

Universidade de Évora - Escola de Ciências e Tecnologia

Mestrado em Biologia da Conservação

Dissertação

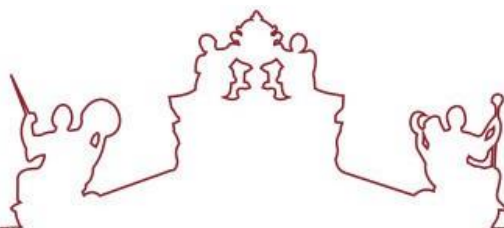
**Factores que influenciam a ocupação de olivais do NE de
Portugal por pequenos mamíferos**

Isabel Martins Barão

Orientador(es) | Ricardo Pita

Joana Maria Jorge Pereira de Castro Paupério

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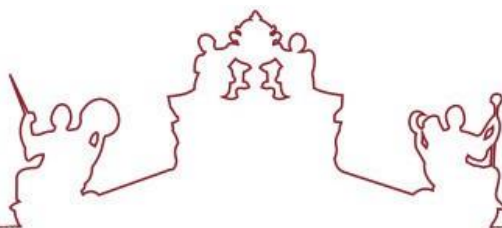
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A dissertação foi objeto de apreciação e discussão pública pelo seguinte júri nomeado pelo Diretor da Escola de Ciências e Tecnologia:

Presidente | Paulo Sá Sousa (Universidade de Évora)

Vogais | Ricardo Pita (Universidade de Évora) (Orientador)
Sara Santos (Universidade de Évora) (Arguente)

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RESUMO

Factores que influenciam a ocupação de olivais do NE de Portugal por pequenos mamíferos

Conhecer a relação entre a comunidade de pequenos mamíferos e os agroecossistemas associados aos olivais é fundamental para a conservação. Este estudo aborda esta temática relativamente aos olivais do NE de Portugal, avaliando os atributos da paisagem que determinam a ocorrência das espécies, e focando-se numa espécie protegida (*Microtus cabreræ*) e noutra considerada potencial praga agrícola (*M. lusitanicus*). Recorrendo a amostragem genética não-invasiva, estimou-se a probabilidade de ocupação das espécies relativamente às variáveis da paisagem. *M.cabreræ* ocorre sobretudo em mosaicos de usos do solo incluindo zonas agrícolas com elevada densidade de orlas, enquanto *M.lusitanicus* tem maior probabilidade de ocorrência em mosaicos com elevada densidade de pastagens. Globalmente, os resultados sugerem que a heterogeneidade dos agroecossistemas de olival do NE de Portugal estão associados a uma comunidade rica de pequenos mamíferos, com probabilidades de ocupação relativamente altas para espécies com problemas de conservação e relativamente baixas para espécies consideradas potenciais pragas.

Palavras-chave: Amostragem genética não-invasiva; Biodiversidade agrícola; Espécies ameaçadas; Espécies praga; *Microtus cabreræ*; *Microtus lusitanicus*; Modelos de deteção-ocupação.

ABSTRACT

Factors driving the small mammal occupancy of olive groves in NE Portugal

How small mammals associate to environmental characteristics in olive groves is an important conservation question. Here are provided first insights on this topic applied to NE Portugal olive groves, by assessing the landscape attributes that determine the occurrence of small mammals, focusing on one protected species (*Microtus cabreræ*) and one potential pest (*M. lusitanicus*). Species occupancy probabilities relative to landscape variables were estimated based on genetic non-invasive sampling. *M. cabreræ* was more likely to occur in land mosaics including high edge density of agricultural fields, while *M. lusitanicus* was more likely to occur in land mosaics with high density of pasture lands. Overall, the results suggest that the heterogeneous olive grove agroecosystems from NE Portugal allow for the occurrence of a species-rich small mammal community, including species of conservation concern at relatively high occupancy rates and potential pests at relatively low occupancy rates.

Keywords: Agricultural biodiversity; Detection-occupancy modelling; Genetic non-invasive sampling (gNIS); *Microtus cabreræ*; *Microtus lusitanicus*; Pest species; Threatened species;

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I. INTRODUÇÃO GERAL

1. Intensificação agrícola em oliveais da região Mediterrânica

A perturbação do habitat, seja por destruição total, alteração, simplificação ou fragmentação, é das maiores ameaças à biodiversidade global (Ehrlich & Ehrlich, 1981), sendo um dos principais temas de investigação em Biologia da Conservação (Haila, 2002; Fazey, Fischer & Lindenmayer, 2005). No passado, as paisagens agrícolas europeias consistiam tradicionalmente em mosaicos complexos de culturas extensas e que sustentavam elevados níveis de biodiversidade (Potter, 1997; Walk & Warner, 2000) desenvolvida e moldada pela história do uso do solo agrícola (Emmerson *et al.*, 2016). No entanto, estas paisagens foram sofrendo alterações dramáticas ao longo dos anos, principalmente devido à intensificação das técnicas agrícolas (Björklund, Limburg & Rydberg, 1999; Siriwardena, Crick, Baillie & Wilson, 2000; Robinson & Sutherland, 2002), conduzindo ao declínio acentuado de inúmeras espécies de fauna e flora (Donald, Green & Heath, 2001; Stoate *et al.*, 2001; Tilman *et al.*, 2001; Kleijn *et al.*, 2009).

A intensificação agrícola, apesar de contribuir para a alimentação da crescente população mundial (Kleijn *et al.*, 2009), é uma das principais ameaças à biodiversidade das zonas rurais (Stoate *et al.*, 2001; Robinson & Sutherland, 2002; Kleijn *et al.*, 2006). Isto deve-se, principalmente, ao aumento do uso de pesticidas e fertilizantes (Bengtsson, Ahnström & Weibull, 2005; Hole *et al.*, 2005; Geiger *et al.*, 2010) e à perda de habitats semi-naturais (Benton, Vickery & Wilson, 2003; Concepción, Díaz & Baquero, 2008; Firbank, Petit, Smart, Blain & Fuller, 2008). Uma especialização crescente, muitas vezes conduzindo a monoculturas, fertilização, irrigação e uso de pesticidas, juntamente com a fragmentação e perda de habitats semi-naturais, altera substancialmente os padrões de disponibilidade de recursos e interações bióticas nas comunidades locais (Matson, Parton, Power & Swift, 1997; Schweiger *et al.*, 2005). O consequente empobrecimento biológico pode, também, comprometer a prestação de serviços dos agroecossistemas, essenciais para o bem-estar humano, proporcionados por diferentes processos ecológicos associados à biodiversidade, como, por exemplo, o controlo

biológico de pragas agrícolas (Thies & Tscharntke, 1999; Östman, Ekbom & Bengtsson, 2001, 2003), a polinização (Garibaldi *et al.*, 2011; Kennedy *et al.*, 2013), a fertilidade do solo, entre outros (Power, 2010).

Um exemplo de intensificação da agricultura muito conhecido é o olival (Weissteiner, Strobl & Sommer, 2011), um sistema agrícola muito importante na região Mediterrânica a nível ambiental, cultural e socio-económico (Rey, 1993; Loumou and Giourga, 2003; Tartaglini, Calabrese & Servadei 2012). Este sistema resulta da conversão de paisagens aráveis em olivais irrigados com densidades de até 1700 árvores/ha (Ramos & Santos, 2009) e que leva, frequentemente, à remoção da vegetação do solo, causando uma redução na produtividade das culturas (Gómez *et al.*, 2011). Atualmente, os sistemas de produção intensiva e, particularmente, super-intensiva de oliveiras encontram-se em rápida expansão na Região Mediterrânica, substituindo olivais tradicionais ou ocupando áreas previamente usadas para produzir outras culturas (Herrera, Costa, Medinas, Marques & Mira, 2015; Sánchez-Martínez & Cabrera, 2015; Infante-Amate *et al.*, 2016). Estes olivais tendem a ser muito maiores que os olivais tradicionais, resultando em paisagens mais homogêneas e simplificadas, com potenciais efeitos negativos sobre a biodiversidade, não só na que tipicamente ocorre nos olivais (Rey *et al.*, 2019), mas também das zonas agrícolas convertidas em olivais (Santos & Cabral, 2004; Santana *et al.*, 2017a, b; Santos, Silva, Bastos, Carvalho & Cabral, 2018), assim como nos habitats envolventes. A perda de heterogeneidade, que consiste na diversidade de elementos da paisagem e na complexidade das suas relações espaciais (Clergue, Amiaud, Pervanchon, Lasserre-Joulin & Plantureux, 2005), consequente da intensificação agrícola, contribuiu para a perda de habitats adequados para muitas espécies e teve implicações significativas para a fauna e flora (Benton *et al.*, 2003). Desta forma, a obtenção de conhecimentos de base sobre a relação entre a heterogeneidade da paisagem e a biodiversidade em zonas agrícolas, nomeadamente nos olivais, será crítico para uma efetiva conservação da biodiversidade (Benton *et al.*, 2003; Tscharntke, Klein, Kruess, Steffan-Dewenter & Thies, 2005; Le Roux *et al.*, 2008). Considerando a tendência global para a intensificação agrícola, torna-se de facto essencial avaliar os fatores que determinam a ocorrência de espécies em olivais com

uma gestão mais tradicional e, por norma, com paisagens mais heterogéneas e ricas em biodiversidade (Figura A). Esta informação será crucial para melhor compreender como a heterogeneidade da paisagem pode influenciar uma variedade de respostas ecológicas das espécies, como a dispersão (Fahrig, 2007), persistência das populações (Fraterrigo *et al.*, 2009), interações interespecíficas (Polis *et al.*, 2004) e funções nos ecossistemas (Lovett *et al.*, 2005). Este conhecimento poderá então ajudar a orientar a gestão dos olivais e dos habitats envolventes de modo que, aliado ao crescente desenvolvimento das práticas agrícolas, seja possível manter uma elevada variedade de espécies, trazendo também benefícios para os agroecossistemas (Moreira *et al.*, 2019).



Figura A- Exemplo de mosaico típico de gestão mais tradicional dos agroecossistemas de olival no NE de Portugal (Fotografia de Ricardo Pita).

2. Modelo de Estudo- Pequenos Mamíferos

Os pequenos mamíferos (Ordens Rodentia e Eulipotyphla) são comuns em paisagens agrícolas (Michel, Burel, Legendre & Butet, 2007), onde desempenham um papel importante, com impactos que tanto podem ser positivos como negativos (e.g., Dickman, 1999, Fischer *et al.*, 2017). Alguns dos impactos positivos incluem (i) a sua importante função como consumidores e dispersores de sementes (Ness & Morin, 2008; Baraibar, Westerman, Carrión & Recasens, 2009); (ii) o seu papel no controle de insetos potenciais pragas agrícolas (Gliwicz & Taylor, 2002); (iii) a sua contribuição para a aeração do solo (Laundré & Reynolds, 1993) e (iv) para ajudar a manter fungos ectomicorrízicos (Schickmann, Urban, Kräutler, Nopp-Mayr & Hackländer, 2012); e, finalmente, (v) o seu papel fundamental enquanto presas para mamíferos carnívoros e aves de rapina (Aschwanden, Birrer & Jenni, 2005; Arlettaz, Krahenbuhl, Almasi, Roulin & Schaub, 2010; Sálek, Kreisinger, Sedlacek & Albrecht, 2010). No entanto, enquanto dispersores de sementes, os pequenos mamíferos também podem, por vezes, disseminar sementes de ervas daninhas (Kiviniemi & Telenius, 1998). Para além disso, em determinadas condições podem constituir pragas agrícolas de várias culturas, podendo também ser vetores de disseminação de doenças (Jacob, 2003; Brown, Huth, Banks & Singleton, 2007; Renwick & Lambin, 2013; Delibes-Mateos, Mougeot, Arroyo & Lambin, 2015).

As recentes mudanças climáticas em combinação com outros fatores antropogénicos constituem potencialmente uma ameaça para as espécies de pequenos mamíferos e podem alterar gravemente as estruturas destas comunidades no futuro (Blois, McGuire & Hadly, 2010). Apesar disso, os pequenos mamíferos raramente são considerados em estudos de conservação de biodiversidade (Butet, Paillat & Delettre, 2006). No caso dos roedores sabe-se que, do ponto de vista demográfico, estes têm o potencial de responder de forma rápida às alterações ambientais (Singleton, Belmain, Brown & Hardy, 2010). Assim, a intensificação agrícola tende a favorecer espécies mais comuns, generalistas e, por vezes, com crescimento populacional muito acelerado, em detrimento de espécies raras e ameaçadas, que têm preferência por sistemas agrícolas tradicionais menos modificados (Millán de la Peña *et al.*,

2003). Consequentemente, algumas espécies podem constituir pragas agrícolas altamente prejudiciais e difíceis de controlar (e.g., Singleton, Sudarmaji, Jacob & Krebs, 2005; Delattre & Giraudoux, 2009; Brown & Khamphoukeo, 2010; Jokic, P. Vukša & M. Vukša, 2010). Portanto, torna-se importante conhecer a relação entre as comunidades de pequenos mamíferos e os diferentes usos do solo, com vista à gestão e conservação efetiva das suas populações (Kleijn *et al.*, 2009), e do funcionamento dos agroecossistemas.

Este estudo foca duas espécies em particular, *Microtus cabrerae* (Thomas, 1906) (Figura A) e *Microtus lusitanicus* (Gerbe, 1879) (Figura B), tratando-se de espécies com diferentes estatutos de conservação, sendo a segunda considerada uma potencial praga de zonas agrícolas. *M. cabrerae* é uma espécie endémica da Península Ibérica com uma distribuição bastante fragmentada (Fernández-Salvador, 2007), estando a população portuguesa inserida no núcleo populacional mais extenso (Luso-Carpetano) e ocorrendo numa faixa bem delimitada, embora fragmentada, do sudoeste ao nordeste do país (Garrido-Garcia *et al.*, 2013). Esta espécie semi-fossorial é considerada especialista no que respeita ao habitat (Pita, Mira & Beja, 2007, 2014), ocorrendo em formações de gramíneas perenes, juncais, nas proximidades de áreas com nível freático elevado e na orla de campos agrícolas com elevada humidade no solo, maioritariamente devido a lençóis freáticos ou sistemas de irrigação (e.g., Fernández-Salvador, 1998; Pita, Mira & Beja, 2006, 2007, 2011; Santos, Mathias, Mira & Simões, 2006, 2007; Luque-Larena & Lopez, 2007). É uma espécie ameaçada, considerada “Vulnerável” em Portugal (Cabral *et al.*, 2005), e encontra-se abrangida pela Diretiva de Habitats (Diretiva 92/43/EEC), integrando os anexos II e IV, e pela Convenção de Berna (Anexo II). Por outro lado, *M. lusitanicus* é endémico do sudoeste europeu (Santos, Lourenço, Mathias & Mira, 2010), ocorrendo desde o quadrante noroeste do território peninsular até ao extremo sudoeste de França (Mira & Mathias, 2007). Em Portugal, a sua distribuição concentra-se sobretudo a norte do rio Tejo, sendo relativamente comum a norte e no centro do país (Santos, Mira & Mathias, 2009a) e detém o estatuto de conservação de “Pouco Preocupante” (Cabral *et al.*, 2005). É uma espécie com hábitos fossoriais (Giannoni, Borghi & Martínez-

Rica, 1993) e que ocorre em múltiplos habitats, desde áreas abertas, como prados e pastagens, a zonas agrícolas (Madureira, 1984 ; Vinhas, 1993; Mira & Mathias, 2007), onde se pode considerar uma potencial praga pois, em elevada densidade populacional, pode causar danos às culturas (Ponsà, Cabré, Ferrando, Bosch & Ventura, 2011).



Figura B- Rato-de-Cabrera (*Microtus cabreræ*). (Fotografia de Joaquim Pedro Ferreira, retirado de <https://www.wilder.pt/>).



Figura C- Rato-cego (*Microtus lusitanicus*). (Fotografia de José Ramón Pato Vicente, retirado de Atlas de Mamíferos de Portugal).

3. Amostragem genética não-invasiva

Os pequenos mamíferos são um grupo difícil de observar diretamente e a sua captura implica um esforço e custos significativos, especialmente para algumas espécies mais elusivas (como *M. cabreræ*, Alasaad *et al.*, 2011), podendo ser também prejudicial para os indivíduos capturados (Moncrief *et al.*, 2008). Assim, torna-se preferível recorrer a métodos de amostragem não-invasivos, como a prospeção de indícios de presença e posterior identificação genética das espécies. Este tipo de métodos permite uma amostragem mais facilitada e torna-se mais económico (Giraudoux *et al.*, 1995; Battersby & Greenwood, 2004; Santos, Mira & Mathias, 2009b) quando comparado com os métodos tradicionais de amostragem (e.g. armadilhagem). A amostragem genética não-invasiva baseia-se no princípio de que o DNA pode ser extraído de várias fontes de material derivado de animais (dejetos, pelos, regurgitações, entre outros) obtidas sem a captura de indivíduos (Barbosa, Paupério, Searle & Alves, 2013). Neste estudo recorreu-se à utilização de dejetos para a identificação das espécies, pois são considerados a fonte não-invasiva de DNA mais óbvia e que se encontra facilmente na área de ocupação de pequenos

mamíferos (Beja-Pereira, Oliveira, Alves, Schwartz & Luikart, 2009; Centeno-Cuadros & Godoy, 2010).

A amostragem genética não-invasiva tem sido cada vez mais utilizada para estimar parâmetros demográficos de espécies difíceis de capturar devido, principalmente, ao menor esforço de amostragem necessário, ao melhor custo-benefício laboratorial e ao maior sucesso da amplificação de DNA (Beja-Pereira, *et al.*, 2009; Marucco, Boitani, Pletscher & Schwartz, 2011). Porém, torna-se limitante na recuperação de informações sobre as características individuais relevantes (idade, massa corporal ou condição reprodutiva) (Cheng, Hodges, Sollmann & Mills, 2017; Ferreira *et al.*, 2018). No geral, este tipo de amostragem tem um grande potencial para programas de monitorização de roedores, incluindo aqueles focados em áreas geográficas consideradas hotspots de biodiversidade, como a Península Ibérica, (Barbosa, *et al.*, 2013) pois permite estudar vários indivíduos e populações sem contactar ou perturbar os indivíduos (Beja-Pereira *et al.*, 2009).

4. Objetivos

Nesta dissertação, pretende-se demonstrar que uma gestão mais tradicional dos olivais, associada a uma elevada heterogeneidade e complexidade de habitats (em contraste com a crescente tendência de intensificação agrícola), promove a existência de uma comunidade de pequenos mamíferos rica em número de espécies, incluindo espécies com problemas de conservação, permitindo simultaneamente manter taxas de ocupação relativamente baixas de espécies que podem tornar-se potenciais pragas.

A investigação integrou-se no projeto 'AGRIVOLE' - *The role of voles in agroecosystems: linking pest management to biodiversity conservation under environmental change* (PTDC/BIA-ECO/31728/2017), cujo objetivo geral é identificar os mecanismos reguladores das comunidades de pequenos mamíferos em olivais, à escala regional, paisagística, e local, com base em amostragem genética não-invasiva e técnicas moleculares de sequenciação de nova geração.

No âmbito desta dissertação, o principal objetivo consistiu em analisar as características da paisagem que afetam a presença de espécies de pequenos mamíferos com distintos estatutos de conservação e potencial de gerar conflitos em olivais e habitats envolventes. Em particular, pretendeu-se analisar as características da paisagem que podem afetar a presença de *Microtus cabrerae*, uma espécie protegida, e *Microtus lusitanicus*, espécie que, quando em elevadas densidades populacionais, pode tornar-se uma potencial praga agrícola. Para isso, a região escolhida foi o nordeste (NE) de Portugal, onde predominam os olivais tradicionais (Duarte, Jones, Lúcio & Nunes, 2006) e onde *Microtus cabrerae* e *Microtus lusitanicus* estão presentes. Provando-se como um método vantajoso para a amostragem de pequenos mamíferos (Giraudoux *et al.*, 1995; Battersby & Greenwood, 2004; Santos *et al.*, 2009b), a prospeção foi realizada através da procura de indícios de presença, nomeadamente dejetos, apoiada de posterior identificação da espécie por análise molecular. A informação obtida foi complementada com análises geográficas dos locais de amostragem e analisada com base em modelos de ocupação das espécies (MacKenzie *et al.*, 2002).

Pretendeu-se, então, testar as seguintes hipóteses:

1. Pequenos mamíferos em olivais no nordeste de Portugal são geralmente menos conspícuos no olival do que nos habitats envolventes;
2. A geometria complexa dos diferentes usos do solo circundantes aos olivais determina criticamente a ocorrência de espécies que diferem no estatuto de conservação e potencial de causar danos nos olivais:
 - 2.1. Os olivais rodeados por um mosaico de zonas agrícolas e orlas de habitats devem estar associados a uma elevada probabilidade de ocorrência da espécie considerada vulnerável, *Microtus cabrerae*;
 - 2.2. Os olivais rodeados por um mosaico de zonas de pastagem e manchas de outros olivais devem estar associados a uma elevada probabilidade de ocorrência da espécie com estatuto de conservação pouco preocupante, *Microtus lusitanicus*.

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II. SCIENTIFIC RESEARCH PAPER

Landscape characteristics affecting the occurrence of small mammals in heterogeneous olive grove agroecosystems

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Abstract

Understanding how small mammals associate to environmental characteristics in olive groves is important to identify potential threats to agriculture and assess the overall conservation value and functioning of agroecosystems. Here, we provide first insights on this topic applied to NE Portugal olive groves, by assessing the landscape attributes that determine the occurrence of small mammals in olive groves, focusing on one protected species (*Microtus cabreræ*) and one species often perceived as a potential pest of olive trees (*M. lusitanicus*). Based on genetic non-invasive sampling (gNIS) of small mammal faeces in 51 olive groves and surrounding habitats, we estimated species occupancy probability relative to compositional and structural landscape variables, while accounting for imperfect detection. The high species identification success rate obtained from gNIS allowed the detection of seven rodent species and one insectivore. Occupancy modelling indicated that, in general, small mammals were more easily detected in marginal habitats than within olive groves. Cabrera voles were more likely to occur in land mosaics including high edge density of agricultural fields, while the Lusitanian pine vole was more likely to occur in land mosaic with high density of pasture lands. Taken together, these results suggest that heterogeneous mosaics of different land uses associated with olive groves in NE Portugal allows the occurrence of a species-rich small mammals community, possibly including well-established populations of the species of conservation concern, while keeping the potential pest species at relatively low occupancy rates. Further studies focusing on species abundance and population dynamics are needed to fully understand the overall conservation value and resilience of NE Portugal olive groves relative to the small mammals' community.

Keywords: Agricultural biodiversity; Detection-occupancy modelling; Genetic non-invasive sampling (gNIS); *Microtus cabreræ*; *Microtus lusitanicus*; Pest species; Threatened species;

Introduction

During the last century, the transition from traditional to intensive agriculture has caused drastic changes in the environmental conditions of agricultural landscapes (Matson, Parton, Power & Swift, 1997; Tilman *et al.*, 2001; Green, Cornell, Scharlemann & Balmford, 2005), being associated with a fast decline in agroecosystems' biodiversity (Andrén, 1997; Stoate *et al.*, 2001; Robinson & Sutherland, 2002; Foley *et al.*, 2005). In the Mediterranean region, for instance, traditional olive farming has been considered a crucial system due to its environmental, cultural and socio-economic importance (Rey, 1993; Loumou & Giourga, 2003; Tartaglini, Calabrese & Servadei, 2012). However, during the last decades there has been an increasing intensification in olive farming with olive groves tending to be much larger and with less ground vegetation than traditional orchards (Herrera, Costa, Medinas, Marques & Mira, 2015; Sánchez-Martínez & Cabrera, 2015; Infante-Amate *et al.*, 2016; Morgado *et al.*, 2020). This leads to more homogeneous and simplified landscapes that likely support much lower biodiversity (Gómez *et al.*, 2011; Weissteiner, Strobl & Sommer, 2011; Rey *et al.*, 2019). Therefore, understanding the relationships between landscape spatial heterogeneity and biodiversity in agricultural landscapes, such as olive farming systems, is of major importance for conserving biodiversity (Benton, Vickery & Wilson, 2003; Tscharntke, Klein, Kruess, Steffan-Dewenter & Thies, 2005; Le Roux *et al.*, 2008).

Small mammals (Rodentia and Eulipotyphla) are considered keystone species in agroecosystems (Fitzgibbon, 1997; Ouin, Paillat & Burel, 2000; Delibes-Mateos, Smith, Slobodchikoff & Swenson, 2011), including olive groves, where they play crucial ecological functions like promoting plant community productivity and diversity, including soil aeration, organic turn-over, and mineralization (Laundré & Reynolds, 1993), seed dispersal (Kiviniemi & Telenius, 1998) and the maintenance of ectomycorrhizal fungi (Schickmann, Urban, Kräutler, Nopp-Mayr & Hackländer, 2012). Small mammals also contribute to support trophic networks, for instance by consuming insects (Gliwicz & Taylor, 2002) and by affecting the diversity of higher trophic levels, as prey species for mammalian and avian predators (e.g., Giraudoux *et al.*, 1997; Moore, Askew & Bishop, 2003; Aschwanden, Birrer & Jenni, 2005;

Arlettaz, Krahenbuhl, Almasi, Roulin & Schaub, 2010; Sálek, Kreisinger, Sedlacek & Albrecht, 2010). However, small mammals may also contribute to spread zoonotic diseases, and, in case of rodents, they may also become agricultural pests (Jacob, 2003; Renwick & Lambin, 2013; Delibes-Mateos, Mougeot, Arroyo & Lambin, 2015). For instance, in fruit tree orchards from southwestern Mediterranean Europe, species such as the Lusitanian pine vole (*Microtus lusitanicus*), are often perceived as a potential pest (Miñarro, Somoano & Ventura, 2017), because they may feed on tree roots, with their negative effects being more noticeable in more intensive farming systems compared to more traditional farming, in which tree root systems tend to be larger and, therefore, more resilient to vole attacks (Miñarro, Montiel & Dapena, 2012). On the other hand, more traditional farming might allow the occurrence of small mammal species that are more vulnerable to land use intensification, such as the Cabrera vole (*Microtus cabreræ*), thus potentially supporting significantly higher concentration of biodiversity values and related ecosystem services (European Commission, 2010). Understanding how olive groves landscape composition and structure affect small mammals' occurrence is therefore a main research topic to obtain first insights on their ability to provide key ecosystem services and unwanted disservices in olive grove landscapes (Fischer *et al.*, 2017). However, the elusive and secretive behaviour of most small mammal species often challenges their study based on traditional sampling techniques (e.g., life-trapping, telemetry) (De Bondi, White, Stevens & Cooke, 2010). This may prevent the completion of basic information on their occurrence patterns across space and time, making the use of molecular tools based on non-invasive sampling a suitable and reliable alternative (Cheng, Hodges, Sollmann & Mills, 2017; Ferreira *et al.*, 2018). Genetic non-invasive sampling is based on the principle that DNA can be extracted from multiple sources of animal-derived material (like faeces, owl pellets and others) without trapping individuals (Barbosa, Paupério, Searle & Alves, 2013). This method has great potential for rodent monitoring programs (Barbosa, *et al.*, 2013), allowing the study of several individuals and populations without disturbing or even see them (Beja-Pereira, Oliveira, Alves, Schwartz & Luikart, 2009).

In this study, we analyse the landscape characteristics that affect the occurrence of small mammal species in Mediterranean olive groves and their surrounding habitats. To do so, we focused on northeast (NE) Portugal olive groves, where traditional farming represents the most common system, despite the recent trends for more intensive farming (larger fields, higher tree densities, and use of chemical fertilizers and herbicides) aiming a higher yield and economic profitability, at least in short-term (e.g., Duarte, Jones, Lúcio & Nunes, 2006). We focused on two rodent species with contrasting conservation status and potential to generate conflicts related to olive tree damage, the Cabrera vole and the Lusitanian pine vole. The Cabrera vole is a “Vulnerable” species in Portugal (Cabral *et al.*, 2005), endemic to the Iberian Peninsula, with a very fragmented distribution (Fernández-Salvador, 2007). This species, considered a habitat specialist with semi-fossorial habits (Pita, Mira & Beja, 2007; 2014), is restricted to habitats dominated by wet perennial mixed grasses with abundant sedge–rush communities, near small streams, ponds, and agricultural field margins with high soil moisture conditions, which in most cases are fed by the water table, or by irrigation systems (e.g., Fernández-Salvador, 1998; Pita, Mira & Beja, 2006; 2007, 2011; Santos, Mathias, Mira & Simões, 2006, 2007; Luque-Larena & Lopez, 2007). The Lusitanian pine vole (*M. lusitanicus*) is a southwestern European endemism (Santos, Lourenço, Mathias & Mira, 2010) considered of “Least Concern” (Cabral *et al.*, 2005), with fossorial habits (Giannoni, Borghi & Martínez-Rica, 1993). This species occurs in many types of habitats, from open habitats such as meadows and pastures, to agricultural areas such as commercial orchards and irrigated crops (Madureira, 1984; Vinhas, 1993; Mira & Mathias, 2007), where, if present at high density, they can be harmful for orchards and crops (Ponsà, Cabré, Ferrando, Bosch & Ventura, 2011).

Based on genetic non-invasive sampling (in alternative to more traditional survey techniques) of the species in selected olive groves and surrounding landscape, we tested the following main hypothesis: (i) small mammals in olive farms from NE Portugal are generally less conspicuous in planted olive groves than in surrounding semi-natural habitats along farm fields and (ii) the complex geometry of different land uses surrounding olive groves critically determines

the occurrence of species differing in their conservation status and potential to cause damage to olive groves. In particular, we predicted that olive groves surrounded by a mosaic of agricultural fields and habitat edges should increase the probability of occurrence of the “Vulnerable” Cabrera vole, while olive groves surrounded by a mosaic of pasture lands and other olive patches should increase the probability of occurrence of the potential olive tree pest, the Lusitanian pine vole. Overall, we expect that the highly heterogeneous mosaics of different land uses associated to olive groves in NE Portugal allows the occurrence of a rich small mammal community, including well-established populations of species of conservation concern, while keeping potential pest species below potential harmful levels.

Methods

❖ Study area and design

The study was conducted in Trás-os-Montes region (Figure 1a), located in NE Portugal. This region represents one of the most important national areas of olive oil production, representing ca. 12 to 15% of total production (Voz do Campo, 2021), most of which provided by traditional olive farming (Duarte *et al.*, 2006). Olive groves from NE Portugal thus hold great importance, not only economically but also from a social, landscape and environmental perspective (Voz do Campo, 2021). Climatically, this region is at the confluence of Atlantic and Mediterranean type environments, which together with the presence of a mountainous system, the distance from the ocean, latitude and physiography, determines the appearance of several local micro-climates, allowing the maintenance of high biodiversity across different taxonomic levels (ICNF, 2017), as seems to be the case of small mammals (e.g., Bencatel *et al.*, 2019).

In order to relate small mammals with landscape characteristics in olive grove agroecosystems from NE Portugal, we selected a total of 51 sampling units, each with a focal olive grove patch and including its surrounding habitats (50m-buffers around the focal patch) (Figure 1b). The minimum distance between selected sampling units was always higher than 1200m, thereby guarantying their independence, given to the typical dispersal distances of small mammal species (e.g., Sutherland Harestad, Price & Lertzman, 2000; Bowman, Jaeger & Fahrig 2002). Between May and July 2020, each sampling unit was

surveyed for small mammals based on genetic non-invasive sampling (gNIS) and relevant landscape metrics were extracted from land use mapping based on satellite images and ground surveys (see sections below).

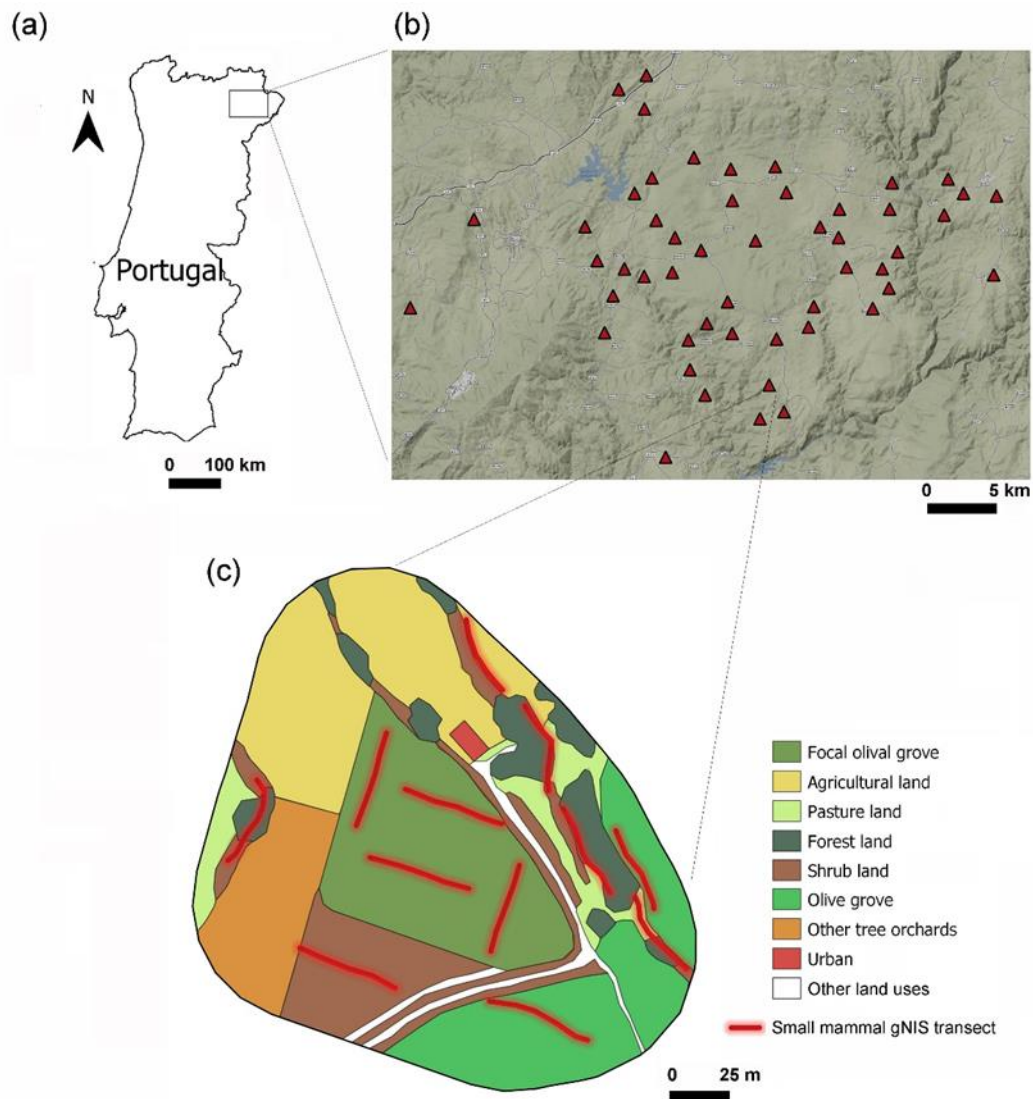


Figure 1- (a) Map of the study area in Trás-os-Montes, NE Portugal; (b) Location of the 51 sampling units centered in olive grove patches distributed across Trás-os-Montes region, selected to survey small mammals and main land uses (red triangles); (c) Example of sampling unit showing the location of transects for small mammal genetic non-invasive sampling, and the main land uses surrounding the focal olive grove (50m-buffers).

❖ Small mammal genetic non-invasive sampling

Small mammal gNIS in each sampling unit consisted in conducting repeated 40m-long transects for detecting their presence signs and collect faecal samples for subsequent molecular identification of the species (see section Molecular analyses). The number of transects in each sampling was defined accordingly to its size (4 transects/ha), ranging between 7 and 19

transects per sampling unit. In each case, ca. 22% of the transects were located within the focal olive grove patch and 78% were distributed preferentially across surrounding semi-natural habitats, with their location chosen according to the availability of adjacent land uses identified (see Figure 1c). The transects were geo-referenced in the field using a GPS device. All faeces identified in the field as belonging to small mammals were collected into microtubes with alcohol 98% identified with a sample number. In order to avoid contamination of the samples, the faeces were collected as isolated faeces or from small latrines and latex gloves were used and the sampling material was sterilized between each sample collection (Ferreira *et al.*, 2018).

❖ Molecular analyses

DNA was extracted from the faeces collected using a magnetic-based enzymatic protocol, following the protocol described by Martins *et al.* (2019) with Agencourt®AMPure®XP beads (Beckman Coulter Company, Massachusetts, United States) and Qiagen® Buffer solutions, adjusted by changing the volumes of ATL solution (400µl) and Proteinase K (25µl). The DNA extract obtained was then transferred to two new microplates for long-term storage (40µl and 30µl each). DNA extractions were conducted in a physically isolated room, where all the equipment was sterilized with bleach and ethanol and exposed to UV light before and after its usage to maintain conditions to monitor and reduce the risk of DNA contamination (Beja-Pereira *et al.*, 2009; Barbosa, *et al.*, 2013; Costa, Rosenbom, Monteiro, O'Rourke & Beja-Pereira, 2017). Negative controls were included in each manipulation. Then, we applied a DNA metabarcoding protocol, which consists in the taxonomic identification of multiple species extracted from a mixed sample (community DNA or environmental DNA), through PCR-amplification and sequencing on a high-throughput platform (e.g., Illumina, Ion Torrent) (Deiner *et al.*, 2017). DNA metabarcoding can complement and overcome the limitations of conventional methods by targeting different species, sampling greater diversity and increasing the resolution of taxonomic identifications (Deiner *et al.*, 2017). A two-step PCR approach was used, where in the first PCR amplification the primer 12S-V5.1F (AGACTGGGATTAGATACCCC) and 12S-V5.1AND2R (TAGAACAGGCTCCTCTAG) from Riaz *et al.* (2011) were used. Each 10µl

PCR mixture contained 5µl of hotstart master mix (Multiplex PCR Kit, QIAGEN), 0.3µl of each primer, 3.4µl ultrapure water and 1µl of diluted DNA. After an initial denaturation cycle at 95°C for 15 min, 35 cycles of 30s at 95°C, 30s annealing at 47°C and 30s extension at 72°C were performed, followed by a final elongation at 72°C for 10 min. Each sample, including extraction negative controls, was replicated three times, and PCR negative controls containing no template DNA were also included. PCR amplicons were then visualized on a 2% agarose gel and the PCR product was purified using AMPure XP beads (to remove primers and primer dimer). A second PCR was performed for attaching the unique dual indexes selected to each sample, using the PCR product diluted ten times. The 14µl indexing PCR mixture contained 7µl 2x KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Cape Town, South Africa), 1,4µl of mixed indexing primer, 2,8µl ultrapure water and 2,8µl of diluted first-round PCR product. Indexing thermal cycling conditions were 95°C, for 3 min; followed by 10 cycles of 95°C for 30s, 55°C for 30s, 72°C for 30s, with an extension of 72°C for 5 min. A second PCR Clean-up with AMPure XP beads was performed and each sample library was quantified with Epoch and normalized before pooling. The final library was then validated in the TapeStation system (High Sensitivity D1000 ScreenTape Assay) and normalized to 4 nM after quantification in qPCR using KAPA Library Quantification Kit for Illumina platforms. Dual-indexed PCR amplicons were sequenced in an Illumina MiSeq System using one MiSeq V2 500-cycle reagent kit (Illumina, California, USA).

❖ Bioinformatics

DNA sequence data were processed using the OBITools program suite (Boyer *et al.*, 2016). First, the paired end reads were aligned and each sequence was assigned to the respective sample. Then, the unaligned sequences records were removed (“obigrep”), the reads were dereplicated into unique sequences across samples and filtered according to size (95-115 bp reads were kept) and removing all sequences with read counts lower than 10. To clean sequences from PCR or sequencing errors (sequence variants) we used “obiclean” with a 50% threshold for the abundance ratio.

The resulting exact sequence variants (ESVs) obtained were then compared with two reference libraries for species identification, namely, the

CIBIO's reference database of Iberian small mammals and the reference nucleotide database (NCBI) using *blastn* (Camacho *et al.*, 2008). Then, the results were condensed to attribute a taxonomic identification to each ESV. Species-level results were attributed to ESVs where a unique species was identified with a percentage identify equal or higher than 98%. If more than one species showed similar high percentage identities, the ESVs would be assigned to a genus or to a higher taxonomic level. Also, when percentage identities were lower than 98%, the ESV was assigned to the taxonomic level that was shared by the most ranked taxa considering the following identity thresholds: $\geq 95\%$ for Genus; $\geq 92\%$ for Family or higher taxonomic levels. Assignments produced by the two databases were cross-checked, and the best taxonomic assignment was selected for each sequence. The resulting taxonomic identifications were then merged with the database containing the number of reads per sample. These analyses were performed using the R packages "maptools" (Bivand & Lewin-Koh, 2021), "rgdal" (Bivand, Keitt & Rowlingson, 2021), "dplyr" (Wickham, François, Henry & Müller, 2021), "taxonomizr" (Sherrill-Mix, 2021) and "tibble" (Müller & Wickham, 2019) in RStudio (Version 1.2.5019) (RStudio Team, 2020).

Finally, the reads obtained for each taxon per sample were summed and, if a taxon was detected within the negative controls (from extraction and PCR), the number of reads was subtracted in the relevant samples. The sample was considered as identified if only one species was detected, with a minimum of 50 reads (though for ca. 97% of the data, a minimum of 500 reads were obtained). In samples where two or more species were detected, the species with highest read count was considered if other species were detected with a number of reads lower than 10% of the read count from the highest-valued species. If those species presented a number of reads higher than 10% of the highest-valued species, the sample was considered contaminated.

❖ **Landscape metrics**

Land uses in each sampling unit were mapped as vector layers in QGIS (version 3.10.0, A. Coruña, QGIS Development Team, 2021), combining information from satellite images obtained from google maps, the map of land uses in NE Portugal COS (Carta de Ocupação de Solos, COS 2018, 2019), and field validation (ground truth). Eight main land use classes were initially

considered: olive groves, pasture lands, shrub lands, agricultural lands, forest lands, other fruit tree orchards, urban and other land uses (Table 1).

Table 1-Main land uses in the study area in NE Portugal and its descriptions.

Land use	Description
Olive grove	plantations of <i>Olea europaea ssp. europaea</i> , including the more or less scattered patches of bare soil and herbaceous vegetation among olive trees.
Pasture land	areas of herbaceous vegetation traditionally used for livestock grazing.
Shrub land	woody vegetation dominated by shrubs, bushes, and young generation trees, generally less than 2m height.
Agricultural land	cultivated areas of cereal crops, vegetables and other horticulture.
Forest land	areas dominated by mature trees, mostly composed by native cork and holm oaks, or occasionally by exotic coniferous and/or broad-leaved trees.
Other fruit tree orchards	plantations of cherry, almond, chestnuts, apple orchards.
Urban	human buildings, social areas, isolated farmhouses and infrastructures.
Other land uses	Roads, fences, ponds, small streams and undetermined areas.

From the maps produced, we extracted for each sampling unit a set of landscape-level variables focusing on the composition and configuration of those land use classes showing a mean cover of at least 5%. Specifically, using the R package “landscapemetrics” (Hesselbarth, Sciaini, With, Wiegand & Nowosad, 2019), we estimated for each of these land use classes the percentage of cover (%); the total edge density (m/ha); the density of habitat patches (nr/100ha); and the normalized landscape shape index (NLSI). The density of edges and habitat patches measure the patchiness of sampling units, with higher values indicating higher patchiness (Hesselbarth *et al.*, 2019). The NLSI provides a simple measure of class aggregation or clumpiness adjusted for the size of sampling units, with higher NLSI values indicating more dispersed or scattered patches (Shetty *et al.*, 2012). In addition, we also estimated the richness of land uses, and the Shannon diversity and evenness indices, in these cases considering all initial main land use classes. The Shannon diversity index measures the probability that two patches randomly selected from a sampling unit will belong to the same land use class and the Shannon evenness index provides information on land use composition, taking the relative abundances of different land uses into account. Before extracting the landscape variables, we converted vector geometries of each sampling unit into raster

images using the R packages “rdgal” (Bivand *et al.*, 2021) and “raster” (Hijmans & Etten, 2021) considering a pixel size of 1m. The R package “landscapetools” was then used to visualize the data (Sciaini, Fritsch & Scherer, 2018).

❖ Modelling

We used single season, single species occupancy-detection models (Mackenzie *et al.*, 2002) to first examine how the location of sampling transects affected the detection probability (p) of those species for which a sufficient number of sightings could be obtained. Specifically, we tested whether the detectability of small mammals was lower within olive groves than in surrounding seminatural habitats, while assuming occupancy probability (Ψ) as constant. Then, we modelled Ψ relative to landscape variables with the best submodel structure of p , focusing on species with different conservation status and pest risk potential, and for which naïve occupancy estimates were less than 85% (higher values were considered indicative of lack of variability in occupancy status across sampling units). Candidate variables explaining the occupancy of each species were identified by running single covariate submodels of Ψ and comparing model AICc scores with that of the model with no covariates affecting Ψ . All covariates were standardized by rescaling their ranges, which consists in subtracting the mean and dividing by the standard deviation. Such transformations make the variables more numerically manageable throughout the model fitting process (Fiske & Chandler, 2011). Single-covariate models with lower AICc than the model with no covariates on Ψ , were indicative of some support of the included variable (Burnham & Anderson, 2002). In that case, the variable was retained for subsequent analyses. Pearson’s correlations among supported variables were then estimated in order to identify those that were highly correlated ($|\text{corr}| > 0.6$, Dormann *et al.*, 2012). To avoid multicollinearity in the multi-model building and selection stages, we implemented the most parameterized model alternatives including different main effect combinations of uncorrelated variables only. From these, the one resulting in lower AICc scores was retained for subsequent steps. The final candidate model set for each species was built by starting from the best full model and implementing simplified versions including all possible combinations of covariates. The best ranking model was then identified based

on AICc (deltaAICc, weighted-AICc) (Burnham & Anderson, 2002). Model averaging was conducted using the subsample of models exhibiting $\text{deltaAICc} < 2$ relative to the top-ranked model, if more than one. We used the 5th and 95th percentiles (90% Confidence Intervals) as the lower and upper bounds of effect size estimates to infer on the effects of landscape covariates included in the best model on species occupancy probability. Estimates including 0 were considered as equivocal (Burnham & Anderson 2002). All models were executed in RStudio (RStudio Team, 2020) using the R packages “unmarked” (Fiske & Chandler, 2011) and “AICcmodavg” (Mazerolle, 2020). Finally, to confirm that any potential spatial autocorrelation in occupancy between sampling units had been controlled for in the analysis (Legendre, 1993), we conducted Moran’s I tests based on the inverse distance matrix between sampling units as an indicator of proximity, using the package ‘ape’ (Paradis & Schliep, 2019). We considered the existence of spatial autocorrelation at $p < 0.05$.

Results

➤ Small mammals’ detection and occupancy based on genetic non-invasive sampling

We surveyed a total of 613 transects across the 51 sampling units, with a mean $\pm$ se (range) of 12 ± 0.38 (7-19) per sampling unit. Within each sampling unit a mean $\pm$ se (range) of 2.65 ± 0.14 (1-5) of the transects were located within the focal olive grove and 9.00 ± 0.28 (6-15) were located at surrounding seminatural areas.

We collected a total of 1953 non-invasive samples (faeces) judged to pertain to small mammals across all 51 sampling units. From these, we were able to identify to the species-level 1621 samples, corresponding to an overall success rate of 83%. 1% of the samples collected were from species of other taxonomic groups (Passeriformes (0.5%); Chalcides (0.1%); *Tarentola mauritanica* (0.1%); Alaudidae (0.05%); Sturnidae (0.05%); Jynx (0.05%); *Gallus gallus* (0.05%); *Garrulus glandarius* (0.05%); *Timon Lepidus* (0.05%)). A total of 5.6% were contaminated and 10.4% failed.

Considering the detection/non-detection of small mammals at the level of each individual transect, a total of 497 records of small mammals were

obtained. The wood mouse (*Apodemus sylvaticus*) was the species most frequently detected (36.6% of overall detections, in a total of 49 sampling units), followed by the Cabrera vole (21.3%, in 37 sampling units), the greater white-toothed shrew (*Crocidura russula*) (16.1%, in 42 sampling units), the Algerian mouse (*Mus spretus*) (15.9%, in 39 sampling units), and the Lusitanian pine vole (8.5%, in 18 sampling units). Less frequently detected species were the southern water vole (*Arvicola sapidus*) (1%), the house mouse (*Mus musculus*) (0.4%) and the Lusitanian field vole (*Microtus rozianus*) (0.2%), respectively detected in 3, 2, and 1 sampling units. These species were therefore discarded from the analyses.

Occupancy-detection modelling for estimating small mammal detection probability (p) relative to transect position, while keeping occupancy (Ψ) constant, revealed that, for all species, estimates of p within olive groves was significantly lower than that estimated at surrounding seminatural habitats (Table 2 and 3). Occupancy probability estimates were equivocal for *C. russula*, ca. 98% for *A. sylvaticus* and ca. 87% for *M. spretus*, followed by the Cabrera vole (77%) and the Lusitanian pine vole (37%) (Table 3).

Table 2- Selection of the best submodel of detectability, assuming constant occupancy for each small mammal species ($\Psi=1$), based on comparisons between AICc values of models considering the effects of transect type (within focal olive groves versus at neighbouring semi-natural marginal habitats) and the model with no effects on detectability. In each case the submodel including the transect type ($p \sim \text{TransType}$) received greater support (AICc, Wt-AICc) than the model with constant detectability ($p=1$), and, therefore, this submodel structure was carried out in subsequent analyses focusing occupancy.

Species	p submodel	AICc	delta AICc	Wt-AICc
<i>M. cabrerae</i>	~ 1	553.916	24.783	0.00
	$\sim \text{TransType}$	529.133		1.00
<i>M. lusitanicus</i>	~ 1	268.363	7.040	0.03
	$\sim \text{TransType}$	261.323		0.97
<i>A. sylvaticus</i>	~ 1	750.621	32.786	0.00
	$\sim \text{TransType}$	717.835		1.00
<i>M. spretus</i>	~ 1	467.628	7.495	0.02
	$\sim \text{TransType}$	460.133		0.98
<i>C. russula</i>	~ 1	486.666	18.523	0.00
	$\sim \text{TransType}$	468.144		1.00

Table 3- Estimates of detectability (p) and occupancy (Psi) of small mammals in NE Portugal according to the models including the effects of transect location, i.e., within focal olive groves (Plot) *versus* at surrounding seminatural habitats (Marginal).

Species	Mean (95%CI) Predicted p (Marginal T)	Mean (95%CI) Predicted p (plot T)	Mean (95%CI) Predicted Psi
<i>M. cabrerae</i>	0.267 (0.223 – 0.317)	0.037 (0.012 - 0.108)	0.765 (0.611 - 0.871)
<i>M. lusitanicus</i>	0.219 (0.158 - 0.296)	0.029 (0.004 - 0.181)	0.366 (0.236 - 0.519)
<i>A. sylvaticus</i>	0.347 (0.304 - 0.392)	0.085 (0.045 - 0.156)	0.984 (0.654 - 0.999)
<i>M. spretus</i>	0.163 (0.126 - 0.210)	0.066 (0.030 - 0.141)	0.868 (0.635 - 0.961)
<i>C. russula</i>	0.158 (0.129 – 0.193)	0.019 (0.005 - 0.072)	1.000 (0.000 - 1.000) *

*Estimates with equivocal meaning.

➤ Landscape variability

Land uses with mean cover over 5% included olive groves, pasture lands, shrub lands, agricultural lands and forest lands. Olive groves were, by far, the most extensive land use class, with a mean cover percentage higher than 50% and ranging from 11,5% to 100%, while forests were the least representative land use, with a mean value of 6,17%, ranging from 0 to 63,8% (Table 4). In addition, we found high variability in all landscape metrics related to edge, density, and aggregation metrics for each of these land uses classes across sampling units. Patch and edge density metrics revealed relatively high patchiness as regards to olive groves, pasture lands and shrub lands across sampling units, in contrast with values found for agricultural and forest lands (Table 4). In general, the values of NLSI showed mostly aggregated patches of land use classes because their mean values are closer to 0 (Table 4). These, together with the high variability observed as regards to diversity and evenness indices (Table 4), suggests high heterogeneity in the geometry and complexity of land mosaic composition and structure across the study area.

Table 4-Summary of descriptive statistics of covariables of species occupancy in 51 olive farms from NE Portugal.

Covariable (units)	code	Mean $\pm$ se	Range
Cover metrics			
Olive grove cover (%)	<i>OliveCover</i>	53.16 $\pm$ 3.26	11.5 - 100
Pasture land cover (%)	<i>PastCover</i>	15.84 $\pm$ 1.82	0– 47.6
Shrub cover (%)	<i>ShrubCover</i>	13.28 $\pm$ 2.08	0 – 58.6
Agricultural land cover (%)	<i>AgricCover</i>	7.01 $\pm$ 1.30	0 – 37.7
Forest cover (%)	<i>ForestCover</i>	6.17 $\pm$ 1.75	0 – 63.8
Edge metrics			
Olive edge density (m/ha)	<i>OliveEdgeD</i>	202.05 $\pm$ 11.95	0 – 443
Pasture land edge density (m/ha)	<i>PastEdgeD</i>	114.16 $\pm$ 12.25	0 – 353
Shrub edge density (m/ha)	<i>ShrubEdgeD</i>	124.60 $\pm$ 16.65	0 – 483
Agricultural land edge density (m/ha)	<i>AgricEdgeD</i>	64.48 $\pm$ 11.21	0 – 303
Forest edge density (m/ha)	<i>ForestEdgeD</i>	46.37 $\pm$ 9.99	0 – 293
Density metrics			
Olive patch density (number/ha)	<i>OlivePatchD</i>	65.18 $\pm$ 5.34	21.7 – 189
Pasture patch density (number/ha)	<i>PastPatchD</i>	61.70 $\pm$ 7.05	0 – 218
Shrub patch density (number/ha)	<i>ShrubPatchD</i>	60.89 $\pm$ 5.56	0 – 203
Agricultural patch density (number/ha)	<i>AgricPatchD</i>	35.94 $\pm$ 5.56	0 – 172
Forest patch density (number/ha)	<i>ForestPatchD</i>	20.32 $\pm$ 4.27	0 – 110
Aggregation metrics			
Olive normalised landscape shape index	<i>OliveNLSI</i>	0.04 $\pm$ 0.02	0 – 1
Pasture normalised landscape shape index	<i>PastNLSI</i>	0.02 $\pm$ 0.01	0 – 0.27
Shrub normalised landscape shape index	<i>ShrubNLSI</i>	0.02 $\pm$ 0	0 – 0.06
Agricultural normalised landscape shape index	<i>AgricNLSI</i>	0.01 $\pm$ 0	0 – 0.1
Forest normalised landscape shape index	<i>ForestNLSI</i>	0.01 $\pm$ 0	0 – 0.07
Diversity metrics			
Number of habitat types	<i>PatchRichness</i>	4.67 $\pm$ 0.20	1 – 7
Shannon diversity index	<i>ShanDiv</i>	1.04 $\pm$ 0.05	0 – 1.73
Shannon evenness index	<i>ShanEvn</i>	0.68 $\pm$ 0.03	0 – 0.91

➤ **Landscape effects on Cabrera and Lusitanian pine voles' occupancy**

Based on the results of single covariate models for each species, while keeping the best structure in the detectability submodel (Table A1-Anexo), candidate landscape variables for model building and selection were AgricCover, AgricEdgeD, AgricPatchD, AgricNLSI and PastNLSI in case of the Cabrera vole, and PastCover, PastEdgeD, PastPatchD, OliveNLSI and ForestNLSI for the Lusitanian pine vole.

Because AgricCover, AgricEdgeD and AgricPatchD were highly correlated (Table A2-Anexo), 3 alternative versions of a full model for the Cabrera vole were implemented, including one of these variables at the time, together with the others uncorrelated variables (AgricNLSI and PastNLSI) (Table A4-Anexo). From these models, the one including AgricEdgeD, AgricNLSI and PastNLSI showed the lowest AICc score (Table A4-Anexo) and was therefore considered in remaining model building and selection procedures. On the other hand, high correlations were also found among PastCover, PastEdgeD, and PastPatchD (Table A3-Anexo), resulting in 3 alternative full models for the Lusitanian pine vole (Table A4-Anexo). From these, the model including PastPatchD, OliveNLSI and ForestNLSI showed a lower AICc than competing alternatives (Table A4-Anexo), being therefore the model considered in subsequent analytical steps.

Model building and selection revealed that, among the 7 final candidate models for the Cabrera vole, the model including the additive effects of AgricEdgeD and PastNLSI received the highest support, being the first ranking model, with an AICc more than 2 units below the second ranked model ($\Delta AICc > 2$) (Table 5). This model had a higher probability of being the best approximating model describing the data, given the candidate set of models considered, and was therefore taken as the best model for the Cabrera vole. This model suggested that Cabrera voles' occupancy probability in NE Portugal increases with the density of agricultural edges, tending to decrease where pastures are more dispersed and scattered (Table 6).

As for the Lusitanian pine vole, the model building and selection revealed that among the 7 final candidate models for explaining this species occupancy, the model including the additive effects of PastPatchD and OliveNLSI was the

best ranked model. However, there was also high support for more four candidate models, with $\Delta AICc < 2$ relative to the top ranked models (Table 5). Model average estimates of the landscape effects included in each of these competing models, revealed that occupancy probability by the Lusitanian pine vole increased with the density of pasture habitats, tending to decrease where olive grove patches are more disaggregated across the landscape and where forests lands are more clumped (Table 6).

Overall, we found no evidence for spatial autocorrelation in occupancy patterns of Cabrera and Lusitanian pine voles, with a very low and non-significant observed Moran's I values ($I=0.01$, $p=0.22$ for the Cabrera vole; and $I=0.01$, $p=0.16$ for the Lusitanian pine vole).

Table 5- AICc-based ranking of candidate models built to assess the effects of landscape variables on Cabrera and Lusitanian pine voles. In each case the submodel structure for detectability included the effects of transect location ($\sim TransType$). Most supported models for each species ($\Delta AICc < 2$) are indicated in bold red.

Species	Psi submodel	AICc	delta AICc	Wt-AICc
Cabrera vole	$\sim AgricEdgeD + PastNLSI$	522.87	0	0.55
	$\sim AgricEdgeD + AgricNLSI + PastNLSI$	525.22	2.35	0.17
	$\sim AgricNLSI + PastNLSI$	525.95	3.08	0.12
	$\sim AgricEdgeD$	527.25	4.38	0.06
	$\sim AgricNLSI$	528.08	5.21	0.04
	$\sim PastNLSI$	528.35	5.48	0.04
	$\sim AgricEdgeD + AgricNLSI$	529.22	6.35	0.02
Lusitanian pine vole	$\sim PastPatchD + OliveNLSI$	258.1	0	0.24
	$\sim PastPatchD$	258.28	0.18	0.22
	$\sim PastPatchD + ForestNLSI$	258.7	0.6	0.18
	$\sim PastPatchD + OliveNLSI + ForestNLSI$	259.31	1.21	0.13
	$\sim OliveNLSI$	259.57	1.47	0.11
	$\sim OliveNLSI + ForestNLSI$	260.52	2.41	0.07
	$\sim ForestNLSI$	261.2	3.1	0.05

Table 6- Estimates of main effects on detection and occupancy obtained from the best model identified for the Cabrera vole and from model averaging of best models identified for the Lusitanian vole Model. Variables with 90% Confidence Interval (CI) of estimates not including 0 are shown in bold red.

Species	Parameter	Covariable	Effect size	Unconditional SE	90% Unconditional CI
Cabrera vole	Detection	<i>TransType</i>	2.26	0.60	1.27, 3.25
	Occupancy	<i>AgricEdgeD</i>	1.68	0.83	0.31, 3.05
		<i>PastNLSI</i>	-2.23	1.68	-5, 0.54
Lusitanian pine vole	Detection	<i>TransType</i>	2.24	1.03	0.54, 3.93
	Occupancy	<i>PastPatchD</i>	0.75	0.41	0.07, 1.43
		<i>OliveNLSI</i>	-7.89	-6.03	-17.8, 2.03
		<i>ForestNLSI</i>	0.44	0.36	-0.15, 1.03

Discussion

Understanding biodiversity patterns in agroecosystems is a main goal to improve conservation management, particularly in areas where farming practices tend to shift from traditional subsistence systems to market-oriented agriculture, primarily aiming at increasing agricultural productivity (Montoya, Gaba, Mazancourt, Bretagnolle & Loreau, 2020). As regards to small mammals, studies aiming to assess species occurrence patterns in agroecosystems have been mostly based on the habitat-level, while fewer have emphasised the landscape scale (e.g., Millán de la Peña *et al.*, 2003; Pita, Lambin, Mira & Beja, 2016). Our study, based on small mammal genetic non-invasive sampling across olive grove agroecosystems from NE Portugal, showed that the high heterogeneity in the cover and geometry of different land uses across the region allows the occurrence of a rich small mammal community at the landscape scale, supporting the idea that spatial diversity in habitat composition and structure increases mammal species richness (e.g., B. Fox & M. Fox, 2000). This was particularly noticeable among ground-dwelling rodents, for which a total of 7 out of the 8 species predicted for the region by Bencatel *et al.* (2019) were confirmed to occur in the study area (only *Rattus rattus* remained undetected), including species differing in their conservation status and perceived pest risk by local farmers. This suggests that the complex land

mosaics, mostly resulting from the still predominating traditional farming in the region (Duarte *et al.*, 2006), should provide a high diversity of micro-habitats that allow the coexistence of different small mammal species, even those that might in some way overlap in their niches, such as voles (family Arvicolinae), which are often associated to marginal grassland habitats in agroecosystems (Rodríguez-Pastor, Luque-Larena, Lambin & Mougeout, 2016). It's, therefore, likely that the high spatial heterogeneity and landscape patchiness of NE Portugal olive grove agroecosystems might provide opportunities for multiple species to spatially segregate and locally coexist under equilibrium (Cramer & Willig, 2002).

While our results provided support for a high small mammal richness occurring in NE Portugal olive grove agroecosystems, the recent trends in olive farming intensification (Weissteiner *et al.*, 2011) raises relevant conservation concerns, as they might compromise the existing ecological equilibrium, for example, by favouring the predominance of potential pest species (Balestrieri, Gazzola, Formenton & Canova, 2019). Decreases in biodiversity levels and ecosystem functioning have been observed in other Mediterranean regions where olive groves are more intensively managed, particularly among bats (Herrera *et al.*, 2015), birds (Morgado *et al.*, 2020), invertebrate species (Castro, Tortosa & Carpio, 2021) and plants (Tarifa *et al.*, 2021). Therefore, preserving the more traditional farming practices in NE Portugal olive groves should be crucial to guarantee the provision of food, shelter and protection for many small mammal species, as well as their predators, thus contributing for overall ecosystem health (Paz *et al.*, 2013; Benedek & Sîrbu, 2018; Serafini, Priotto & Gomez, 2019).

According to our predictions, small mammal activity signs (mainly faeces) were, in general, more conspicuous in seminatural habitats surrounding olive groves than within olive groves, suggesting a general low potential for crop damage by the species in the study region. This is also supported by the relatively low occupancy rates predicted for the Lusitanian pine vole, the species that is often perceived by farmers as a potential pest of olive trees (Ponsà, *et al.*, 2011; Miñarro, *et al.*, 2012). According to our predictions, the probability of occupancy by this species increased with the density of pasture

habitats, which is, in general, consistent with previous literature showing the association of this species to open habitats, like meadows or pastures (e.g., Santos *et al.*, 2010; Bencatel *et al.*, 2019). In addition, there was some tendency for higher occupancy probability where olive patches were more clumped and where forest patches were more disaggregated, which suggests that eventual expansion of olive farming into surrounding semi-natural habitats (landscape homogenization) might create favourable conditions for an eventual predominance of Lusitanian pine voles, via reduced competition or predation pressures (Paz *et al.*, 2013), which could increase their potential for causing damages in olive groves. On the other hand, our results also indicated relatively high occupancy rates by the Cabrera vole, suggesting that agroecosystems from NE might support well-established populations of this “Vulnerable” species (Cabral *et al.*, 2005), thus highlighting its high value in terms of conservation. In particular, the occupancy of the Cabrera vole increased with the density of agricultural edges, which is compatible with the existing literature, associating this species with wet herbaceous vegetation cover that typically occurs along agricultural field margins in agroecosystems (Pita *et al.*, 2006). In addition, there was some tendency for higher occupancy estimates where pastures were more aggregated, which agrees with the idea that this land use might favour landscape connectivity for this species in farmed areas (Pita *et al.*, 2007). Overall, our study suggests that spatial heterogeneity in olive grove agroecosystems should be key to the occurrence of either rare and specialist species, or more common and generalist species, thus contributing to the maintenance of high biodiversity levels.

We acknowledge that more research focusing on more detailed demographic parameters is needed to fully understand the processes driving small mammal populations and community structuring and the overall conservation value and resilience of NE Portugal olive groves relative to small mammals’ community. This should also include, for instance, the assessment of possible competition-based mechanisms of population regulation involving potential pest species (Cramer & Willig 2002), which would support the value of a rich small mammal community in providing ecosystem services, such as biocontrol services (e.g., Gomez, Provencal & Polop, 2008). While the use of

multispecies occupancy modelling could have provided important first insights on interspecific relationships at the landscape level, our sample size did not allow the implementation of such approach. Despite these limitations, we believe, however, that this study provides important first insights on the landscape characteristics that determine the occurrence of small mammals in olive grove ecosystems for NE Portugal. This also highlights the utility of non-invasive genetic sampling methods at the landscape scale for informing and improving agroecosystem management towards more biodiverse and healthy environments (e.g., Waits, 2004; Beja-Pereira *et al.*, 2009; Barbosa *et al.*, 2013). Importantly, our results suggest that traditional olive farming maintaining a complex matrix of small patches of crops interspersed with other land-uses should provide ideal conditions to guarantee the continued coexistence of small mammals with different biological and ecological traits in NE Portugal, thus resulting in a more diverse community (Benedek & Sîrbu, 2018), likely to provide important ecosystem services contributing to the long-term sustainability olive farming in the region (e.g. Moreira *et al.*, 2019).

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III. DISCUSSÃO GERAL

Considerando a crescente tendência de intensificação agrícola verificada em muitas regiões, torna-se importante compreender o modo como as características da paisagem influenciam a presença de pequenos mamíferos em agroecossistemas dominados por olival. Esta informação é fundamental para a implementação de uma melhor gestão das zonas agrícolas, tendo em vista a conservação da sua biodiversidade.

Este estudo sugere que, provavelmente, a heterogeneidade espacial elevada e a complexidade do mosaico da paisagem em olivais no Nordeste (NE) de Portugal podem favorecer a ocorrência de múltiplas espécies em habitats diferentes, mas que coexistem em equilíbrio ao nível da paisagem (Cramer & Willig, 2002). Esta afirmação é suportada pela elevada heterogeneidade observada na cobertura e geometria dos diferentes usos do solo, permitindo, ao nível da paisagem, a ocorrência de uma comunidade rica em pequenos mamíferos. Com efeito, tendo como base o uso de técnicas de amostragem genética não-invasiva, detetou-se a presença de 7 de 8 espécies de roedores previstas de ocorrer na área de estudo (Bencatel *et al.*, 2019), incluindo espécies com diferentes estatutos de conservação e potenciais pragas agrícolas. Além disso, os resultados sugerem que paisagens com mosaicos complexos, maioritariamente resultantes da predominância de uma gestão agrícola mais tradicional na região (Duarte, Jones, Lúcio & Nunes, 2006), poderão oferecer uma elevada diversidade de micro-habitats que permitem a coexistência de diferentes espécies. Inclusivamente, espécies que possam apresentar sobreposição de nichos, como a família Arvicolinae que está muitas vezes associada a pastagens marginais nos ecossistemas (Rodríguez-Pastor, Luque-Larena, Lambin & Mougeout, 2016).

De acordo com as hipóteses testadas, os indícios de presença de pequenos mamíferos (fundamentalmente dejetos) foram mais notórios nos habitats semi-naturais envolventes, do que dentro dos olivais, sugerindo que existe pouca probabilidade de causarem danos nas culturas. Inclusive, *Microtus lusitanicus*, que é comumente considerado uma praga agrícola (Ponsà, Cabré, Ferrando, Bosch & Ventura, 2011; Miñarro, Montiel & Dapena, 2012),

mostrou uma probabilidade de ocupação relativamente baixa. De acordo com as previsões e literatura existente (e.g., Santos, Lourenço, Mathias & Mira, 2010; Bencatel *et al.*, 2019), a probabilidade de ocupação desta espécie aumenta com a densidade de pastagens. Adicionalmente, existe uma tendência para ocupação em manchas de olival mais agregadas e manchas de florestas mais separadas, sugerindo que uma eventual expansão do olival, que conduz à homogeneização da paisagem e consequente diminuição dos efeitos de predação e competição, pode criar condições favoráveis para um forte aumento populacional desta espécie e, consequentemente, uma amplificação de potenciais danos causados nos olivais. Por outro lado, os resultados indicam uma elevada probabilidade de ocupação por *Microtus cabrerae*, sugerindo que os agroecossistemas do NE de Portugal podem suportar uma população bem-estabelecida desta espécie “vulnerável” (Cabral *et al.*, 2005) e salientando o valor de conservação desta região. Em particular, a probabilidade de ocupação por esta espécie aumenta de acordo com a densidade das orlas de zonas agrícolas, sendo consistente com pesquisas anteriores que associam esta espécie a zonas com vegetação herbácea relacionada a uma maior humidade que tipicamente ocorre ao longo das orlas agrícolas nos agroecossistemas (Pita, Mira & Beja, 2006). Para além disso, observa-se uma tendência para uma maior possibilidade de ocupação por esta espécie em zonas onde as pastagens estão mais agregadas, estando de acordo com a ideia de que este uso do solo poderá favorecer a conectividade da paisagem para esta espécie em zonas agrícolas (Pita, Mira & Beja, 2007). No geral, este estudo sugere que a heterogeneidade espacial nos agroecossistemas dos olivais pode ser a chave para a ocorrência de múltiplas espécies, desde raras ou consideradas especialistas até às mais comuns e generalistas, contribuindo assim para manter elevados níveis de biodiversidade.

Apesar dos resultados indicarem, que os olivais tradicionais no NE de Portugal podem comportar uma elevada riqueza específica de pequenos mamíferos, este tipo de sistema tende para uma crescente intensificação (Weissteiner, Strobl & Sommer, 2011), podendo trazer preocupações relevantes para a conservação de determinadas espécies e, no geral, do equilíbrio ecológico. Os olivais intensivos, que conduzem à simplificação da

paisagem e remoção de habitats semi-naturais, reduzem a capacidade de manter espécies que dependem de habitats naturais e semi-naturais (Concepción *et al.*, 2012), favorecendo, assim, potenciais pragas agrícolas (Balestrieri, Gazzola, Formenton & Canova, 2019). A perda de biodiversidade e de funcionalidade dos agroecossistemas tem vindo a ser observada noutros locais da região Mediterrânica onde os olivais estão a ser geridos de modo mais intensivo (e.g., Herrera, Costa, Medinas, Marques & Mira, 2015; Morgado *et al.*, 2020; Castro, Tortosa & Carpio, 2021; Tarifa *et al.*, 2021). Assim, é essencial preservar a gestão mais tradicional dos olivais no NE de Portugal para garantir a existência de condições ecológicas para pequenos mamíferos, e, também, para os seus predadores, para a existência de um ecossistema mais equilibrado (Paz *et al.*, 2013; Benedek & Sîrbu, 2018; Serafini, Priotto & Gomez, 2019).

Apesar das limitações existentes, como a impossibilidade de avaliar as relações interespecíficas através de modelos de ocupação multi-espécies devido ao tamanho de amostragem, este estudo permitiu dar os primeiros passos no conhecimento das características da paisagem que podem determinar a ocorrência de pequenos mamíferos nos olivais do NE de Portugal. Para além disso, este estudo destaca também a utilidade de métodos de amostragem não-invasivos, neste caso com base na identificação molecular das espécies, para conhecer e melhorar a gestão dos olivais, de modo a manter uma elevada biodiversidade e ecossistemas equilibrados (e.g., Waits, 2004; Beja-Pereira, Oliveira, Alves, Schwartz & Luikart, 2009; Barbosa, Paupério, Searle & Alves, 2013). Pesquisas futuras são necessárias e devem focar-se num estudo mais detalhado dos parâmetros demográficos, de modo a compreender os processos que levam à estruturação das populações e comunidades de pequenos mamíferos. e, também, entender o valor de conservação e resiliência dos olivais do NE de Portugal. Deve incluir-se, também, a avaliação de possíveis mecanismos de regulação populacional baseados na competição envolvendo potenciais pragas (Cramer & Willig 2002).

Concluindo, os resultados sugerem que os olivais tradicionais do NE de Portugal, ao manterem uma matriz complexa de pequenas zonas cultivadas intercaladas com outros usos do solo, criam as condições ideais para garantir a

coexistência de pequenos mamíferos com marcadas diferenças biológicas e ecológicas, resultando numa comunidade bastante rica (Benedek & Sîrbu, 2018) e que, provavelmente, contribui com importantes serviços ecossistêmicos que favorecem a sustentabilidade dos olivais na região (e.g. Moreira *et al.*, 2019). Desta forma, medidas que favoreçam este tipo de sistemas e que preservem a complexidade e heterogeneidade da paisagem constituem um importante passo para travar a perda de biodiversidade e do funcionamento dos agroecossistemas fruto da crescente intensificação agrícola, que é, conseqüentemente, um grande problema para a Conservação (Benton, Vickery & Wilson, 2003). Futuramente, deverá ser também criada legislação específica focada na prevenção do domínio excessivo de olivais (e outras culturas) intensivos e que priorizem a conservação da biodiversidade nos agroecossistemas pois prevê-se que, a longo prazo, a perda de biodiversidade afete a produtividade dos solos e das culturas (e.g., Frison, Cherfas & Hodgkin, 2011).

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ANEXOS

Table A1- Support for the effects of each landscape variable alone on Cabrera and Lusitanian pine voles relative to the model with constant occupancy, while keeping detectability as a function of transect location ($p \sim \text{TransType}$) (see Table 1). AICc scores of models with constant occupancy were 529.13 and 261.33 for the Cabrera and Lusitanian vole, respectively. Supported effects are identified in bold red.

Psi Submodel	Cabrera vole			Lusitanian pine vole		
	AICc	DeltaAICc	Wt-AICc	AICc	DeltaAICc	Wt-AICc
Cover metrics						
~OliveCover	530.99	-1.86	0.28	262.76	-1.42	0.33
~PastCover	531.45	-2.32	0.24	260.76	0.57	0.57
~ShrubCover	531.35	-2.22	0.25	262.68	-1.34	0.34
~AgricCover	528.11	1.03	0.63	262.68	-1.34	0.34
~ForestCover	531.49	-2.36	0.24	263.60	-2.26	0.24
Edge metrics						
~OliveEdgeD	530.27	-1.13	0.36	262.81	-1.48	0.32
~PastEdgeD	531.18	-2.05	0.26	260.29	1.04	0.63
~ShrubEdgeD	531.46	-2.33	0.24	262.80	-1.47	0.32
~AgricEdgeD	527.25	1.88	0.72	263.65	-2.32	0.24
~ForestEdgeD	531.36	-2.23	0.25	262.86	-1.52	0.32
Density metrics						
~OlivePatchD	530.27	-1.13	0.36	263.69	-2.35	0.24
~PastPatchD	531.28	-2.14	0.26	258.28	3.05	0.82
~ShrubPatchD	531.46	-2.33	0.24	263.32	-1.98	0.27
~AgricPatchD	526.91	2.22	0.75	263.48	-2.15	0.25
~ForestPatchD	531.35	-2.22	0.25	263.24	-1.91	0.28
Aggregation metrics						
~OliveNLSI	530.71	-1.57	0.31	259.57	1.76	0.71
~PastNLSI	528.35	0.78	0.60	264.96	-3.62	0.14
~ShrubNLSI	530.86	-1.72	0.30	261.69	-0.36	0.46
~AgricNLSI	528.08	1.05	0.63	262.04	-0.70	0.41
~ForestNLSI	531.49	-2.36	0.24	261.20	0.13	0.52
Diversity metrics						
~PatchRichness	530.56	-1.43	0.33	263.39	-2.05	0.26
~ShanDiv	530.40	-1.26	0.35	263.00	-1.67	0.30
~ShanEvn	531.11	-1.98	0.27	262.93	-1.59	0.31

Table A2- Pearson's correlations among covariates receiving some support regarding their effects on Cabrera vole occupancy. Correlations >0,6 or <-0,6 are indicated in bold red and from each of the corresponding pair of variables only the one yielding the lower AICc in single covariate occupancy models was retained in subsequent analyses.

	<i>AgricCover</i>	<i>AgricEdgeD</i>	<i>AgricPatchD</i>	<i>AgicNLSI</i>	<i>PastNLSI</i>
<i>AgricCover</i>	1				
<i>AgricEdgeD</i>	0.89	1			
<i>AgricPatchD</i>	0.72	0.88	1		
<i>AgicNLSI</i>	0.18	0.36	0.46	1	
<i>PastNLSI</i>	0.49	0.48	0.25	0.06	1

Table A3- Pearson's correlations among covariates receiving some support regarding their effects on Lusitanian pine vole occupancy. Correlations >0,6 or <-0,6 are indicated in bold red and from each of the corresponding pair of variables only the one yielding the lower AICc in single covariate occupancy models was retained in subsequent analyses.

	<i>PastCover</i>	<i>PastEdgeD</i>	<i>PastPatchD</i>	<i>OliveNLSI</i>	<i>ForestNLSI</i>
<i>PastCover</i>	1				
<i>PastEdgeD</i>	0.80	1			
<i>PastPatchD</i>	0.69	0.87	1		
<i>OliveNLSI</i>	-0.22	-0.23	-0.21	1	
<i>ForestNLSI</i>	0.17	0.19	0.04	-0.10	1

Table A4- Alternative versions of the full model for each species, accounting for those covariates that are highly correlated and therefore may not be included in the same model. In each case the model indicated in bold red was the alternative with lower AICc scores, and therefor was carried forward to the model building and selection procedure.

Species	Psi submodel	AICc
Cabrera vole	~Agric_EdgeD + Agric_NLSI + Past_NLSI	525.22
	~Agric_Cover + Agric_NLIS + Past_NLSI	525.25
	~Agric_Patch_D + Agric_NLSI + Past_NLSI	526.03
Lusitanian pine vole	~ Past_Patch_D + Forest_NLSI + Olive_NLSI	259.31
	~ Past_Cover + Forest_NLSI + Olive_NLSI	261.84
	~ Past_EdgeD+ Forest_NLSI + Olive_NLSI	261.53