.86 ± 5.45) and was more relevant than flux ability at the surrounding area (% dPC intra = 78.66 ± 7.28; % dPC flux = 21.34 ± 7.28). At the road and in the virtual roadless scenario, node dPC connector values were very close to or equal to zero and, therefore, were not considered for further analysis.

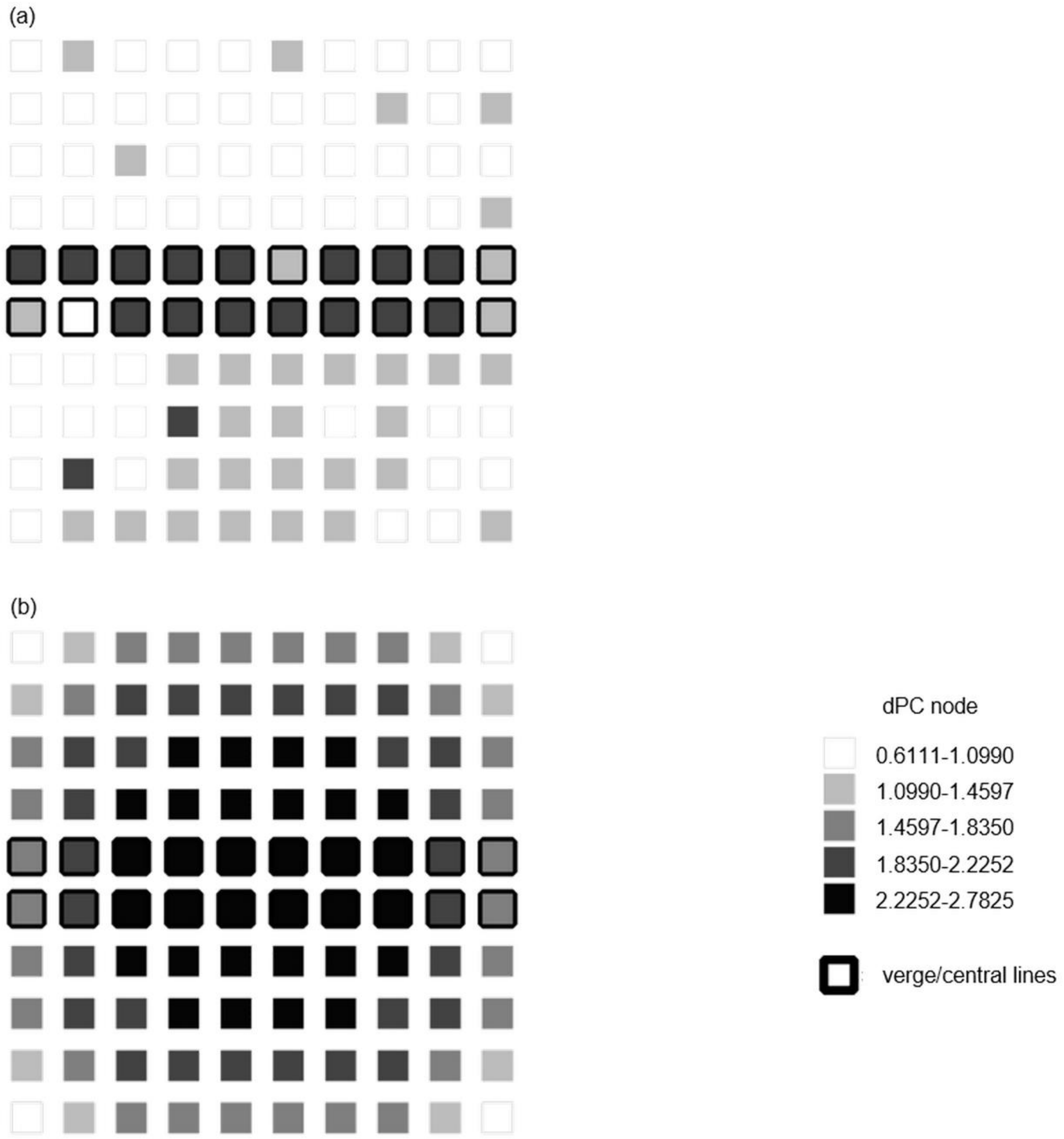

Figure 4.3 - Relative importance of nodes for connectivity (dPC node) in square grids (10 x 10 traps) for (a) road and (b) virtual roadless scenario. dPC values correspond to session one and are classified in quantiles.

### 4.3.2 Road verge and management effects on wood mice flow

The metrics derived from the PC index suggested that connectivity at the road area was higher on verges than on the surrounding farmland on both sides of the road. At the North section, mean dPC node and mean dPC link were, respectively, 2.2 and 21.3 times higher at the verge (dPC node = 1.90 ± 0.23; dPC link = 0.196 ± 0.014) than at the surrounding farmland (dPC node = 0.86 ± 0.16; dPC link = 0.009 ± 0.001) (Figure 4.4, Figure 4.5). At the South section, mean dPC node and mean dPC link were, respectively, 1.5 and 6.9 times higher at the verge (dPC node = 1.74 ± 0.28; dPC link = 0.183 ± 0.021) than at the surrounding farmland (dPC node = 1.16 ± 0.19; dPC link = 0.026 ± 0.004) (Figure 4.4, Figure 4.5).

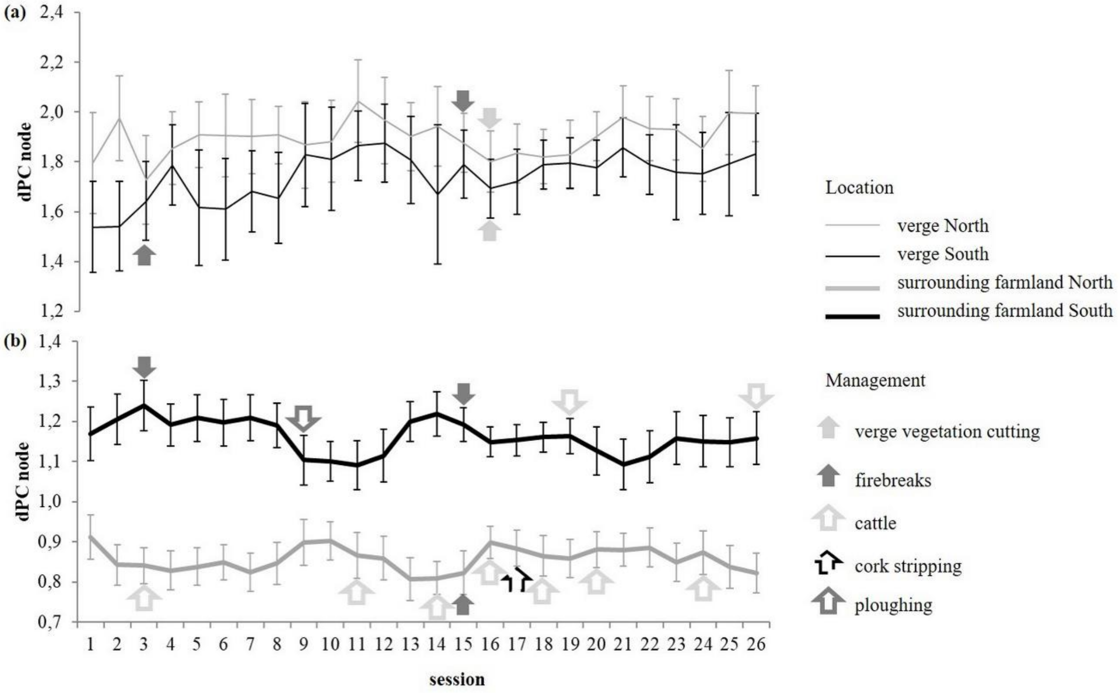

Figure 4.4 - Relative importance of nodes for connectivity (dPC node). We show mean dPC values in the verge (10 nodes; a) and in the surrounding farmland (40 nodes; b) for North and South patches, along 26 sessions. Bars represent 95% confidence intervals. Arrows represent management related to road (verge vegetation cutting and firebreaks) and farmland (cattle grazing, cork stripping and ploughing).

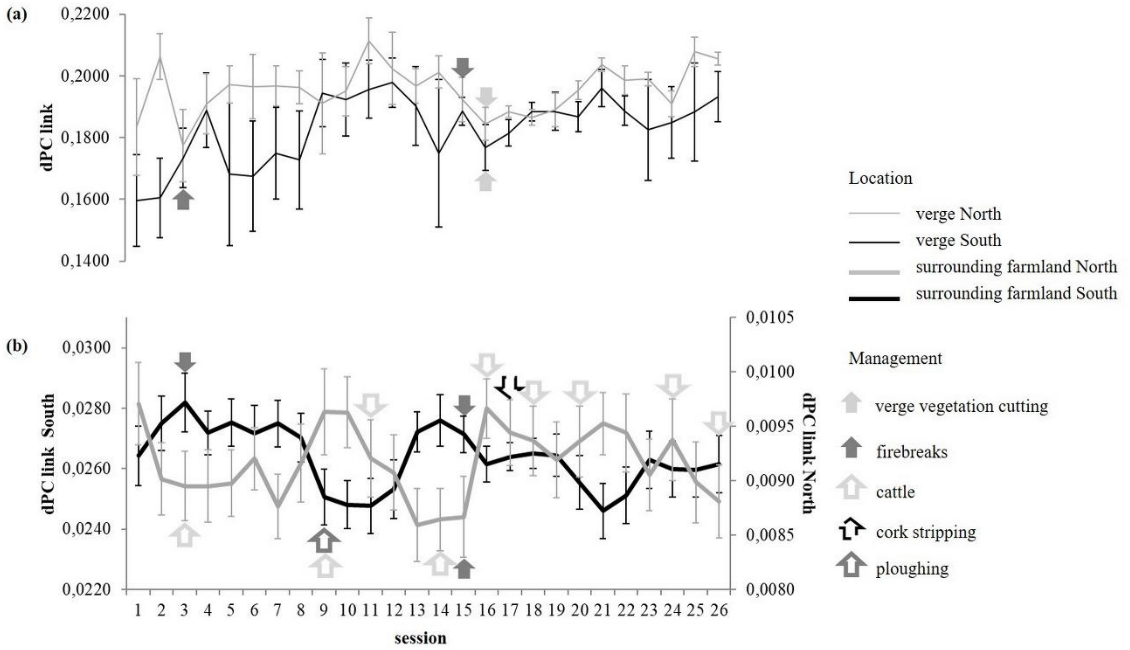

Figure 4.5 - Relative importance of links for connectivity (dPC link). We show mean dPC values in the verge (9 links; a) and in the surrounding farmland (66 links; b) for North and South patches, along 26 sessions. Bars represent 95% confidence intervals. Arrows represent management related to road (verge vegetation cutting and firebreaks) and farmland (cattle grazing, cork stripping and ploughing).

Model averaging explaining the importance of nodes for connectivity (dPC nodes) included 17 models out of 64 possible (Supplementary materials 4.C; Table 4.C.1). The averaged model for the connectivity provided by nodes had strong support for location (W+ = 1.00), and less support for ploughing (W+ = 0.75) and vegetation cutting at verges (W+ = 0.39) (Table 4.1). Nodes at verges contributed more to connectivity than nodes located at the surrounding farmland. Nodes at the southern part of the farmland area have a significantly higher contribution than the nodes located at the northern part (Table 4.1, Figure 4.4). The effects of ploughing and vegetation cutting at verges on node importance show a similar trend to that described below for links, however, 95% confidence intervals for their estimated coefficients include zero and therefore were considered non-significant (Table 4.1).

Table 4.1 – Averaged model of the effects of location (verge or surrounding farmland) and five management actions on the mean importance of nodes for functional connectivity (dPC nodes). Model-averaging is based on the 95% confidence set of best models. We show standard errors (SE), lower and upper boundaries of 95% confidence intervals (LCI and UCI) and relative importance ($w_+$) *per* class of each variable involved. Estimates whose 95% CI excluded zero are in bold. The reference categories are "surrounding farmland South" for Location and "absence in the session" for the remaining variables.

| | Estimate | SE | CI | w+ |
|---|---|---|---|---|
| **(Intercept)** | **1.164** | **0.006** | **(1.151, 1.176)** | |
| Location | | | | 1.00 |
| **surrounding farmland North** | **-0.308** | **0.009** | **(-0.325, -0.291)** | |
| **verge South** | **0.578** | **0.014** | **(0.551, 0.605)** | |
| **verge North** | **0.734** | **0.014** | **(0.707, 0.761)** | |
| Ploughing | | | | 0.75 |
| at node | -0.060 | 0.031 | (-0.121, 0.001) | |
| Elsewhere | 0.039 | 0.025 | (-0.011, 0.088) | |
| Verge vegetation cutting | | | | 0.39 |
| at node | -0.074 | 0.044 | (-0.160, 0.013) | |
| Elsewhere | 0.015 | 0.022 | (-0.029, 0.058) | |
| Cork stripping | | | | 0.21 |
| at node | 0.030 | 0.031 | (-0.031, 0.091) | |
| Elsewhere | -0.022 | 0.025 | (-0.071, 0.028) | |
| Cattle | | | | 0.11 |
| at node | 0.005 | 0.012 | (-0.018, 0.028) | |
| Elsewhere | -0.003 | 0.010 | (-0.023, 0.017) | |
| Firebreaks | | | | 0.09 |
| at node | -0.008 | 0.028 | (-0.062, 0.046) | |
| Elsewhere | 0.002 | 0.016 | (-0.030, 0.034) | |

Model averaging explaining the importance of links for connectivity (dPC link) included 8 models out of 64 possible (Supplementary materials 4.C; Table 4.C.2). The averaged model for the connectivity provided by links had strong support for the effects of location (W+ = 1.00), ploughing (W+ = 1.00) and vegetation cutting at verges (W + = 0.91) (Table 4.2). The importance of links significantly increased at the verges (and between them) when compared to the surrounding farmland South (reference category). Moreover, the importance of links was significantly higher on the southern than on the northern part of the surrounding farmland (Table 4.2, Figure 4.5). Connectivity provided by the links significantly decreased where vegetation cutting (at verges) or ploughing occurred, but increased in neighboring areas, where these management actions were absent (Table 4.2, Figure 4.5).

Averaged models for nodes and links showed little support for the effects of firebreaks, cork stripping and cattle (95% confidence intervals for estimates include zero; Tables 4.1 and 4.2).

Models for nodes and links had no collinearity problems (VIF<1.5). Patterns were absent in the residuals of models including all variables accounted in model averaging for nodes and links (Supplementary materials 4.C; Figs.4. C.1 to 4.C.6).

Table 4.2 – Averaged model of the effects of location (patches linked) and five management actions on the mean importance of links for functional connectivity (dPC links). Model-averaging is based on the 95% confidence set of best models. We show standard errors (SE), lower and upper boundaries of 95% confidence intervals (LCI and UCI) and relative importance ($w_{+}$) per class of each variable involved. Estimates whose 95% CI excluded zero are in bold. The reference categories are "surrounding farmland South" for Location and "absence in the session" for the remaining variables. Exponent notation means multiplication by: a=$10^{-1}$; b=$10^{-2}$; c=$10^{-3}$; d=$10^{-4}$; e=$10^{-5}$; f=$10^{-7}$.

| | **Estimate** | **SE** | **CI** | **w+** |
|---|---|---|---|---|
| **(Intercept)** | **$2.64^{b}$** | **$8.86^{e}$** | **($2.62^{b}$, $2.66^{b}$)** | |
| Location | | | | 1.00 |
| **surrounding farmland - verge South** | **$-2.00^{b}$** | **$9.87^{e}$** | **($-2.02^{b}$, $-1.98^{b}$)** | |
| **surrounding farmland North** | **$-1.73^{b}$** | **$9.53^{e}$** | **($-1.75^{b}$, $-1.71^{b}$)** | |
| **verge South** | **$1.57^{a}$** | **$1.37^{c}$** | **($1.54^{a}$, $1.59^{a}$)** | |
| **verge North - verge South** | **$1.32^{c}$** | **$1.99^{d}$** | **($9.30^{d}$, $1.71^{c}$)** | |
| **surrounding farmland -verge North** | **$-1.49^{b}$** | **$1.28^{d}$** | **($-1.51^{b}$, $-1.46^{b}$)** | |
| **verge North** | **$1.69^{a}$** | **$8.96^{d}$** | **($1.67^{a}$, $1.71^{a}$)** | |
| Ploughing | | | | 1.00 |
| **at one node** | **$-1.22^{d}$** | **$2.22^{d}$** | **($-5.57^{d}$, $-3.13^{d}$)** | |
| **at both nodes** | **$-1.35^{c}$** | **$4.47^{d}$** | **($-2.23^{c}$, $-4.76^{d}$)** | |
| **Elsewhere** | **$4.59^{d}$** | **$1.63^{d}$** | **($1.39^{d}$, $7.78^{d}$)** | |
| Verge vegetation cutting | | | | 0.91 |
| at one node | $-1.39^{d}$ | $2.01^{d}$ | ($-5.32^{d}$, $2.54^{d}$) | |
| at both nodes | $-1.50^{c}$ | $8.81^{d}$ | ($-3.23^{c}$, $2.24^{d}$) | |
| **Elsewhere** | **$4.23^{d}$** | **$1.65^{d}$** | **($9.90^{e}$, $7.47^{d}$)** | |
| Cork stripping | | | | 0.57 |
| at one node | $6.00^{d}$ | $4.65^{d}$ | ($-3.11^{d}$, $1.51^{c}$) | |
| at both nodes | $3.16^{d}$ | $1.77^{d}$ | ($-3.14^{e}$, $6.64^{d}$) | |
| Elsewhere | $-2.50^{d}$ | $1.94^{d}$ | ($-6.30^{d}$, $1.30^{d}$) | |
| Firebreaks | | | | 0.19 |
| at one node | $-5.93^{e}$ | $1.86^{d}$ | ($-4.23^{d}$, $3.04^{d}$) | |
| at both nodes | $1.90^{e}$ | $1.80^{d}$ | ($-3.34^{d}$, $3.72^{d}$) | |
| Elsewhere | $-2.28^{d}$ | $1.25^{d}$ | ($-4.72^{d}$, $1.62^{e}$) | |
| Cattle | | | | 0.04 |
| at one node | $3.95^{e}$ | $1.27^{d}$ | ($-2.10^{d}$, $2.89^{d}$) | |
| at both nodes | $4.44^{e}$ | $7.98^{e}$ | ($-1.12^{d}$, $2.01^{d}$) | |
| Elsewhere | $-1.42^{f}$ | $7.20^{e}$ | ($-1.41^{d}$, $1.41^{d}$) | |

## 4.4. Discussion

The presence of a medium traffic road sharply decreased the overall landscape functional connectivity for wood mice's foraging movements when compared with a virtual roadless scenario. However, road verges (about 20% of the road area) improved functional connectivity. Links at road verges were significantly more important than in equivalent lines at the virtual roadless scenario. Road verges included most of the important microhabitats for maintaining connectivity for wood mice. Connectivity was significantly reduced immediately after vegetation cutting on verges or land ploughing. However, fine-scale connectivity simultaneously increased in adjacent unmanaged areas, suggesting a compensation effect.

### 4.4.1 Road and road verges affect functional connectivity

Road presence drastically reduced landscape connectivity for wood mice's foraging movements. Specifically, paved lanes reduced the importance of crossings between the two verges and, consequently, reduced connectivity across the road area. Additionally, links along the verges were much more important than at the surrounding farmland or than at the virtual roadless scenario, where all links had similar value for connectivity. These results were expected because only 5% of wood mice cross roads (Ascensão et al., 2016). Although they are amongst the most road-killed mammals in Portugal (Carvalho and Mira, 2011), their survival is very similar in road and roadless areas (Galantinho et al., 2017). Therefore, most of the animals might not attempt to cross the paved lanes and probably adjust their foraging behaviour in the area surrounding the road. Indeed, this species avoids gaps in vegetation cover to decrease predation risk (Díaz et al., 2005). For the same reason, paved roads are strong barriers for wood mouse's ecological equivalent in America, *Peromiscus leucopus* (Marrotte et al., 2014). Previous studies about the road barrier (or filter) effect on small mammals already discarded avoidance of traffic (Ford and Fahrig, 2008; McGregor et al., 2008; Ascensão et al., 2016). We also discarded the lack of motive to reach the opposite side of the road because suitable habitat exists on both sides (Riley et al., 2006; Carvalho et al. 2018), and road crossings were detected. Wood mice use or disperse through a wide range of habitats but are more abundant and move easily in microhabitats of dense vegetation cover (Montgomery, 1985; Rosalino et al., 2011). In fact, road verges provided better microhabitats conditions for functional connectivity (dPC node) than the surrounding farmland. At road verges the flux between microhabitat patches (dPC flux) was as important as microhabitat itself (dPC intra), while at the surrounding farmland these connections (dPC flux) were much less relevant. The value of microhabitat itself (dPC intra) was also higher at road verges than at the equivalent central lines of the virtual roadless scenario. Thus, the importance of verges and surrounding land management on functional connectivity could result from a combination of: (1) retreat from managed land into undisturbed areas (Tew and MacDonald, 1993; Ouin et al., 2000; Wilson et al., 2014), (2) higher suitability of microhabitat at verges (Ascensão et al., 2012; Encarnação and Becker, 2015) and (3) the road as filter to movement (Ascensão et al., 2016). Other causes for higher connectivity along the verge require further testing, but they may include a greater availability of seeds and invertebrates (Bellamy et al., 2000) or specific consequences of gap avoidance: territory pile-up (Riley et al., 2006), confinement (Marrotte et al., 2014) or detour to find safe passage (Bakker and Van Vuren, 2004; D'Amico et al., 2015). Our findings show that both good microhabitat conditions and linear structure allow for safe movements along road verges and confirm their role as habitat corridors for small mammals at fine scale.

### 4.4.2 Management effects on functional connectivity

All management practices induced gaps in vegetation (or changes in structure such as cork stripping); however, only ploughing and vegetation cutting at verges affected functional connectivity for wood mice's foraging movements significantly.

Ploughing was the only agricultural management practice that decreased functional connectivity for wood mice. The effects of grazing and cork stripping on functional connectivity had little support. Grazing affects small mammals according to its intensity and their dependence on shrub cover (Johnston and Anthony, 2008). Thus, results in heavily grazed *montado* systems where wood mice are more abundant at fenced verges (Ascensão et al., 2012) could differ from ours in a lightly grazed area, where shrub and herbaceous cover were never depleted. On the other hand, in all *montado* systems, cork stripping only occurs for a short period of time (1 to 2 hours per tree) and usually every

9 years (Costa et al., 2004). Indeed, our results suggest that cork stripping does not hamper functional connectivity.

Vegetation cutting at verges was the only road related management activity that affected fine-scale connectivity. The absence of an effect of firebreak opening on functional connectivity was surprising because it also generates a gap in vegetation. However, firebreaks were opened on two occasions and never affected both verges simultaneously, while cutting was conducted on both verges. Therefore, we believe that connectivity is affected not only by the size of the vegetation gap, but also by the specific area affected by the gap. In fact, verges were the most important sections of the road area for fine-scale connectivity. However, firebreaks and vegetation cutting at verges may have had an additive effect on connectivity. Both verges were cut one month after the firebreak opening, generating a total vegetation gap of 60 meters (including paved lanes).

Altogether, vegetation cutting at verges, firebreaks and ploughing were the main drivers of change on vegetation structure. These changes explain the higher relative importance of microhabitat quality (dPCintra) at the road area than at the virtual roadless scenario.

More importantly, we have shown that the role of verges for fine-scale connectivity depends on management actions on verges and surrounding areas, allowing animals to retreat from managed land into undisturbed areas.

### 4.4.3 Strengths and potential limitations

Our data allowed us to analyze how the presence of a road, verges and management affected landscape functional connectivity for wood mice’s foraging movements. This was possible since we gathered movement and microhabitat (vegetation) data at a scale adjusted to the species (Rosalino et al. 2011) and compared it to a virtual roadless scenario. Our study design is not a traditional experimental setting that would test independent effects of each management action. Even so, in a typical well-preserved *montado*, all management actions occur in the same area and therefore they are not independent. Also, we did not study social differences in behavior that could affect functional connectivity (females vs. males, adults vs. juveniles or residents vs. dispersers; Fey et al., 2015; Garrido-Garduño et al., 2016). However, these individual differences would be particularly relevant for studying connectivity and/or gene flow between isolated areas (Fey et al., 2015). Nonetheless, detailed studies on individual movements for resident and transient animals (Galantinho et al, in prep), will allow us to assess more thoroughly the role of road verges as corridors for small mammals.

Our study was conducted only in one plot what limits certain inferences due to pseudoreplication. However, inferences from studies that account for pseudoreplication should not be neglected (Davies and Gray, 2015). In our study site, we analyzed 100 nodes and 180 links along 26 sessions and compared our results with a virtual roadless scenario. Moreover, our modelling approach accounts for temporal and spatial autocorrelation. Altogether, our work provides scientific evidence that may guide solutions for roadside management in other areas.

### 4.4.4 Implications for verge management and future research

Our results highlight the importance of road verges for the flow of wood mice, even when roads are embedded in well preserved habitats. Our findings emphasize the importance of verge management to conserve landscape connectivity for small mammals' foraging movements. This is particularly relevant because roads are major threats to many small mammal species of conservation concern worldwide (e.g., the Endangered *Callistomys pictus*, Roach and Naylor, 2016), but its verges may also be valuable remnant habitats for these species (e.g., the Near Threatened *Microtus cabrerae*, Pita et al., 2006).

Therefore, verge management should be carefully planned considering its importance in providing refuge habitat and functional connectivity for small mammals. Management should either: (1) promote refuge and/or movement for species of conservation concern (e.g., *Microtus cabrerae*; Pita et al., 2006) and for key prey species as the wood mouse, or (2) deter fluxes of species that may become pests (e.g., *Microtus arvalis*; Redon et al., 2010). In the first case, management at verges and at surrounding areas should be articulated to ensure that microhabitat features essential to functional connectivity are maintained near roads. If management at road surroundings involves vegetation clearing, and safety (including fire risk) is not compromised, we suggest implementing firebreaks exclusively and maintaining at least one of the road verges unharmed. In places where both firebreaks and vegetation cutting in verges are mandatory for safety reasons, we recommend that together they should occupy a maximum width of 10 m (current minimum width allowed by law). However, these recommendations must consider the vulnerability to roadkills of local predators that may be attracted to denser verges where preys are more abundant (Ascensão et al., 2012).

Quantifying the effects of culverts and underpasses on functional connectivity (Ascensão et al., 2016) and the efficiency of the detour to find a safe passage across the road (Bakker and Van Vuren, 2004) would improve conservation measures. These two effects should be further analyzed for specialist species. Specialist small mammals' use of underpasses depends on appropriate vegetation structure (D'Amico et al., 2015). Thus, the importance of verge vegetation management for an efficient use of culverts may be critical. On the other side, Sullivan et al. (2012) highlighted that abundance (and diversity) of small mammals in hedgerows does not increase beyond an optimum value of shrub biomass. Thus, road verges may still provide refuge and corridors for movement when the vegetation is partially cut. However, studies aiming to define minimum thresholds of cover needed for small mammals' movements are still lacking. Only with this information will be possible to define a level of vegetation cover and height on road verges that allows for a compromise between road safety (including fire ignition risk) and promotion of small fauna movements.

## 4.5 Conclusions

Altogether, we showed that the presence of a national road on a well-preserved habitat can decrease overall landscape connectivity for wood mice's foraging movements. However, road verges enhance movement (comparing with the surrounding areas) and therefore, can partially counteract this negative effect. Nonetheless, the increase in connectivity provided by road verges depends on the amount of vegetation cover available after management on verges and surrounding areas. Our conclusions could be applied to small mammals that avoid crossing vegetation gaps such as paved lanes or ploughed areas (Oxley et al., 1974; Macpherson et al., 2011). Our results for a common species in a well-preserved habitat strengthen the global concerns of road effects on more pristine environments (Laurance and Balmford, 2013).

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# Supplementary materials 4.A

## Details for node parameterization

In order to parameterize nodes (traps) for the connectivity analysis we assumed that they were differentiated by the relation between the capture index and the nine microhabitat variables sampled. Generalized linear models were used to model node value for the road area. Generalized least squares estimation was used to account for heterogeneity and correlation structures when necessary (package nlme 3.1.-113; Pinheiro et al., 2013). We followed Zuur et al. (2009) for model building and validation and Burnham and Anderson (2002) for model selection. All statistical analysis was performed on R version 3.0.2 (R Core Team, 2013). The capture index was log transformed to approach normality. We checked for collinearity between microhabitat variables using the variation inflation factor (VIF) (excluded variables with VIF > 3; package AED 1.0, Zuur, 2010). Our candidate model set included all possible combinations of the non-collinear variables. We ranked models using AIC and obtained averaged parameter estimates and associated unconditional standard errors (SE) of all models in the 95% confidence set (sum of Akaike weights ≥ 0.95; package MuMIn 1.10.5; Barton, 2014). The relative importance of each variable (*w*+) was assessed by the sum of Akaike weights (*w*) of all models of the set where the variable occurred. Model validation consisted of plotting model residuals against fitted values and each of the explanatory variables (Zuur et al., 2009). We checked for autocorrelation in model residuals using the autocorrelation function for temporal patterns (ACF; package nlme 3.1.-113, Pinheiro et al., 2013) and bubble plot for spatial patterns (packages *sp* 1.0-14, Pebesma and Bivand, 2005; and *gstat* 1.0-19, Pebesma, 2004).

These analyses excluded 3 from the 9 variables initially sampled due to: correlation problems for tree height and tree cover (VIF > 4; after elimination, VIF < 3 for all variables) and lack of variability for bare ground and rock cover. Model averaging included all models retained in the 95% confidence set from the top models (16 of 64 models) (Table 4.A.1). Averaged estimates stress that shrub height was the most important variable related to wood mice captures (w+=1.00) (Table 4.A.2). Traps with higher shrubs (class 4) captured more mice, but confidence intervals of estimates for the remaining variables included zero. Model validation is acceptable (Figures 4.A.1 and 4.A.2).

Table 4.A.1 – Model selection for node value at the road area. Models relate six microhabitat variables (ShrC – shrub cover; TreC – tree cover; LitC – litter cover; HerC – herbaceous cover; ShrH- shrub height; HerH – herbeceus height) to the index of animals captured. Models are ranked by AIC. We present AIC differences to the best model (delta) and Akaike weights ($w_i$) based on the entire set of models. Models accounted for model averaging (Akaike weights sum > 0.95; 95% confidence set) are highlighted in bold.

| Model | (Int) | ShrC | TreC | LitC | HerC | ShrH | HerH | df | logLik | AIC | delta | $w_i$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **49** | **0.794** | | | | | **+** | **+** | **7** | **-108.73** | **231.5** | **0.00** | **0.237** |
| **17** | **1.113** | | | | | **+** | | **5** | **-111.02** | **232.0** | **0.58** | **0.178** |
| **57** | **0.832** | | | | **+** | **+** | **+** | **10** | **-106.61** | **233.2** | **1.76** | **0.098** |
| **19** | **1.014** | | **+** | | | **+** | | **8** | **-108.92** | **233.8** | **2.37** | **0.072** |
| **51** | **0.763** | | **+** | | | **+** | **+** | **10** | **-107.03** | **234.1** | **2.59** | **0.065** |
| **21** | **1.032** | | | **+** | | **+** | | **8** | **-109.05** | **234.1** | **2.64** | **0.063** |
| **53** | **0.751** | | | **+** | | **+** | **+** | **10** | **-107.26** | **234.5** | **3.05** | **0.052** |
| **25** | **0.875** | | | | **+** | **+** | | **8** | **-109.40** | **234.8** | **3.34** | **0.045** |
| **59** | **0.819** | | **+** | | **+** | **+** | **+** | **13** | **-104.86** | **235.7** | **4.25** | **0.028** |
| **50** | **0.756** | **+** | | | | **+** | **+** | **10** | **-108.25** | **236.5** | **5.03** | **0.019** |
| **27** | **0.822** | | **+** | | **+** | **+** | | **11** | **-107.35** | **236.7** | **5.23** | **0.017** |
| **61** | **0.782** | | | **+** | **+** | **+** | **+** | **13** | **-105.35** | **236.7** | **5.24** | **0.017** |
| **18** | **1.145** | **+** | | | | **+** | | **8** | **-110.38** | **236.8** | **5.29** | **0.017** |
| **29** | **0.830** | | | **+** | **+** | **+** | | **11** | **-107.49** | **237.0** | **5.50** | **0.015** |
| **58** | **0.854** | **+** | | | **+** | **+** | **+** | **13** | **-105.67** | **237.3** | **5.88** | **0.013** |
| **23** | **0.999** | | **+** | **+** | | **+** | | **11** | **-107.83** | **237.7** | **6.19** | **0.011** |
| 55 | 0.736 | | + | + | | + | + | 13 | -106.17 | 238.3 | 6.87 | 0.008 |
| 52 | 0.670 | + | + | | | + | + | 13 | -106.28 | 238.6 | 7.09 | 0.007 |
| 22 | 0.390 | + | | + | | + | | 11 | -108.48 | 239.0 | 7.50 | 0.006 |
| 20 | 0.370 | + | + | | | + | | 11 | -108.50 | 239.0 | 7.54 | 0.005 |
| 54 | 0.689 | + | | + | | + | + | 13 | -106.60 | 239.2 | 7.74 | 0.005 |
| 26 | 0.950 | + | | | + | + | | 11 | -108.68 | 239.3 | 7.88 | 0.005 |
| 60 | 0.744 | + | + | | + | + | + | 16 | -103.80 | 239.6 | 8.13 | 0.004 |
| 63 | 0.791 | | + | + | + | + | + | 16 | -104.22 | 240.4 | 8.97 | 0.003 |
| 62 | 0.755 | + | | + | + | + | + | 16 | -104.27 | 240.5 | 9.07 | 0.003 |
| 31 | 0.823 | | + | + | + | + | | 14 | -106.30 | 240.6 | 9.13 | 0.002 |
| 28 | 0.806 | + | + | | + | + | | 14 | -106.74 | 241.5 1 | 0.01 | 0.002 |
| 30 | 0.838 | + | | + | + | + | | 14 | -106.75 | 241.5 1 | 0.03 | 0.002 |
| 56 | 0.624 | + | + | + | | + | + | 16 | -105.24 | 242.5 1 | 1.02 | 0.001 |
| 24 | 0.997 | + | + | + | | + | | 14 | -107.34 | 242.7 1 | 1.21 | 0.001 |
| 64 | 0.673 | + | + | + | + | + | + | 19 | -102.94 | 243.9 1 | 2.41 | 0.000 |
| 32 | 0.756 | + | + | + | + | + | | 17 | -105.54 | 245.1 1 | 3.61 | 0.000 |
| 34 | 0.357 | + | | | | | + | 7 | -117.41 | 248.8 1 | 7.36 | 0.000 |
| 41 | 0.807 | | | | + | | + | 7 | -118.05 | 250.1 1 | 8.63 | 0.000 |
| 42 | 0.320 | + | | | + | | + | 10 | -115.19 | 250.4 1 | 8.91 | 0.000 |
| 36 | 0.249 | + | + | | | | + | 10 | -115.92 | 251.8 2 | 0.37 | 0.000 |
| 38 | 0.207 | + | | + | | | + | 10 | -116.01 | 252.0 2 | 0.56 | 0.000 |
| 45 | 0.500 | | | + | + | | + | 10 | -116.21 | 252.4 2 | 0.96 | 0.000 |
| 44 | 0.169 | + | + | | + | | + | 13 | -113.70 | 253.4 2 | 1.93 | 0.000 |
| 46 | 0.140 | + | | + | + | | + | 13 | -113.85 | 253.7 2 | 2.22 | 0.000 |
| 43 | 0.756 | | + | | + | | + | 10 | -117.34 | 254.7 2 | 3.22 | 0.000 |
| 37 | 0.453 | | | + | | | + | 7 | -120.49 | 255.0 2 | 3.52 | 0.000 |
| 33 | 0.894 | | | | | | + | 4 | -123.83 | 255.7 2 | 4.18 | 0.000 |
| 40 | 0.139 | + | + | + | | | + | 13 | -115.11 | 256.2 2 | 4.75 | 0.000 |

|  |  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 47 | 0.481 |  | + | + | + | + | 13 | -115.85 | 257.7 2 | 6.23 | 0.000 |
| 48 | 0.413 | + | + | + | + | + | 16 | -112.92 | 257.8 2 | 6.36 | 0.000 |
| 35 | 0.838 |  | + |  |  | + | 7 | -122.75 | 259.5 2 | 8.04 | 0.000 |
| 39 | 0.423 |  | + | + |  | + | 10 | -119.77 | 259.5 2 | 8.08 | 0.000 |
| 10 | 0.303 | + |  |  | + |  | 8 | -123.62 | 263.2 3 | 1.77 | 0.000 |
| 12 | 0.123 | + | + |  | + |  | 11 | -120.93 | 263.9 3 | 2.39 | 0.000 |
| 14 | 0.882 | + |  | + | + |  | 11 | -121.19 | 264.4 3 | 2.90 | 0.000 |
| 16 | 0.244 | + | + | + | + |  | 14 | -119.63 | 267.3 3 | 5.79 | 0.000 |
| 2 | 0.198 | + |  |  |  |  | 5 | -129.08 | 268.2 3 | 6.70 | 0.000 |
| 4 | 0.986 | + | + |  |  |  | 8 | -126.28 | 268.6 3 | 7.09 | 0.000 |
| 13 | 0.460 |  |  | + | + |  | 8 | -127.03 | 270.1 3 | 8.60 | 0.000 |
| 6 | 0.220 | + |  | + |  |  | 8 | -127.17 | 270.3 3 | 8.86 | 0.000 |
| 9 | 0.849 |  |  |  | + |  | 5 | -130.75 | 271.5 4 | 0.03 | 0.000 |
| 11 | 0.747 |  | + |  | + |  | 8 | -128.30 | 272.6 4 | 1.13 | 0.000 |
| 8 | 0.944 | + | + | + |  |  | 11 | -125.33 | 272.7 4 | 1.20 | 0.000 |
| 15 | 0.453 |  | + | + | + |  | 11 | -125.60 | 273.2 4 | 1.74 | 0.000 |
| 5 | 1.037 |  |  | + |  |  | 5 | -132.38 | 274.8 4 | 3.30 | 0.000 |
| 1 | 1.384 |  |  |  |  |  | 2 | -135.57 | 275.1 4 | 3.68 | 0.000 |
| 3 | 1.230 |  | + |  |  |  | 5 | -132.72 | 275.4 4 | 3.98 | 0.000 |
| 7 | 0.982 |  | + | + |  |  | 8 | -130.45 | 276.9 4 | 5.42 | 0.000 |

Table 4.A.2 - Averaged model for node value at the road area. Modelling relates microhabitat variables to the index of animals captured. Model-averaging is based on the 95% confidence set of best models. We show standard errors (SE), lower and upper boundaries of 95% confidence intervals (LCI and UCI) and relative importance ($w_+$) per class of each variable involved. Estimates whose 95% CI excluded zero are in bold.

| | Estimate | SE | LCI | UCI | w + |
|---|---|---|---|---|---|
| (Intercept) | 0.90001 | 0.26464 | 0.376248 | 1.423769 | |
| Shrub height | | | | | 1.00 |
| class 2 | -0.29736 | 0.25942 | -0.8114 | 0.216694 | |
| class 3 | 0.36041 | 0.23632 | -0.10852 | 0.829327 | |
| **class 4** | **1.09232** | **0.24676** | **0.603198** | **1.581452** | |
| Herbaceus height | | | | | 0.56 |
| class 2 | 0.12964 | 0.22544 | -0.31801 | 0.577289 | |
| class 4 | 0.48572 | 0.25849 | -0.02702 | 0.998454 | |
| Shrub cover | | | | | 0.05 |
| class 2 | 0.0204 | 0.28679 | -0.54837 | 0.58917 | |
| class 3 | -0.18998 | 0.31932 | -0.82354 | 0.443579 | |
| class 4 | -0.17811 | 0.45584 | -1.08274 | 0.726518 | |
| Herbaceus cover | | | | | 0.25 |
| class 2 | 0.27332 | 0.25823 | -0.23938 | 0.786019 | |
| class 3 | -0.12814 | 0.30161 | -0.72601 | 0.46974 | |
| class 4 | -0.02378 | 0.37074 | -0.75735 | 0.709786 | |
| Tree cover | | | | | 0.20 |
| class 2 | 0.4347 | 0.28095 | -0.12329 | 0.992689 | |
| class 3 | 0.05377 | 0.22939 | -0.40168 | 0.509219 | |
| class 4 | 0.2171 | 0.19886 | -0.17745 | 0.611652 | |
| Litter cover | | | | | 0.17 |
| class 2 | -0.11453 | 0.2972 | -0.70468 | 0.47561 | |
| class 3 | 0.237 | 0.31473 | -0.38798 | 0.861971 | |
| class 4 | 0.22826 | 0.25858 | -0.28529 | 0.741805 | |

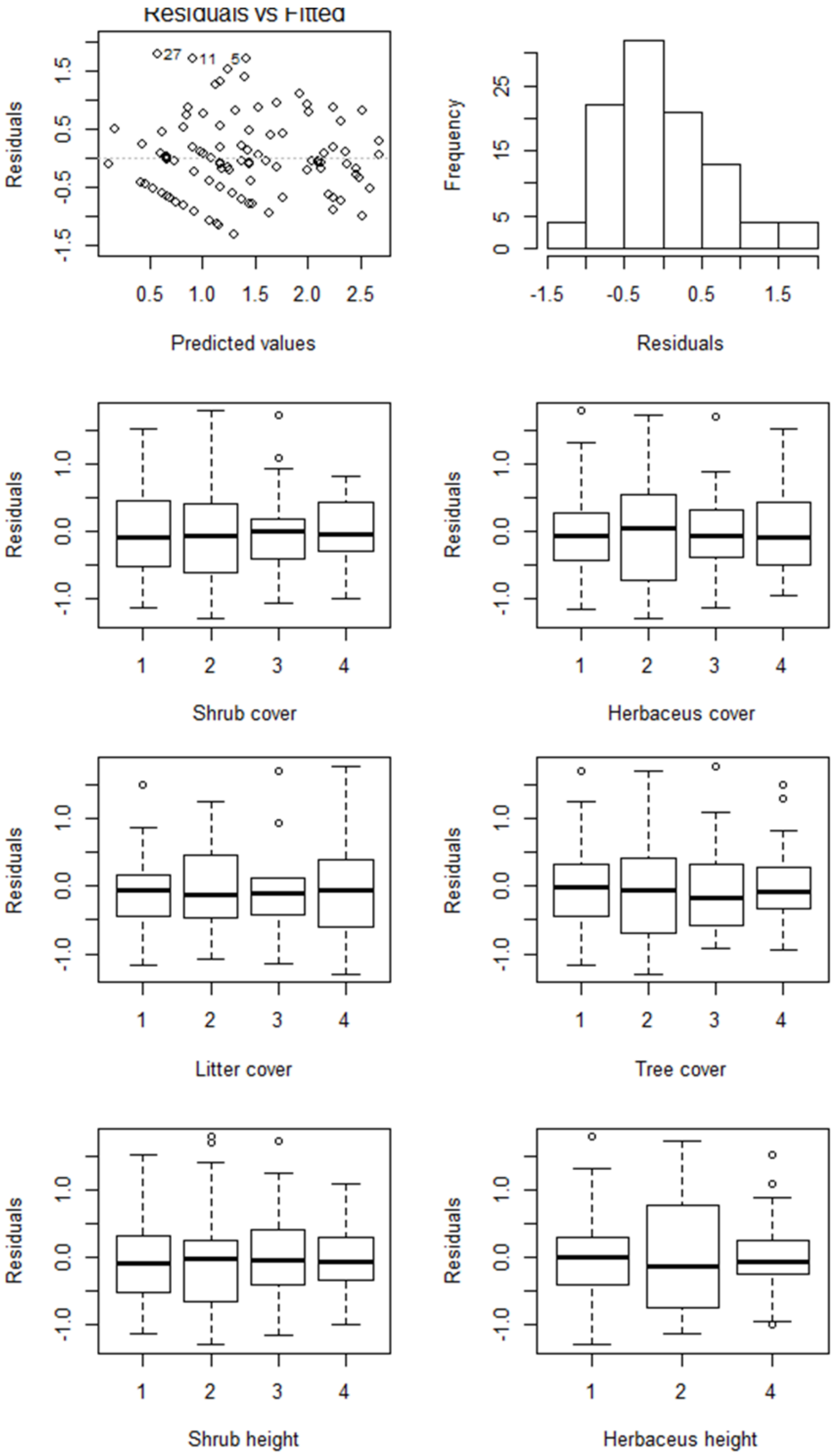

Figure 4.A.1 – Model validation graphs for node valorization at the road area. The model relates six microhabitat variables to the index of animals captured. The model validated accounts for all variables included in the averaged model.

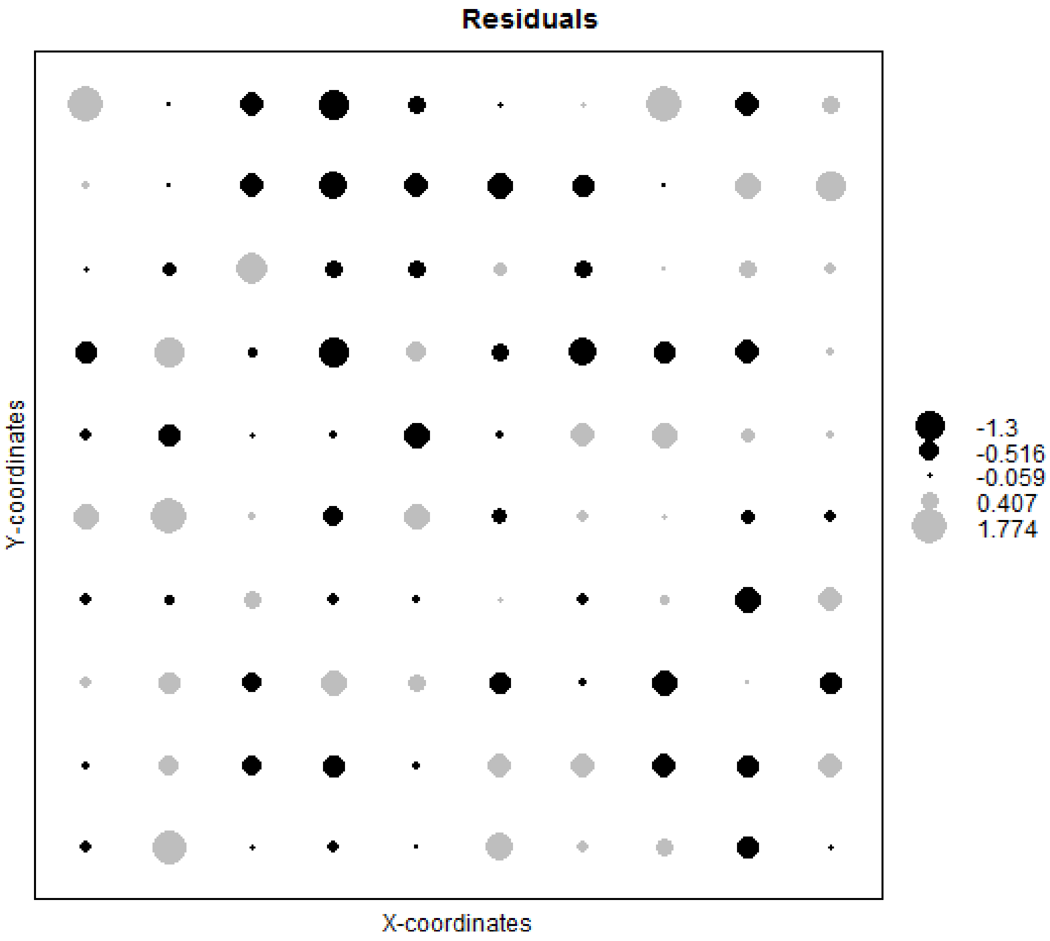

Figure 4.A.2 – Bubbleplot for the model explaining node valorization at the road area. We show the standardized residuals obtained by the model with all the variables accounted in averaging plotted versus their spatial coordinates. The model relates six microhabitat variables to the index of animals captured.

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# Supplementary materials 4.B

## Distances travelled in consecutive nights

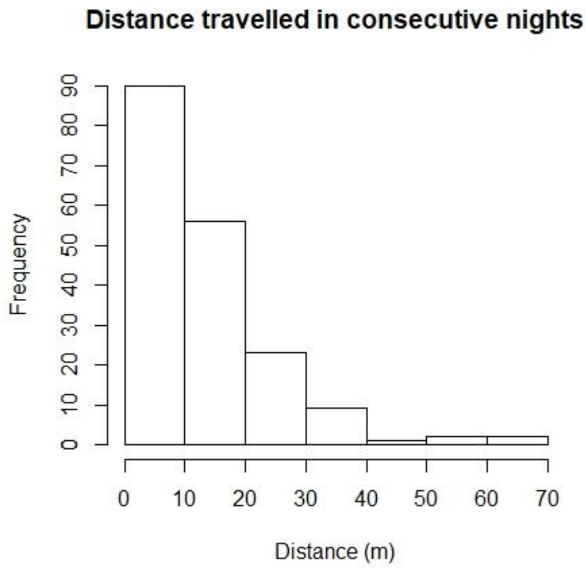

Figure 4.B.1- Distances travelled by the 77 wood mice captured in consecutive nights.

# Supplementary materials 4.C

## Details for model selection, averaging and validation for the effects of management and location on the importance of nodes and links for connectivity

Table 4.C.1 – Model selection for node importance (dPC node) for connectivity at the road area. Models relate five management actions (cat - cattle, fir - firebreaks, plo - ploughing, str – cork stripping, vpa - verge cutting) and location (pat) to the index of animals captured. Models are ranked by AIC. We present AIC differences to the best model (delta) and Akaike weights ($w_i$) based on the entire set of models. Models accounted for model averaging (Akaike weights sum > 0.95; 95% confidence set) are highlighted in bold.

| model | (Int) | cat | fir | pat | plo | str | vpa | df | logLik | AIC | Delta |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **13** | **1.164** | | | **+** | **+** | | | **7** | **593.31** | **-1172.60** | **0.00** |
| **45** | **1.164** | | | **+** | **+** | | **+** | **9** | **594.91** | **-1171.80** | **0.79** |
| **5** | **1.162** | | | **+** | | | | **5** | **590.27** | **-1170.50** | **2.08** |
| **29** | **1.165** | | | **+** | **+** | **+** | | **9** | **594.13** | **-1170.30** | **2.36** |
| **37** | **1.161** | | | **+** | | | **+** | **7** | **591.94** | **-1169.90** | **2.73** |
| **61** | **1.164** | | | **+** | **+** | **+** | **+** | **11** | **595.77** | **-1169.50** | **3.07** |
| **14** | **1.165** | **+** | | **+** | **+** | | | **9** | **593.49** | **-1169.00** | **3.63** |
| **15** | **1.164** | | **+** | **+** | **+** | | | **9** | **593.35** | **-1168.70** | **3.91** |
| **46** | **1.164** | **+** | | **+** | **+** | | **+** | **11** | **595.01** | **-1168.00** | **4.60** |
| **21** | **1.163** | | | **+** | | **+** | | **7** | **591.00** | **-1168.00** | **4.61** |
| **47** | **1.164** | | **+** | **+** | **+** | | **+** | **11** | **594.96** | **-1167.90** | **4.68** |
| **53** | **1.162** | | | **+** | | **+** | **+** | **9** | **592.72** | **-1167.40** | **5.18** |
| **6** | **1.162** | **+** | | **+** | | | | **7** | **590.40** | **-1166.80** | **5.82** |
| **30** | **1.166** | **+** | | **+** | **+** | **+** | | **11** | **594.38** | **-1166.80** | **5.85** |
| 7 | 1.162 | | + | + | | | | 7 | 590.31 | -1166.60 | 5.99 |
| 31 | 1.165 | | + | + | + | + | | 11 | 594.18 | -1166.40 | 6.26 |
| 38 | 1.161 | + | | + | | | + | 9 | 592.00 | -1166.00 | 6.62 |
| 39 | 1.161 | | + | + | | | + | 9 | 591.99 | -1166.00 | 6.63 |
| 62 | 1.165 | + | | + | + | + | + | 13 | 595.92 | -1165.80 | 6.78 |
| 63 | 1.164 | | + | + | + | + | + | 13 | 595.83 | -1165.70 | 6.95 |
| 16 | 1.165 | + | + | + | + | | | 11 | 593.52 | -1165.00 | 7.57 |
| 22 | 1.163 | + | | + | | + | | 9 | 591.19 | -1164.40 | 8.23 |
| 23 | 1.163 | | + | + | | + | | 9 | 591.05 | -1164.10 | 8.51 |
| 48 | 1.164 | + | + | + | + | | + | 13 | 595.05 | -1164.10 | 8.51 |
| 54 | 1.162 | + | | + | | + | + | 11 | 592.81 | -1163.60 | 8.99 |
| 55 | 1.162 | | + | + | | + | + | 11 | 592.77 | -1163.50 | 9.07 |
| 8 | 1.162 | + | + | + | | | | 9 | 590.43 | -1162.90 | 9.75 |
| 32 | 1.166 | + | + | + | + | + | | 13 | 594.42 | -1162.80 | 9.78 |
| 40 | 1.161 | + | + | + | | | + | 11 | 592.04 | -1162.10 | 10.53 |
| 64 | 1.165 | + | + | + | + | + | + | 15 | 595.97 | -1161.90 | 10.68 |
| 24 | 1.163 | + | + | + | | + | | 11 | 591.23 | -1160.50 | 12.16 |
| 56 | 1.162 | + | + | + | | + | + | 13 | 592.86 | -1159.70 | 12.90 |
| 52 | 1.171 | + | + | | | + | + | 10 | -1170.35 | 2360.70 | 3533.31 |
| 60 | 1.171 | + | + | | + | + | + | 12 | -1169.32 | 2362.60 | 3535.25 |
| 50 | 1.171 | + | | | | + | + | 8 | -1173.93 | 2363.90 | 3536.48 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 58 | 1.172 | + | | + | + | + | 10 | -1172.91 | 2365.80 | 3538.42 |
| 20 | 1.171 | + | + | | + | | 8 | -1185.81 | 2387.60 | 3560.24 |
| 28 | 1.171 | + | + | + | + | | 10 | -1184.79 | 2389.60 | 3562.20 |
| 18 | 1.171 | + | | | + | | 6 | -1189.18 | 2390.40 | 3562.97 |
| 26 | 1.172 | + | | + | + | | 8 | -1188.16 | 2392.30 | 3564.93 |
| 36 | 1.171 | + | + | | | + | 8 | -1189.16 | 2394.30 | 3566.93 |
| 44 | 1.171 | + | + | + | | + | 10 | -1188.14 | 2396.30 | 3568.90 |
| 34 | 1.171 | + | | | | + | 6 | -1192.69 | 2397.40 | 3570.00 |
| 42 | 1.171 | + | | + | | + | 8 | -1191.68 | 2399.40 | 3571.97 |
| 4 | 1.171 | + | + | | | | 6 | -1204.40 | 2420.80 | 3593.42 |
| 12 | 1.171 | + | + | + | | | 8 | -1203.40 | 2422.80 | 3595.41 |
| 2 | 1.171 | + | | | | | 4 | -1207.72 | 2423.40 | 3596.05 |
| 10 | 1.171 | + | | + | | | 6 | -1206.72 | 2425.40 | 3598.05 |
| 51 | 1.172 | | + | | + | + | 8 | -1271.06 | 2558.10 | 3730.73 |
| 59 | 1.172 | | + | + | + | + | 10 | -1270.10 | 2560.20 | 3732.82 |
| 49 | 1.172 | | | | + | + | 6 | -1278.41 | 2568.80 | 3741.44 |
| 57 | 1.172 | | | + | + | + | 8 | -1277.46 | 2570.90 | 3743.54 |
| 35 | 1.172 | | + | | | + | 6 | -1288.48 | 2589.00 | 3761.56 |
| 43 | 1.172 | | + | + | | + | 8 | -1287.53 | 2591.10 | 3763.68 |
| 33 | 1.172 | | | | | + | 4 | -1295.73 | 2599.50 | 3772.08 |
| 41 | 1.172 | | | + | | + | 6 | -1294.80 | 2601.60 | 3774.20 |
| 19 | 1.171 | | + | | + | | 6 | -1297.69 | 2607.40 | 3780.00 |
| 27 | 1.172 | | + | + | + | | 8 | -1296.76 | 2609.50 | 3782.13 |
| 17 | 1.171 | | | | + | | 4 | -1304.90 | 2617.80 | 3790.41 |
| 25 | 1.171 | | | + | + | | 6 | -1303.97 | 2619.90 | 3792.55 |
| 3 | 1.171 | | + | | | | 4 | -1314.76 | 2637.50 | 3810.14 |
| 11 | 1.172 | | + | + | | | 6 | -1313.84 | 2639.70 | 3812.29 |
| 1 | 1.171 | | | | | | 2 | -1321.87 | 2647.70 | 3820.36 |
| 9 | 1.171 | | | + | | | 4 | -1320.96 | 2649.90 | 3822.52 |

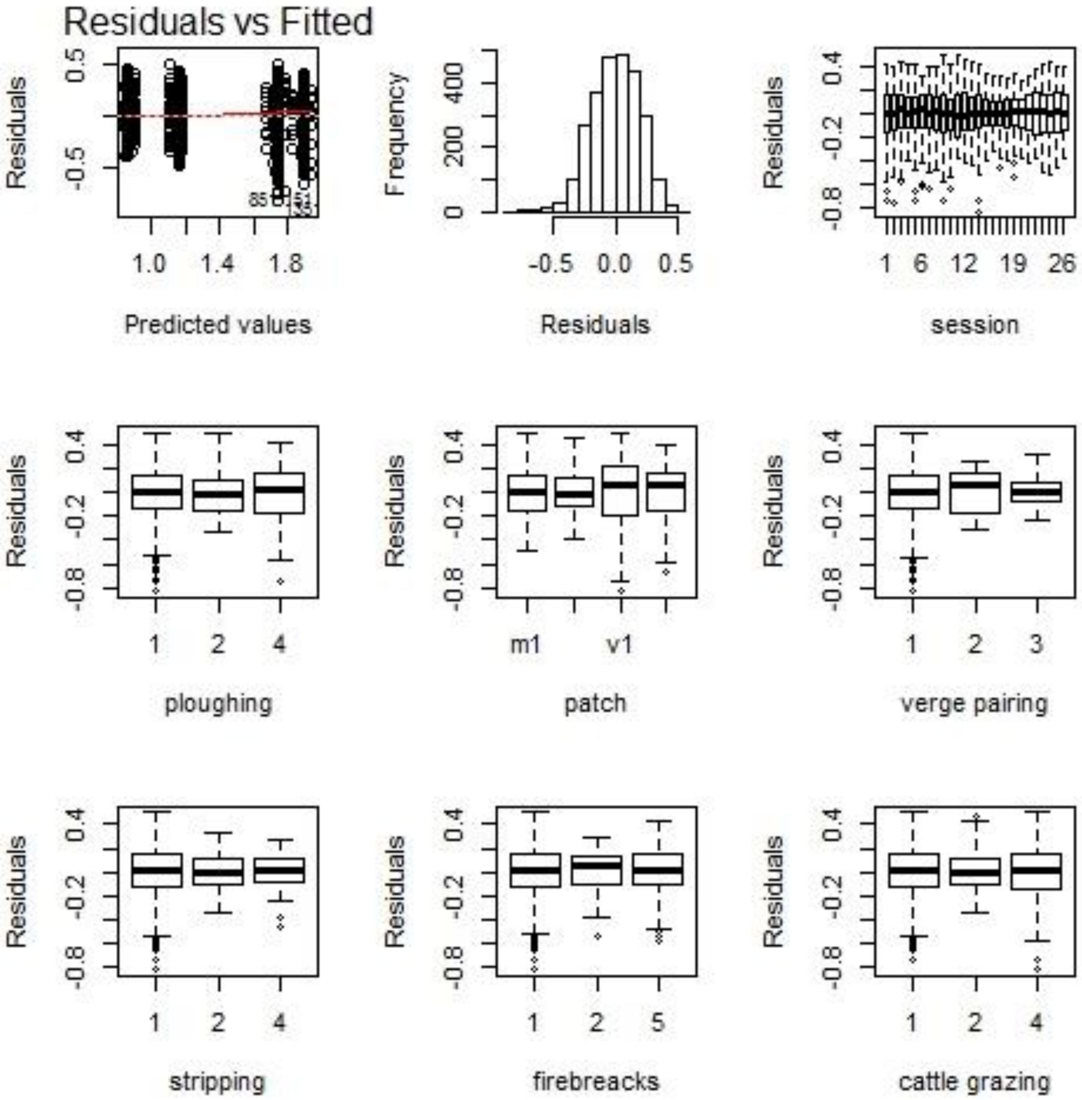

Figure 4.C.1 - Model validation graphs for node importance (dPC node) at the road area. The model relates five management actions (cattle, firebreaks, ploughing, cork stripping, verge vegetation cutting) and location (patch) to dPC node. The model validated accounts for all variables included in the averaged model.

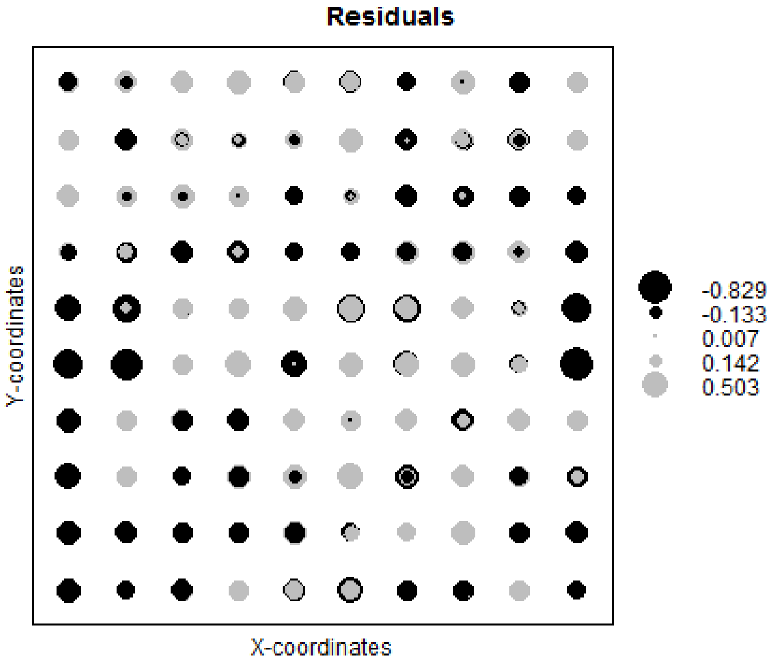

Figure 4.C.2- Bubbleplot for the model explaining node importance (dPC node) for connectivity at the road area. We show the standardized residuals obtained by the model with all the variables accounted in averaging plotted versus their spatial coordinates. The model relates five management actions (cattle, firebreaks, ploughing, cork stripping, verge vegetation cutting) and location (patch) to dPC node.

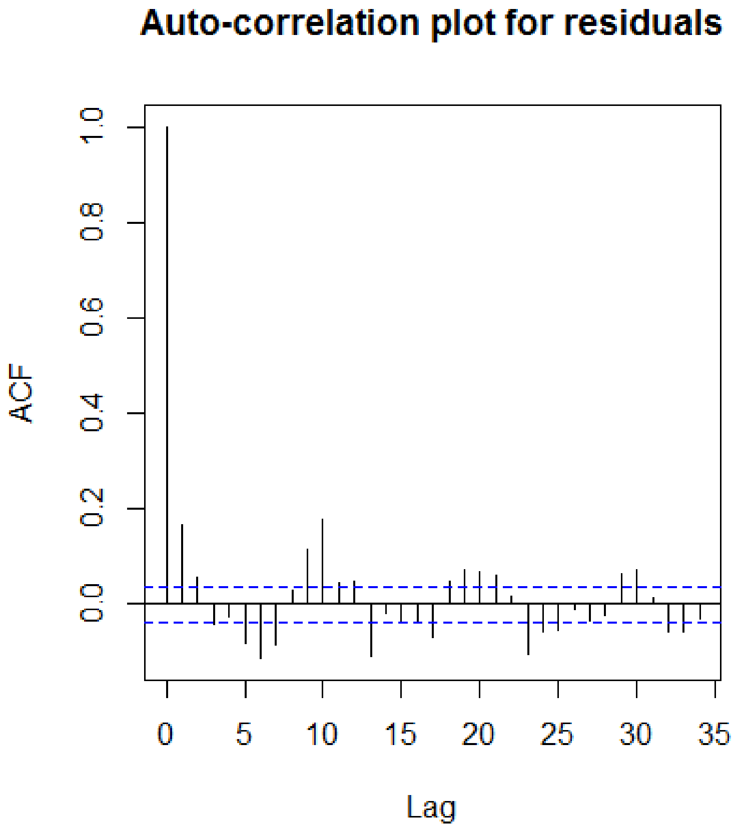

Figure 4.C.3- Auto-correlation function (ACF) for the model explaining node importance (dPC node) for connectivity at the road area. The model relates five management actions (cattle, firebreaks, ploughing, cork stripping, verge vegetation cutting) and location (patch) to dPC node. Residuals were obtained by the model with all the variables accounted in averaging. Time lags are expressed in the horizontal axis and correlation in the vertical axis. Dotted lines represent 95% confidence interval.

Table 4.C.2 - Model selection for link importance (dPC link) for connectivity at the road area. Models relate five management actions (cat - cattle, fir - firebreaks, plo - ploughing, str – cork stripping, vpa – verge vegetation cutting) and location (pat) to the index of animals captured. Models are ranked by AIC. We present AIC differences to the best model (delta) and Akaike weights ($w_i$) based on the entire set of models. Models accounted for model averaging (Akaike weights sum > 0.95; 95% confidence set) are highlighted in grey.

| model | (Int) | cat | fir | pat | plo | str | vpa | df | logLik | AIC | delta |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **61** | **0.02642** | | | **+** | **+** | **+** | **+** | **23** | **21341.88** | **-42637.80** | **0.00** |
| **45** | **0.02641** | | | **+** | **+** | | **+** | **20** | **21338.58** | **-42637.20** | **0.61** |
| **63** | **0.02643** | | **+** | **+** | **+** | **+** | **+** | **26** | **21343.54** | **-42635.10** | **2.68** |
| **47** | **0.02642** | | **+** | **+** | **+** | | **+** | **23** | **21340.39** | **-42634.80** | **2.98** |
| **29** | **0.02643** | | | **+** | **+** | **+** | | **20** | **21336.73** | **-42633.50** | **4.30** |
| **13** | **0.02642** | | | **+** | **+** | | | **17** | **21333.62** | **-42633.20** | **4.52** |
| **62** | **0.02641** | **+** | | **+** | **+** | **+** | **+** | **26** | **21342.15** | **-42632.30** | **5.47** |
| **46** | **0.0264** | **+** | | **+** | **+** | | **+** | **23** | **21338.71** | **-42631.40** | **6.34** |
| 15 | 0.02644 | | + | + | + | | | 20 | 21335.66 | -42631.30 | 6.45 |
| 31 | 0.02645 | | + | + | + | + | | 23 | 21338.61 | -42631.20 | 6.54 |
| 64 | 0.02643 | + | + | + | + | + | + | 29 | 21344.11 | -42630.20 | 7.54 |
| 48 | 0.02642 | + | + | + | + | | + | 26 | 21340.78 | -42629.60 | 8.21 |
| 30 | 0.02643 | + | | + | + | + | | 23 | 21337.74 | -42629.50 | 8.30 |
| 14 | 0.02642 | + | | + | + | | | 20 | 21334.34 | -42628.70 | 9.09 |
| 32 | 0.02644 | + | + | + | + | + | | 26 | 21340.15 | -42628.30 | 9.48 |
| 16 | 0.02643 | + | + | + | + | | | 23 | 21336.86 | -42627.70 | 10.05 |
| 37 | 0.02635 | | | + | | | + | 17 | 21330.01 | -42626.00 | 11.76 |
| 53 | 0.02636 | | | + | | + | + | 20 | 21332.98 | -42626.00 | 11.80 |
| 39 | 0.02637 | | + | + | | | + | 20 | 21331.98 | -42624.00 | 13.80 |
| 55 | 0.02638 | | + | + | | + | + | 23 | 21334.81 | -42623.60 | 14.14 |
| 5 | 0.02637 | | | + | | | | 14 | 21325.16 | -42622.30 | 15.45 |
| 21 | 0.02638 | | | + | | + | | 17 | 21327.96 | -42621.90 | 15.85 |
| 7 | 0.02638 | | + | + | | | | 17 | 21327.35 | -42620.70 | 17.07 |
| 54 | 0.02636 | + | | + | | + | + | 23 | 21333.09 | -42620.20 | 17.59 |
| 38 | 0.02635 | + | | + | | | + | 20 | 21330.05 | -42620.10 | 17.67 |
| 23 | 0.02639 | | + | + | | + | | 20 | 21330.01 | -42620.00 | 17.75 |
| 40 | 0.02637 | + | + | + | | | + | 23 | 21332.19 | -42618.40 | 19.39 |
| 56 | 0.02638 | + | + | + | | + | + | 26 | 21335.14 | -42618.30 | 19.49 |
| 22 | 0.02638 | + | | + | | + | | 20 | 21328.59 | -42617.20 | 20.59 |
| 6 | 0.02637 | + | | + | | | | 17 | 21325.58 | -42617.20 | 20.60 |
| 8 | 0.02638 | + | + | + | | | | 20 | 21328.19 | -42616.40 | 21.39 |
| 24 | 0.02639 | + | + | + | | + | | 23 | 21331.10 | -42616.20 | 21.57 |
| 58 | 0.02558 | + | | | + | + | + | 20 | 14626.83 | -29213.70 | 13424.11 |
| 60 | 0.02554 | + | + | | + | + | + | 23 | 14629.22 | -29212.40 | 13425.33 |
| 26 | 0.02558 | + | | | + | + | | 17 | 14622.96 | -29211.90 | 13425.85 |
| 28 | 0.02553 | + | + | | + | + | | 20 | 14625.81 | -29211.60 | 13426.15 |
| 50 | 0.02555 | + | | | | + | + | 17 | 14616.83 | -29199.70 | 13438.12 |
| 52 | 0.02551 | + | + | | | + | + | 20 | 14619.37 | -29198.70 | 13439.03 |
| 20 | 0.0255 | + | + | | | + | | 17 | 14615.99 | -29198.00 | 13439.78 |
| 18 | 0.02555 | + | | | | + | | 14 | 14612.98 | -29198.00 | 13439.81 |
| 42 | 0.02562 | + | | | + | | + | 17 | 14583.90 | -29133.80 | 13503.96 |
| 44 | 0.02559 | + | + | | + | | + | 20 | 14586.40 | -29132.80 | 13504.96 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 0.02562 | + | | + | | | 14 | 14580.03 | -29132.10 | 13505.71 |
| 12 | 0.02558 | + | + | + | | | 17 | 14582.99 | -29132.00 | 13505.79 |
| 34 | 0.02559 | + | | | | + | 14 | 14574.27 | -29120.50 | 13517.22 |
| 36 | 0.02555 | + | + | | | + | 17 | 14576.92 | -29119.80 | 13517.94 |
| 4 | 0.02555 | + | + | | | | 14 | 14573.55 | -29119.10 | 13518.66 |
| 2 | 0.02559 | + | | | | | 11 | 14570.44 | -29118.90 | 13518.89 |
| 59 | 0.02561 | | + | + | + | + | 20 | 14553.80 | -29067.60 | 13570.17 |
| 57 | 0.02569 | | | + | + | + | 17 | 14548.67 | -29063.30 | 13574.44 |
| 51 | 0.02559 | | + | | + | + | 17 | 14544.65 | -29055.30 | 13582.47 |
| 27 | 0.0256 | | + | + | + | | 17 | 14542.70 | -29051.40 | 13586.37 |
| 49 | 0.02567 | | | | + | + | 14 | 14539.14 | -29050.30 | 13587.49 |
| 25 | 0.02568 | | | + | + | | 14 | 14537.40 | -29046.80 | 13590.97 |
| 19 | 0.02558 | | + | | + | | 14 | 14533.81 | -29039.60 | 13598.15 |
| 17 | 0.02566 | | | | + | | 11 | 14528.15 | -29034.30 | 13603.47 |
| 43 | 0.02564 | | + | + | | + | 17 | 14514.82 | -28995.60 | 13642.14 |
| 41 | 0.02572 | | | + | | + | 14 | 14509.65 | -28991.30 | 13646.48 |
| 35 | 0.02562 | | + | | | + | 14 | 14505.93 | -28983.90 | 13653.91 |
| 11 | 0.02564 | | + | + | | | 14 | 14503.92 | -28979.80 | 13657.94 |
| 33 | 0.0257 | | | | | + | 11 | 14500.39 | -28978.80 | 13658.98 |
| 9 | 0.02572 | | | + | | | 11 | 14498.58 | -28975.20 | 13662.61 |
| 3 | 0.02562 | | + | | | | 11 | 14495.28 | -28968.60 | 13669.20 |
| 1 | 0.0257 | | | | | | 8 | 14489.60 | -28963.20 | 13674.57 |

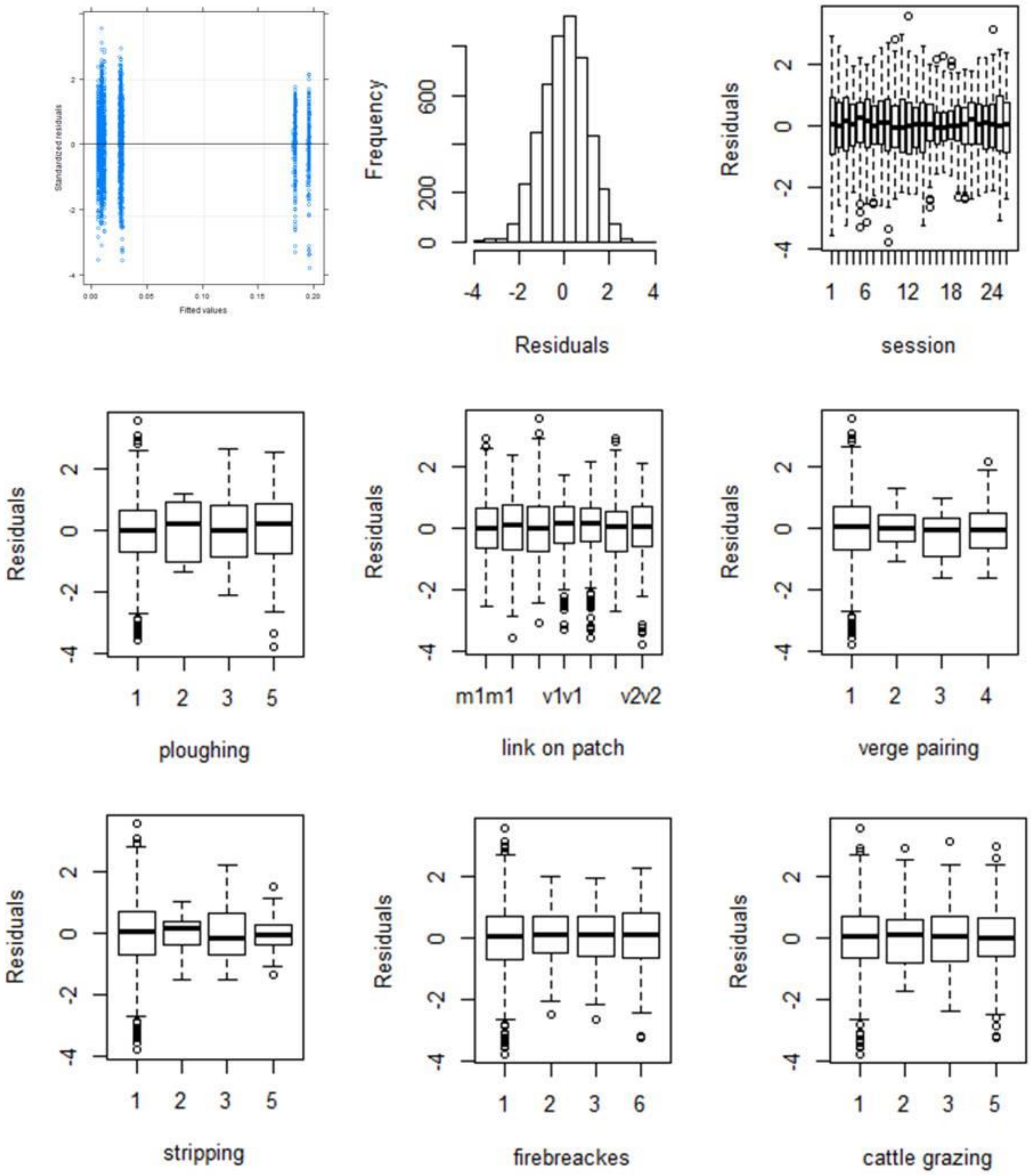

Figure 4.C.4 - Model validation graphs for link importance (dPC link) at the road area. The model relates five management actions (cattle, firebreaks, ploughing, cork stripping, verge vegetation cutting) and location (link on patch) to dPC link. The model validated accounts for all variables included in the averaged model.

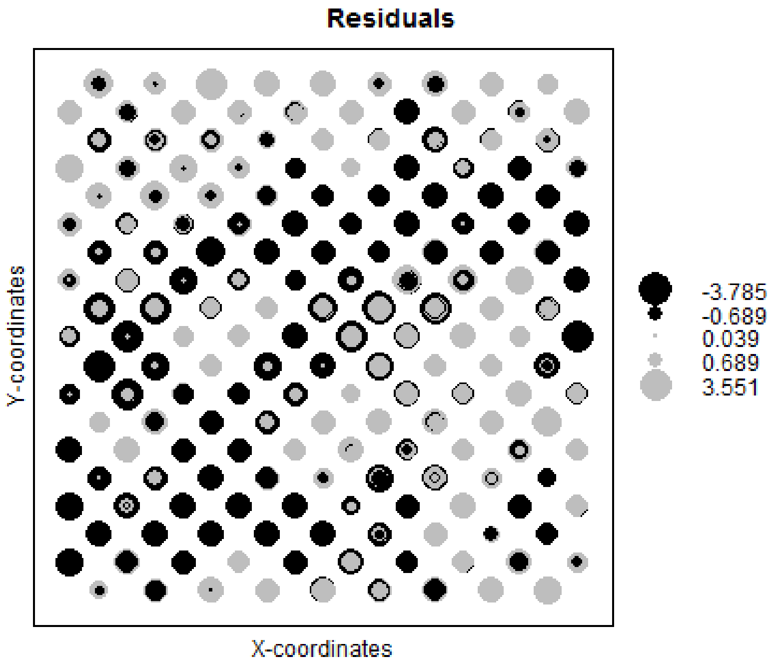

Figure 4.C.5 - Bubbleplot for the model explaining link importance (dPC link) for connectivity at the road area. We show the standardized residuals obtained by the model with all the variables accounted in averaging plotted versus their spatial coordinates. The model relates five management actions (cattle, firebreaks, ploughing, cork stripping, verge vegetation cutting) and location (link on patch) to dPC link.

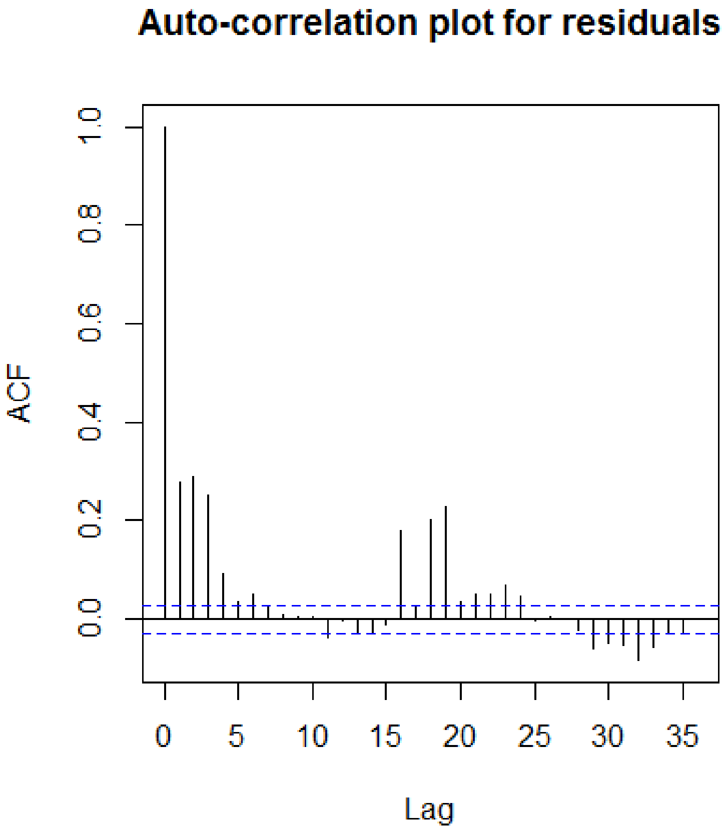

Figure 4.C.6 - Auto-correlation function (ACF) for the model explaining link importance (dPC link) for connectivity at the road area. The model relates five management actions (cattle, firebreaks, ploughing, cork stripping, verge vegetation cutting) and location (link on patch) to dPC link. Residuals were obtained by the model with all the variables accounted in averaging. Time lags are expressed in the horizontal axis and correlation in the vertical axis. Dotted lines represent 95% confidence interval.

# Chapter 5

## General Conclusions

## 5.1. Thesis overview and summary of the main findings

The main goals of this thesis were to assess the effects of roads on the population parameters (chapter 2), movements patterns (chapter 3), and landscape connectivity (chapter 4) for small mammals in a well-preserved habitat that is responsible for high levels of biodiversity in Europe. Accordingly, this thesis also aimed to provide advice for road verge management intended to promote small-mammal conservation using the wood mouse as model species (*Apodemus sylvaticus*).

The study of population parameters (Chapter 2) showed that wood mice's abundance and body condition were significantly lower at the road area when compared to a similar roadless area. However, survival, temporary emigration, capture probability, turnover, sex ratio and age structure were similar on both areas. A smaller population with a lower body condition but similar population dynamics seems to reflect the lower habitat availability and quality at the road area. The presence of paved lanes and of vegetation clearing at road verges were the main causes for the reduction in habitat availability and quality. Vegetation clearing at road verges usually occurs in late spring or early summer, just before the most critical period for survival in the Mediterranean region due to the lower availability of food and water (Rosário and Mathias, 2004). These results show that the increased abundance of small mammals near roads reported in other studies (Fahrig and Rytwinski, 2009; Ascensão et al., 2012) cannot be generalized to all demographic parameters nor to all habitats. More specifically, the effects of roads on small mammal populations depend on the habitat quality and management of road verges and road surrounding areas.

The study of wood mice's movements (Chapter 3) revealed that patterns were clearly different between the road and the roadless areas. Wood mice crossed the road less often, moved away from and parallel to it more frequently and used more road verges when compared with the equivalent locations at the roadless area (virtual road and virtual road verges at the two central lines of the trapping grid). At the road area, firebreak openings and higher shrubs promoted road crossings, and males used more often road verges than females. These results show that roads and roadside management may strongly affect wood mouse movement patterns.

The study of connectivity (Chapter 4) showed that the road decreased overall landscape connectivity and imposed a partial barrier for wood mice's movements. Nevertheless, road verges provided much higher functional connectivity when compared with the surrounding farmland habitat. This result seems to be related to the better microhabitat conditions on road verges at least during part of the year. Annual vegetation cutting on road verges, and land ploughing in the surrounding landscape decreased significantly functional connectivity. Simultaneously, fine-scale connectivity increased in adjacent unmanaged areas, indicating a compensation effect. These results provide further evidence of the important role of road verges as corridors for small mammals. Moreover, this study quantifies the increase in functional connectivity provided by road verges and demonstrates their importance even in well-preserved habitats.

## 5.2. Implications for roadside vegetation management aiming small-mammal conservation

The results from this thesis provide insights for the conservation of small mammals on roadsides. The three studies show that the road and vegetation management (at road verges and at surrounding habitats) strongly affect wood mice's population parameters and movements, and consequently influence overall landscape functional connectivity. Specifically, the most influential management practices were firebreaks openings (at road verges and surrounding habitats), vegetation cutting (at verges), and land ploughing (at surrounding habitats). These results also show the great importance of road verges as habitats and corridors for small mammals even in well-preserved habitats. Moreover, these results suggest that current vegetation management can increase roadkill risk. Therefore, vegetation management for conservation of small mammals on road surroundings must ensure that they provide refuges and/or corridors but also minimize roadkill risk.

Higher shrub cover and height favour the use of verges and road crossings by wood mice (Chapter 3). Thus, where roadkill risk is high for vulnerable species, vegetation should be cut at both road verges simultaneously to avoid small-mammal crossings from lower to higher shrub cover (from cut to uncut road verges) (Chapter 3). Habitat at road verges is particularly valuable for maintaining small-mammal populations during disturbances on private surrounding land (Chapter 4). Therefore, firebreaks should not be opened on the private surrounding land at the same time (or immediately before or after) as verge vegetation cutting to avoid vegetation removal over a wider area. Coordination between management practices is possible through arrangements between landowners and road managers and should be a major goal of small-mammal conservation plans on road dominated areas. If coordination is not possible, the total width of firebreaks and verge vegetation cutting should not exceed 10 meters (minimum prescribed by law). Moreover, land ploughing should not be used because it may eliminate both vegetation and underground nests (Green, 1979). This management practice forces small mammals to leave ploughed patches, increasing roadkill risk and hampering functional connectivity (Chapters 3 and 4). On the other hand, where roadkill risk is considered low, road verge management must preserve a continuous strip of uncut vegetation (Chapters 2, 3 and 4). This vegetation corridor should be at least 1m wide with 50% shrub cover including high shrubs at the side of the verge that is further away from the paved lanes (e.g., along a fence; Chapter 3).

Nevertheless, road management practices must also be adjusted to account for two important risks of maintaining vegetation at road verges: (1) fire risk especially in the Mediterranean region (Parente et al., 2018) and (2) possible attraction and increase of roadkill of small-mammal predators and ungulates (Ascensão et al., 2012; Martinig and McLaren, 2019; Silva et al., 2019). In high fire risk landscapes, the replacement of a continuous strip of tall vegetation near the fence by small natural vegetation “islands” at regular intervals should be considered. These “islands” would act as steeping stones for small-mammal movements. Nevertheless, the minimum size and the maximum distance between “islands” must still be carefully assessed. On roadkill hotspots, road verge vegetation should only be maintained where higher fences with smaller mesh size are implemented to prevent most larger species from moving into the road (e.g., Grilo et al., 2009). In high-speed unfenced roads, vegetation should be completely cleared at road verges.

Altogether, these recommendations are particularly important in the absence of underpasses and overpasses which are the most common management practices implemented so far (Meaney et al., 2007; White and Huges, 2019). However, these recommendations also apply when directing animals to those crossing structures (McDonald and Saint Claire, 2004; D’Amico et al., 2015) and so decrease roadkill risk.

## 5.3. Limitations and future research

The main limitation of this thesis was the use of only two areas to conduct the studies due to budget constraints which may limit some inferences due to potential pseudoreplication. Nevertheless, inferences from studies that account for pseudoreplication should not be discarded (Davies and Gray, 2015). In fact, an effort was made to lessen this limitation by accounting for temporal and spatial autocorrelation (Chapters 2, 3 and 4).

Capture-mark-recapture techniques were used not only to study population parameters but also movement patterns and functional connectivity due also to budget constraints. Although telemetry is one of the best methods to analyse movement, capture-mark-recapture techniques yield similar and consistent results and both have been successfully used in studies of small-mammal movements (Clark et al., 2001; Gomez et al., 2011; Ramsay et al., 2019). Moreover, this thesis used mostly movement patterns (Chapter 3), probability of movement and proportion of use (Chapter 4) rather than exact movement measures. Therefore, the inferences drawn are not compromised. However, telemetry would provide more detailed data on space use (e.g., pathways chosen; velocity and time spent in each section of the study areas). Thus, in the future, telemetry can be used to assess more accurately the effects of residency status and the immediate impact of verge vegetation cutting and land ploughing on movement patterns.

This thesis provides valuable insights about the influence of roads, roadside vegetation characteristics and management on wood mouse population traits and movements. However, besides extending the study to other species with different and specific habitat requirements, further investigation is needed to refine roadside vegetation management to promote small mammal conservation. In particular, assessing the minimum amount of verge vegetation needed to maintain viable populations, minimize movement disturbance, and maximize functional connectivity is extremely important for wildlife conservation management. More specifically, defining the minimum size of vegetation “islands” and the maximum distance between them can be crucial where continuous vegetation corridors along road verges are not possible (e.g., high fire risk or high roadkill risk areas).

## 5.4. Concluding remarks

In a well-preserved habitat, a national road and roadside management negatively affected a wood mice population by: decreasing abundance and body condition (Chapter 2), conditioning road crossings and movement direction (Chapter 3) and decreasing overall landscape functional connectivity (Chapter 4). Nevertheless, road verges had an extremely important role in providing habitat and pathways for movement thereby promoting functional connectivity on road surroundings (Chapter 4). Apparently, wood mice may have perceived the presence of the road and vegetation management as a risk and probably adjusted their movements to reduce it (Chapter 3). Indeed, this species can adjust foraging movements to habitat alterations and probable increases in the perception of risk (see also Tattersall et al., 2001; Díaz et al., 2005). Habitat at road verges eased the adjustment of movements to the presence of the road and management practices. This adjustment may have allowed for a smaller population to persist and to maintain a similar dynamics (e.g., turnover, survival, temporary emigration) than a larger population in a similar roadless habitat.

Nonetheless, the role of road verges depended on the amount of vegetation cover available after management on verges and surrounding areas. Compulsory vegetation cutting to prevent fires may compromise road verge value for wildlife conservation. Road verges' role for conservation is further hampered when landowners open firebreaks adjacent to the road, displacing small mammals and so increasing roadkill risk. Therefore, agreements between road operators and landowners should be part of regular management procedures to decide when and where to cut vegetation on verges and open firebreaks. Ideally, these agreements should allow for the maintenance of strips of undisturbed vegetation on road verges that ensure habitat and pathways for movement and so increase road verges' value for conservation of small fauna. Nevertheless, management practices that increase and maintain road verge vegetation must always consider and prevent the potential increase of the risk of roadkill (of small mammals and their predators) and wildfire ignition and spreading. Despite the study limitations, these recommendations could reasonably be applied to small mammal species that depend on vegetation cover and avoid crossing gaps such as those created by roads and roadside management (Oxley et al., 1974; Macpherson et al., 2011).

This thesis shows that in well preserved habitats, a national road and roadside management can negatively affect a small mammal with high reproductive ability. Therefore, the previously documented positive or neutral effects of roads on small mammals (e.g., Fahrig and Rytwinski, 2009; Benitez-Lopez et al., 2010; Ascensão et al., 2012) do not apply to all circumstances, and especially when roads cross well-preserved habitats. Although small mammal populations can be resilient to roads and verge management locally, even populations of highly abundant species may struggle to persist if management further restricts resource availability across the road network (e.g., *Cricetus cricetus*; Banaszek et al., 2020). This thesis will increase the awareness of the importance of roadless areas and of road verges in road management plans for the conservation of small mammals in well-preserved habitats. These concerns and recommendations for vegetation management on roadsides will be particularly important for the conservation of vulnerable species on road areas, in stretches lacking underpasses or overpasses.

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