Universidade de Évora - Escola de Ciências e Tecnologia Universidade de Lisboa - Instituto Superior de Agronomia

Mestrado em Gestão e Conservação de Recursos Naturais

Dissertação

Wolf breeding sites in human grounds: insights on habitat features and sources of disturbance to support conservation measures

João Pedro Monteiro Cardoso

Orientador(es) | Francisco Jorge Álvares
António Mira
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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:

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Évora 2022

AGRADECIMENTOS

Começo por agradecer aos meus orientadores, Dr. Francisco Álvares, Dr. João Cabral e Dr. António Mira, pelo incansável apoio e constante dedicação em todas as fases desta dissertação. Agradeço também todos os ensinamentos que irei levar para a minha vida profissional e os incentivos que me permitiram a conclusão desta etapa!

Estendo os meus agradecimentos à Patrícia Gil e à Joana Casimiro, que foram fundamentais para que pudesse aprofundar o meu conhecimento e proficiência em técnicas de trabalho de campo na monitorização do lobo-ibérico.

Não deixo por mencionar o enorme apoio que tive da equipa do LEA-UTAD, prestado, concretamente, pelo André Fonseca, na vertente do software SIG, pelo Paulo Barros na análise estatística em R e pela Rita Bastos, na análise de sensibilidade.

Agradeço ainda aos técnicos de campo do CIBIO que obtiveram e organizaram os dados de armadilhagem fotográfica utilizados, nomeadamente aos responsáveis pela amostragem no Alto Minho: Mónia Nakamura, Helena Rio-Maior, Joana Casimiro e Mariana Gonçalves; e a Sul do Douro: Sara Roque, Barbara Martí, Patrícia Gil e Ana Serronha.

É também importante para mim agradecer o constante apoio do João Sousa, e a ajuda e companheirismo do André Reis, Inês Fontes, Sara Cosme e Rita Aranha nesta etapa, e durante todo o mestrado.

Por fim, agradeço à minha família, particularmente à minha mãe Elisa Silva, à minha irmã Elisa Serôdio e ao meu cunhado Ricardo Santos, por toda a compreensão, amparo e incentivo tanto na realização desta dissertação, como em todo o meu percurso académico e pessoal.

Obrigado!

Wolf breeding sites in human grounds: insights on habitat features and sources of disturbance to support conservation measures

ABSTRACT

Disturbance at key areas used by wolves may have negative impacts on population dynamics and species recovery, becoming particularly critical in regions with high human activity, such as the Iberian Peninsula. This study evaluated the sources of human-related disturbances and ecological features in the Iberian wolf key areas, including feeding sites, highway crossing structures and, more exhaustively, breeding sites, using available data from camera trapping obtained in two areas within wolf range in Portugal. Differences at spatial and temporal level were observed in the use of these areas by wolves, other wildlife, domestic animals and humans. A decreasing use of these sites by wildlife with an increase in the use by domestic animals and humans was predicted. However, wild species shown to benefit from areas with intermediate human intervention. The expansion of traditional agriculture and fire mitigation measures seems to mitigate or revert the decreasing trends of wolves, mesocarnivores and wild ungulates.

Key words: Breeding sites; Camera-trapping; Iberian wolf; Human disturbance; Ecological modelling

Locais de reprodução do lobo em territórios humanos: perceções sobre as características do habitat e fontes de perturbação para apoiar medidas de conservação

RESUMO

Perturbações em áreas-chave ocupadas pelos lobos podem causar impactos negativos na dinâmica populacional e na recuperação desta espécie, tornando-se particularmente crítico em regiões com elevada presença humana, como na Península Ibérica. Este estudo avaliou as fontes de perturbações humanas e características ecológicas nas áreas-chave do lobo-ibérico, incluindo locais de alimentação, passagens de autoestradas e, mais exaustivamente, locais de reprodução, utilizando dados de armadilhagem fotográfica obtida em duas áreas de distribuição do lobo em Portugal. Diferenças espaciais e temporais foram observadas nas deteções de lobos, outros animais selvagens, animais domésticos e humanos. Foi prevista uma diminuição do uso desses locais pela fauna selvagem com um aumento do uso por animais domésticos e humanos. No entanto, espécies selvagens demonstraram beneficiar de áreas com intervenção humana intermédia. A expansão da agricultura tradicional e medidas de mitigação de incêndios mostraram desacelerar ou mesmo reverter as tendências decrescentes de lobos, mesocarnívoros e ungulados selvagens.

Palavras-chave: Locais de reprodução; Armadilhagem fotográfica; Lobo ibérico; Perturbação humana; Modelação ecológica

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1. INTRODUCTION

The increase of anthropogenic intervention in the ecosystems, characterized by the widespread presence of people, human activities, and infrastructures, leads to habitat disruption, targeted as one of the most serious threats to biological diversity worldwide (Crooks, 2002; Morales-González *et al.* 2020). Large carnivores, often occurring in areas with high human densities, widespread agricultural activities, livestock grazing, urban development and dense networks of transport infrastructures, are particularly sensitive to landscape change (Huck *et al.* 2010; Støen *et al.,* 2015; Morales-González *et al.* 2020). Large carnivores are especially vulnerable to local extinction, due to their relatively large movement ranges, low population density, and susceptibility to direct persecution by humans (Crooks, 2002; Støen *et al.,* 2015). Therefore, long-term viability of large carnivore populations is largely dependent on their ability to adapt to human-modified landscapes and/or on the application of adequate conservation strategies (Sazatornil *et al*., 2016; Morales-González *et al.* 2020).

1.1. Key areas for large carnivore conservation

Large carnivores that occur in landscapes with high anthropic pressure, adopt behavioural adaptations to minimize risks associated with proximity to humans, while fulfilling their ecological needs such as resting, feeding, or dispersing. Such adaptations are particularly relevant during sensitive periods, as the breeding seasons (Støen *et al*., 2015; Sazatornil *et al*., 2016; Morales-González *et al*., 2020). Thus, the existence of adequate resting, feeding and breeding areas complemented with key locations that allow the movement in fragmented habitats, are crucial factors for their persistence (Huck *et al*., 2010; Ordiz *et al*., 2011; Iliopoulos *et al*., 2014; Planella *et al*., 2016; Martinig & Bélanger-Smith, 2016). Particularly, for large carnivores like wolves, the selection of resting sites can be a critical factor in human-dominated landscapes, as they must offer protection to reduce exposure risk (Llaneza *et al.*, 2016). Wolves are mostly active at night or at twilight, resting and sleeping mainly during daylight, which increases their vulnerability during this time due to the decrease of risk perception. This way, wolves locate their resting sites far away from human-made structures, selecting areas with high availability of refuge, such as dense vegetation cover (Llaneza *et al*., 2016). Although wolves can colonize different types of habitats and tolerate a certain level of human disturbance, their habitat tolerance is shaped by food availability and mortality risk (Capitani *et al.,* 2006; Eggermann *et al.,* 2011; Llaneza *et al.,* 2012; Owens, 2012; Ahmadi *et al*., 2013). Thus, feeding sites, which include killing sites (predation events) or scavenging sites, are particularly important in human-dominated landscapes where these large carnivores feed significantly on anthropogenic food sources such as livestock or human refuse (Álvares *et al.,* 2015, Planella *et al.,* 2016). Furthermore, the barrier effect caused by roads (particularly in high speed and traffic volume fenced highways) restrain wolf movements, inducing limited access to resources, limited gene flow and restricted dispersal movements (Santos, *et al.,* 2007; Zimmermann *et al.,* 2014; Martinig & Bélanger-Smith, 2016). Those negative effects can be mitigated by promoting safe crossings for wolves on existing road crossing structures, such as under and overpasses, or by building specific wildlife passages which enhance ecological connectivity contributing to wolf population viability and conservation (Santos, *et al.,* 2007; Boitani & Powell, 2012; Martinig & Bélanger-Smith, 2016). Nevertheless, another essential key factor for wolves' occurrence and recovery in human dominated landscape consists of the existence of suitable habitat in providing adequate shelter for breeding and pup-rearing, which corresponds to an extremely vulnerable period for this species (Theuerkauf *et al.*, 2003; Capitani *et al*., 2006). The configuration of wolf home-ranges mostly depends on the location of these important breeding areas, being wolf movements a compromise between avoidance of human interference and exploitation of the available resources (Ciucci *et al.,* 1997).

1.2. Wolf breeding sites

Generally, wolves have a well-defined breeding season, which includes birth season (late May-early June) and pup-rearing season (June-September), when the breeding female and the pups, become temporally and spatially predictable around breeding sites, increasing their exposure to disturbance and other human-related risks (Pimenta *et al*., 2005; Sazatornil *et al*., 2016; Rio-Maior *et al*., 2018). During this phenological period, movement patterns of breeding individuals tend to be constrained around breeding sites (or homesites), an area that includes the den, during preweaning, and the rendezvous sites, at postweaning, where pups are fed and protected. Figure 1 represents the annual cycle of social dynamics of a pack, including the birth and pup-rearing seasons (Ruprecht *et al*., 2012; Iliopoulos *et al*., 2014; Rio-Maior *et al*., 2018).

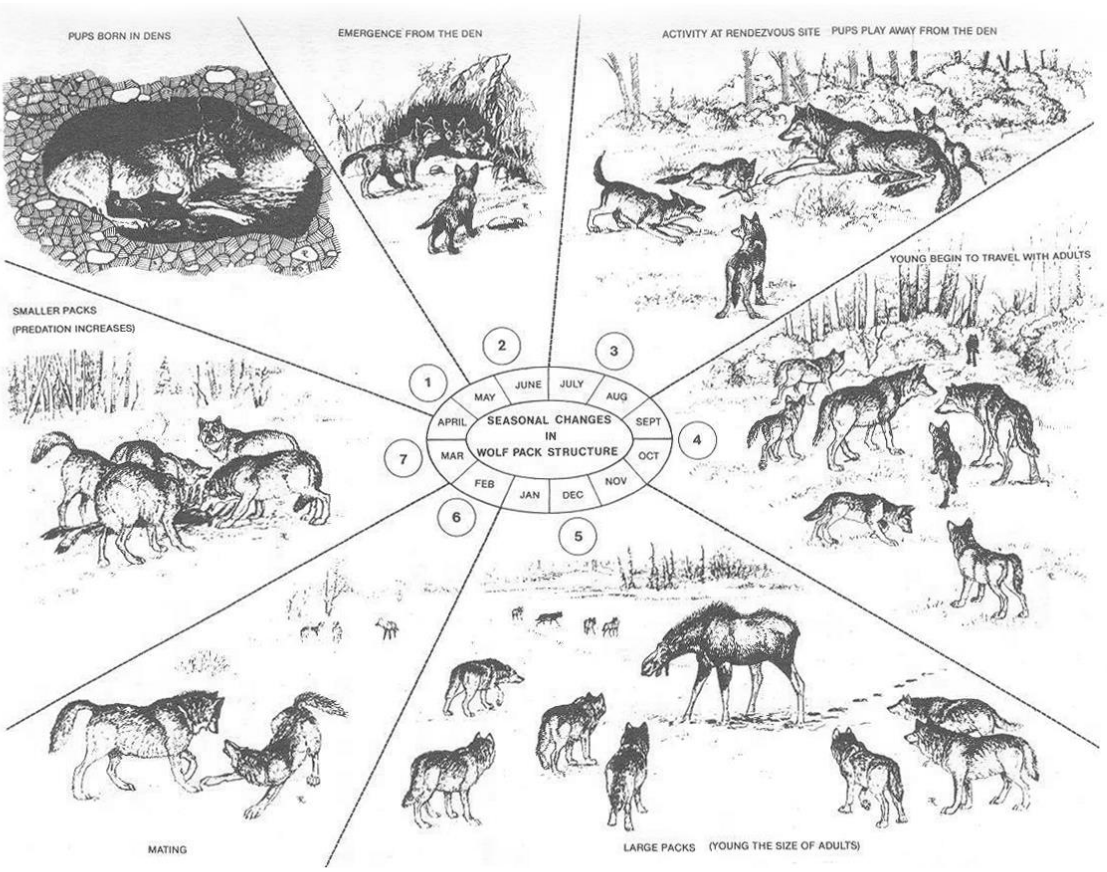

***Figure 1**: Representation of the annual cycle in the social dynamics of a wolf pack, including breeding and pup-rearing season, in the temperate region of North America and Europe (Adapted from Álvares, 2011).*

To compensate their intrinsic vulnerability during breeding period, wolves select areas with low human activity, by avoiding human-related structures and agricultural land, or adjust their temporal activity in response to human presence (Sazatornil *et al*., 2016). Therefore, they seek shelter in poorly accessible places while fulfilling the ecological requirements of the species (Sazatornil *et al*., 2016; Rio-Maior *et al*., 2019). As shown by Sazatornil *et al.* 2016 (Figure 2), significant effects were observed in homesite selection patterns by wolves across study areas in North America and Eurasia. Results from those authors indicated a consistent behavioural response of wolves in avoiding human-made structures, placing their homesites, significantly, further from linear infrastructures and human settlements. This avoidance tended to be stronger for main roads and larger villages than for all-kind of roads and human settlements.

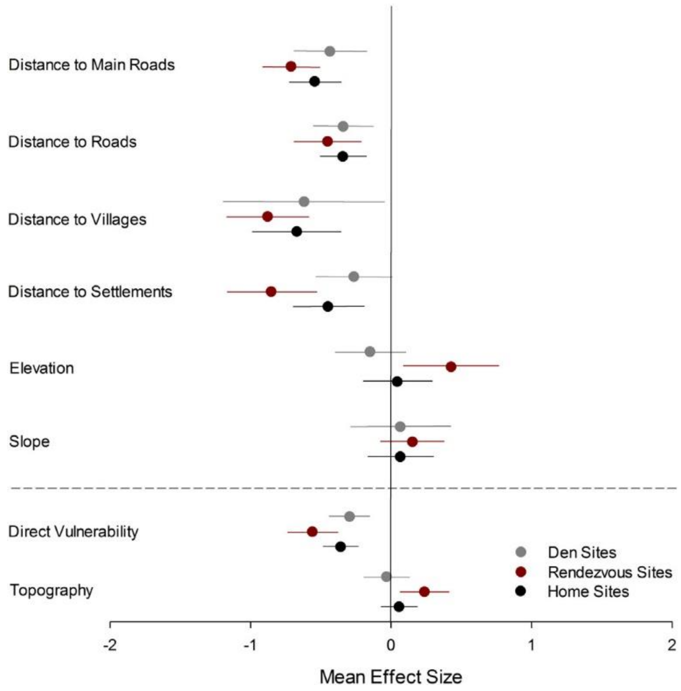

Figure 2: *Results of a meta-analysis on homesite selection patterns by wolves regarding scale-independent variables (above dashed line) and factors (below dashed line). Summary effect sizes are shown for homesites in general, and specifically for den and rendezvous sites. Direct Vulnerability represented the risk of human-caused disturbance and/or mortality (Adapted from Sazatornil et al., 2016).*

Therefore, the choice and reuse of suitable areas for breeding and raising pups plays an extremely important role in wolf persistence, as pup mortality is most frequent during the first six months of life (Capitani *et al*., 2006; Iliopoulos *et al*., 2014). Anthropic disturbance at breeding sites during all pup-rearing season, may have direct effects on pup survival and could result in the abandonment of regularly used breeding sites (Argue *et al*., 2008; Iliopoulos *et al*., 2014). This becomes even more crucial in southern European regions, such as the Iberian Peninsula, where the habitats occupied by wolves are often fragmented and with a high level of human presence and activity (Capitani *et al*., 2006; Sazatornil *et al*., 2016; Grilo *et al*., 2018; Rio-Maior *et al.*, 2019). In the Iberian Peninsula, wolf breeding sites are commonly located far from human settlements and roads, in higher altitude areas with steep slopes and/or in densely forested or shrubby areas, near water lines (Álvares *et al.*, 2015; Grilo *et al*., 2018; Lino *et al.,* 2019). These areas used for wolf breeding are also suitable places for several other wildlife species that profit from the suitable refuge conditions that allow wolf occurrence (Owens, 2012).

1.3. Iberian wolf as a key-species

In the Iberian Peninsula, wolves occur in a human dominated landscape with multiple uses related to livestock grazing, hunting, forestry and infrastructure development such as road network and wind farms (Santos, *et al.*, 2007; Rio-Maior *et al.*, 2019; Eggermann *et al.*, 2011). The Iberian wolf, *Canis lupus signatus*, is a subspecies of grey wolf that is endemic to the Iberian Peninsula. This subspecies is slightly smaller than northern wolves and has distinctive white marks on the upper lips, dark marks on the tail and a pair of dark stripes on its front legs (Torres & Fonseca, 2016). Wolves were still present in almost all Iberian Peninsula until early XXth century, however, in the following decades there was a sharp decline on wolf's distribution range, becoming restricted to the mountainous areas of northern Portugal and Spain (Figure 3) with a population estimated in approximately 65 and 250 breeding packs, respectively (Pimenta *et al.*, 2005).

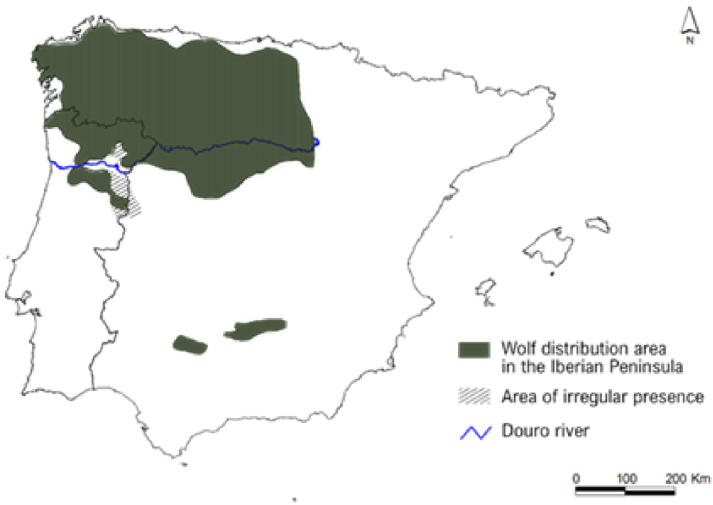

Figure 3*: Current wolf distribution in the Iberian Peninsula (*Adapted from Pimenta *et al.* 2005*)*

The Portuguese wolf range comprises two subpopulations, one at the north of Douro river, in continuity with the Spanish population, which has between 45 and 55 packs and other at the south of Douro river that does not exceed 10 packs (Figure 4; Pimenta *et al.*, 2005). The latter subpopulation is extremely threatened, being one of the few wolf subpopulations in Europe that is considered to be on the verge of extinction (Boitani & Ciucci, 2009). This subpopulation located at south of Douro river, is geographically isolated and has suffered significant reductions in number and range during the last decades, harbouring a lower genetic diversity than the subpopulation at north of Douro river, with evidence of genetic isolation between these two subpopulations

(Álvares *et al.*, 2015). The separation between these two main subpopulations appears to be associated with major river valleys, including the Douro and Tâmega rivers, which also correspond to high levels of human activity, accompanied with a high density of infrastructures (Torres & Fonseca, 2016).

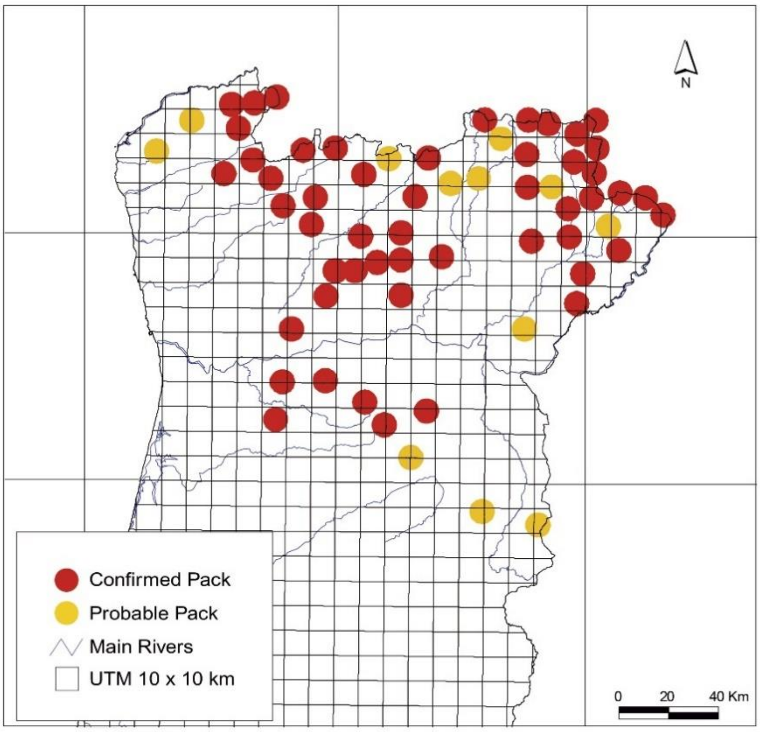

Figure 4*: Confirmed and probable packs in Portugal detected in the 2002/2003 national census (*Adapted from Pimenta *et al.* 2005*)*

As a result of the small population size, Iberian wolves are legally protected both in Portugal and Spain, being considered as a priority species for conservation at European level (Santos, *et al.*, 2007; Álvares *et al.*, 2015). In Portugal, wolves are a strictly protected species by specific legislation since late 1980s (Law No. 90/88; Decree-Law No. 54/2016). This legislation regulates, among other aspects, the system of financial compensation to livestock owners with losses caused by this predator and the protection of important areas for wolf occurrence, including breeding sites (Cabral *et al.*, 2005; Álvares *et al.*, 2015). Therefore, the national authorities in Portugal impose that every new infrastructure projected within the wolf range should be subject to an Environmental Impact Assessment (EIA), to evaluate and minimise potential negative effects on this carnivore. If negative impacts are expected, the project's approval is dependent on the promotion of different mitigation and/or compensation measures, which can range from local layout adjustments, restrictions in construction scheduling or promoting regional habitat management actions. Ultimately, if the predicted impacts are too severe, the entire project may be disregarded (Ferrão da Costa *et al.*, 2018).

There is a great variability in the size of wolf packs, taking into consideration the time of year, the availability of food and the levels of anthropogenic mortality in the area inhabited by them. Thus, Iberian packs in human-dominated landscapes are often relatively small, as a consequence of human-caused mortality (Rio-Maior *et al.*, 2018; Nakamura *et al.*, 2021). Considering these factors, a pack of Iberian wolves can vary, on average, between 2 to 9 individuals, depending on the context where the pack occurs (Pimenta *et al.*, 2005; Álvares *et al.*, 2015; Nakamura *et al.*, 2021). Nevertheless, unlike many other large carnivores, wolves are extremely adaptable, being able to modify pack structure in response to changing levels of mortality and regional prey abundance. Wolves accomplish this through altering fertility levels, promoting dispersion of individuals to other areas, and changing their tolerance towards other wolves in neighbouring areas (Owens, 2012). The wolf is considered an opportunist species, being able to change their diet depending on food availability. Preferentially, it predates wild ungulates such as roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and wild boar (*Sus scrofa).* However, in many regions of the Iberian Peninsula, most of these species have disappeared or are scarce, and wolves often depend on domestic ungulates (Barja, 2009; Torres & Fonseca, 2016; Pimenta *et al.*, 2018. Livestock, an important economic activity in Iberian Peninsula, normally is raised under extensive grazing in the mountains rather than in fenced pastures, often unguarded or with just one shepherd. Therefore, it is common for wolves to predate on sheep (*Ovis aries*), cattle (*Bos taurus*) and horses (*Equus caballus*), resulting in conflicts with human interests (Eggermann *et al.* 2011; Pimenta *et al.*, 2018). Nevertheless, wolf diet can be extremely flexible, and whenever these species are scarce, wolves often feed on other domestic animals, like dogs (*Canis familiaris*) and cats (*Felis catus*), as well as mesocarnivores, like foxes (*Vulpes vulpes*) and badgers (*Meles meles*) and smaller prey such as lagomorphs, rodents or even human refuse, as aforementioned (Álvares *et al.*, 2015, Martins, *et al.*, 2020). The consumption of carnivore species by wolves is driven by fragmented and human-dominated landscapes, where mesopredator densities are often increased and ungulate densities decreased, which intensify competition and the need for alternative food sources (Martins, *et al.*, 2020).

Considering the key habitat factors that determine the Iberian wolf presence, there is a large amount of potentially suitable habitat for wolves in the Iberian Peninsula, including large tracts of still unoccupied areas, being the current wolf range not limited by a lack of suitable habitat (Grilo *et al.*, 2018). However, the human persecution and disturbance, mostly due to the construction of roads and other infrastructures, such as the inland wind farms, dams and respective accessibilities in remote areas, are some of the main factors contributing to the decline of the Iberian wolf (Santos, *et al.*, 2007; Álvares *et al.*, 2015; Nakamura *et al.*, 2021). According to the results obtained from wolves found dead in Portugal between 1997 and 2004, collision with vehicles was the most frequently detected cause of death, followed by snare trapping, shooting and poisoning (Pimenta *et al.*, 2005). Moreover, wind farm development in Portugal has an extensive overlap with the wolf distribution area, causing habitat disturbance during wind turbines installation and operation as well as from increased vehicle circulation on the built road network, leading to a decrease in wolf reproductive success during construction and in the early years of operation (Ferrão da Costa *et al.*, 2018). If these limiting causal factors related to the socio-economic trends in Portugal continue operating, the Iberian wolf persistence within its current range is threatened (Santos, *et al.*, 2007). Also, as wolf breeding-sites in Portugal are often located at steeper slopes dominated by shrublands, mixed conifer forests, and eucalyptus, which are vegetation types with high intrinsic flammability, usually, these places are prone to fire disturbance (Lino *et al.*, 2019). Nevertheless, Iberian wolves demonstrate a remarkable resilience to fire although burnt landscapes may induce higher exposure to human disturbance and persecution due to limited refuge conditions (Lino *et al.*, 2019). Finally, another major threat to wolf conservation is the scarcity of natural prey, essentially caused by human action over the last century, leading to a greater dependence on domestic prey (Pimenta *et al.*, 2005; Santos, *et al.*, 2007; Álvares *et al.*, 2015; Eggermann *et al.*, 2011).

1.4. Methodological approaches to assess wolf requirements

Wildlife monitoring and research on species like the wolf depend on reliable population estimates, which can be challenging to obtain for elusive large carnivores that live in forested habitats without extensive snow cover (Carbone *et al.*, 2001). In Iberian Peninsula, wolf surveys are mostly based on direct and indirect sampling (Blanco & Cortés, 2012). Direct sampling methods consist of direct observation of individuals and howling surveys, where wolf response is elicited via human imitation of howling. Howling sessions start at sunset and are repeated during the early night-time hours, taking place between August and October because, in the Iberian Peninsula, is when pups usually remain at the rendezvous sites and the reply rates are higher (Llaneza *et al.*, 2005). The indirect sampling methods include performing transects to locate scats and other presence signs in areas with high probability of wolf occurrence that, when fresh, are collected for genetic analysis to determine species and individual identification (Llaneza *et al.*, 2014; Nakamura *et al.,* 2017). Besides the genetic data, scats can also provide additional biological information such as the health status, physiological parameters and diet of the individual (Nakamura *et al.*, 2017). Additionally, collected dead animals and livestock killed by wolves are also used to assess wolf presence (Torres & Fonseca, 2016). The increasing development of camera trapping techniques became a complement for wolf monitoring, allowing the estimation of wolf presence, breeding occurrence and pack size, being an effective way to estimate the number of individuals trough a capture-recapture approach (Carbone *et al.*, 2001; Mattioli *et al.*, 2018). Camera trapping is the use of remotely triggered cameras, with an infrared motion sensor, that automatically takes images and/or videos of animals or other subjects passing in front of them (Fonseca *et al.*, 2003; Rovero *et al.*, 2013). Camera traps are instruments that have become a tool of choice in wildlife research and monitoring, being a survey tool that has improved the capacity to infer information not only about the target species but also regarding other components of the environment, such as human disturbance (Meek *et al.*, 2014; Rovero *et al.*, 2013).

The knowledge and prediction of the suitable conditions of a critical resource, such as breeding sites and other key areas for refuge or feeding, is important to support decision-making for the development of conservation strategies, considering the current threats for wolf populations in human-dominated landscapes (Álvares *et al.*, 2015; Norris *et al.*, 2002). However, in order to understand how landscape and environmental features influence selection patterns of wolf breeding sites, it is fundamental to consider the past, current and future landscape changes and socio-economic trends occurring within the range of wolf populations (Santos, *et al.*, 2007). The Iberian wolf is an adequate key-species to address human disturbance in breeding sites and other sensitive areas for wolves, as it occurs in a human dominated landscape with multiple uses (Santos, *et al.*, 2007; Rio-Maior *et al.*, 2019; Eggermann *et al.*, 2011). It is expected that these highly disturbed landscapes induce a significant disturbance in breeding wolves. Nevertheless, there is a lack of knowledge on the interplay between levels of disturbance and socio-ecological features in wolf breeding sites, and the implications for their use by wolves (Theuerkauf *et al.*, 2003; Llaneza *et al.*, 2012). This limited knowledge regarding this crucial topic for the Iberian wolf in Portugal, makes it difficult to evaluate conservation priorities (Álvares *et al.*, 2015; Nakamura *et al.*, 2021).

In this context, ecological models can represent an important contribute to support decision-making through the simulation of alternative environmental and management scenarios that are difficult or impossible to understand otherwise

(Schmolke *et al.* 2010; Santos *et al.* 2013). Since most of the environmental impacts are long-term phenomena or occur after a time lag, early indications of change need to be identified (Bastos *et al.,* 2016). There are a great variety of model types and modelling approaches in order to anticipate ecological trends in complex systems, namely System Dynamics (SDs), Species Distribution Models (SDM), Bayesian Networks (BNs), Couple Component Models (CCMs), Agent-Based Models (ABMs), Knowedge-Based Models (KBMs) and hybrid protocols such as the Stochastic Dynamic Methodology (StDM) (Santos *et al.* 2013; Kelly *et al.* 2013).

Models that simultaneously attempt to capture the functional composition of ecosystems can be important tools in conservation studies (Morinha *et al.* 2017). In fact, the prediction of how anthropogenic environmental changes will affect the ecology of species/populations and composition of biotic communities in disturbed ecosystems is essential to improve the conservation and management planning (Mokany *et al.* 2016). The use of innovative hybrid modelling methodologies, combining process-based models with correlative approaches, is promising to capture and understand the dynamics of changes in complex systems, both at space and time levels (Bastos *et al.* 2016). These powerful modelling techniques can be very useful to anticipate and prioritize conservation efforts by testing hypothetical habitat/landscape management scenarios, which are of particular importance for vulnerable/threatened species, namely in the implementation of effective conservation measures (Morinha *et al.* 2017; Mokany *et al.* 2016). Therefore, ecological modelling has been gradually considered as part of research and conservationist agendas, supporting the design of optimized and cost-efficient management strategies and measures (Santos *et al.* 2013). In this perspective, the stochastic dynamic methodology (StDM) has been developed as a modelling protocol from which management strategies can be designed and tested (Morinha *et al.* 2017).

The StDM main vocation is to provide a mechanistic understanding of ecological cause-effect relationships, based on the premise that holistic emergent patterns of ecosystem phenomena can synthesize the complexity of ecological processes (Cabral *et al.* 2008). The StDM hybrid protocol is a sequential methodological process, combining conventional dynamic modelling techniques with statistical procedures, in order to predict the ecological status of changed ecosystems by taking into account stochastic phenomena that characterize the real ecological processes (Santos *et al.* 2013). This potential was tested by applications of the proposed methodological principles to simulate the wolf use trends under contrasting realistic scenarios in representative Portuguese breeding sites of this threatened species.

1.5. Goals and hypothesis

With the scope of addressing the wolf conservation needs in Portugal that are explicit in the national legislation for the protection of this species, where is highlighted the "prohibition to deteriorate or destroy its habitat, or areas for reproduction and rest" (DRE, 2021), this work has three main goals:

i) Characterize the level of human disturbance and wildlife use in wolf breeding sites and other important areas for wolves (e.g. feeding sites and highway crossings) by resorting to camera trapping data from Alto Minho region in NW Portugal and the region at south of Douro river, collected between 2015 and 2020. We hypothesize that there are regional variations in the levels and types of human disturbance between study areas, with south of Douro river being the area with higher disturbance levels, given its highly human-dominated landscape (Alexandre *et al.*, 2000).

ii) Assess spatial (intrapack and between packs) and temporal variations (circadian, seasonal and yearly) on detection rates of wolves, wildlife and sources of disturbance in wolf breeding sites. We hypothesize that a higher incidence of wildlife use (e.g. wild ungulates and mesocarnivores) is expected in wolf breeding sites from Alto Minho region due to the existence of several protected areas that harbour more suitable habitats and better refuge conditions (Rio-Maior *et al.*, 2019). Temporal variations in the levels and types of human disturbance across circadian period are also expected, with lower disturbance levels occurring at night, when human activity is minimal, compared to the diurnal period (Llaneza *et al.*, 2016; Rio-Maior *et al.*, 2019). Finally, an increase of disturbance levels related to outdoor/touristic activities in the year 2020 is predicted, due to the documented increase of these practices in natural areas during the Covid-19 pandemic (Soga *et al.*, 2021).

iii) Evaluate how landscape/environmental features influence the use of breeding sites by wolves, other wildlife (e.g., mesocarnivores and wild ungulates) and human-related uses, as domestic animals, humans on foot and vehicles, as well as forecast shifts in breeding sites use related with landscape changes, by applying a hybrid correlative/dynamic modelling approach, the StDM protocol. One of the central requirements is that the data set used in the correlative treatment includes relevant gradients of spatiotemporal change (Bastos *et al.*, 2018). Therefore, time and space are implicit in the dynamic model construction and simulations (Bastos *et al.*, 2016), where it is hypothesized that levels of wildlife use and human presence in wolf breeding sites are closely related to their landscape/environmental features. Therefore, dynamic variables such as land cover trends, combined with the proximity to human settlements and other infrastructure, would be expected to shift the suitability and location of breeding sites, given predicted landscape changes and trends in human disturbance (Santos, *et al.*, 2007).

Based on our findings we will discuss conservation implications and suggest possible management measures to minimize human disturbance in sensitive areas for wolves, particularly in breeding sites. In fact, the need for science-based information regarding wolf population dynamics in Portugal is crucial to support the implementation of more effective management measures (Álvares *et al.*, 2015; Nakamura *et al.*, 2021). In this regard, the proposed framework may support informed conservation decision-making, by testing locally tailored management actions and/or by anticipating the consequences of future landscape changes.

2. METHODOLOGY

2.1. Study areas

The present work is focused in two study areas included in the Portuguese wolf range, where this large carnivore occurs under different ecological conditions, namely the Alto Minho region, located in northwest Portugal and comprising seven wolf packs sampled, and the mountainous regions of Beira Alta, located at south of Douro river and comprising three wolf packs sampled (Figure 5).

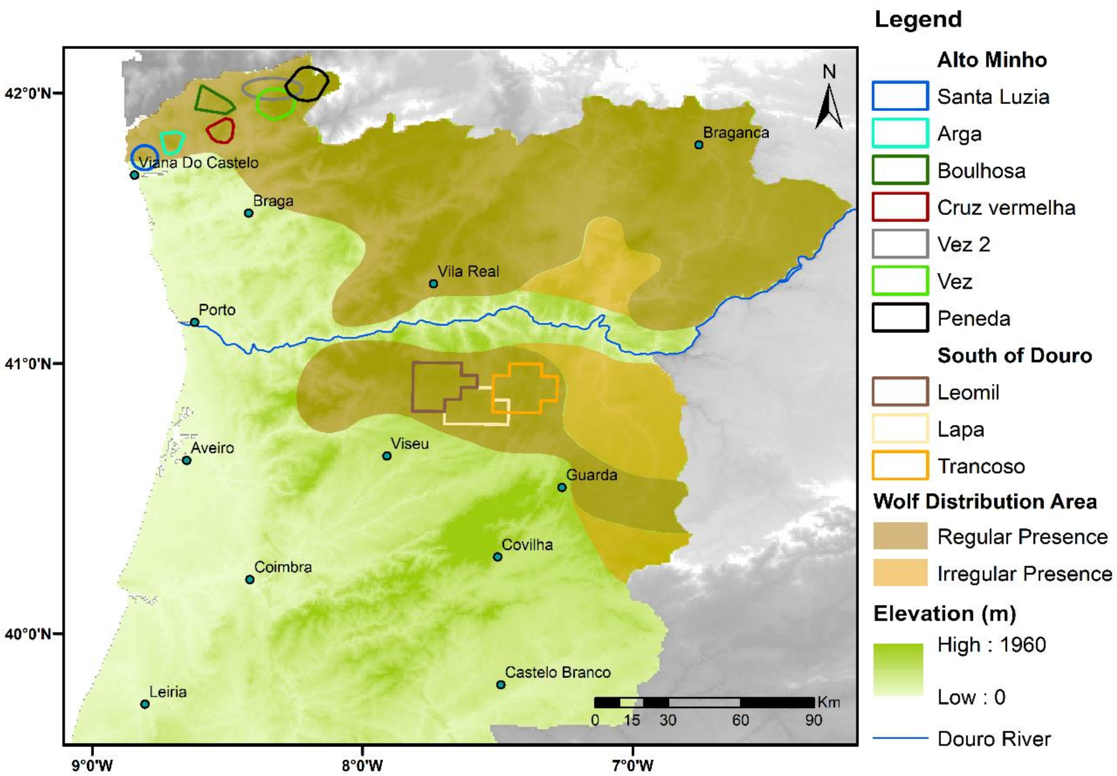

Figure 5: *Location of the sampled packs territory in the two study areas within Portuguese wolf range: Alto Minho and south of Douro regions. The territories of packs in Alto Minho were obtained using the 95% Minimum Convex Polygon method of resident wolves monitored by GPS telemetry, while at south of Douro river corresponds to the pack territories based on sampling squares.*

The Alto Minho region is confined by the Minho river (north), the Lima river (south), the Atlantic Ocean (west) and the Portuguese-Spanish border (east) (Figure 6). The study area is located in a region of high natural richness, highlighting the existence of several classified areas with protection status, such as the Peneda-Gerês National Park, the Corno do Bico Protected Landscape and SAC Serra de Arga, classified as Natura 2000 network (Rio-Maior *et al.* 2020). This region is dominated by mountain massifs, where the highest altitudes are located in Serra d'Arga (825m), Corno do Bico (883m) and Serras do Soajo-Peneda (1416m) (Rio-Maior et al. 2020). Regarding geomorphology, the East (Serra da Peneda) and West (Serra de Arga) areas are dominated by granite outcrops, with an abrupt slope, while the Central area (Serras do Soajo and Boulhosa) is dominated by metamorphic rocks that result in a smoother slope. From a bioclimatic point of view, this study area is included in the Euro-Siberian biogeographic region, which reaches its southern limit here. Thus, a large number of species that are typical from this biogeographic region and have a restricted range in Portugal occur in this area (Rio Maior *et al.* 2020). The annual cumulative rainfall in Alto Minho is one of the highest in Portugal with an average of 2000 mm (IPMA, 2021). The land occupation is dominated by agricultural fields, scrubland, degraded forest (by fires and/or logging) and hardwood or pine forest (Rio-Maior *et al.* 2020). These areas are frequently used by humans, particularly for livestock grazing and infrastructure development, such as wind power farms and a dense road network, including two highways that cross the wolf distribution area and may affect the connectivity between packs (Rio-Maior *et al.* 2020). The human density in rural areas is on average 34 people/Km^2 (INE, 2021). Despite the high presence and human intervention, the wolf population in this region appears to be stable and with a high reproduction rate (Álvares *et al.* 2015; Nakamura *et al.* 2021). The study area currently covers the territories assigned to seven to eight packs, including the ones sampled in this study: Santa Luzia, Arga, Boulhosa, Cruz Vermelha, Vez 2, Vez and Peneda (Rio-Maior *et al.* 2020). In this region of Portugal, particularly inside Peneda-Gerês National Park, wolf diet is based on livestock, mostly free-ranging horses and cattle (Álvares, 2011). Consequently, most of the economic losses caused by wolf predation at the national level occur in this region, generating an accentuated human-wolf conflict (Pimenta *et al*., 2018). Even so, this wolf population reaches, locally, high densities, only possible due to the reduced humanization of the habitat at higher elevations and the great availability of food (Álvares, 2011).

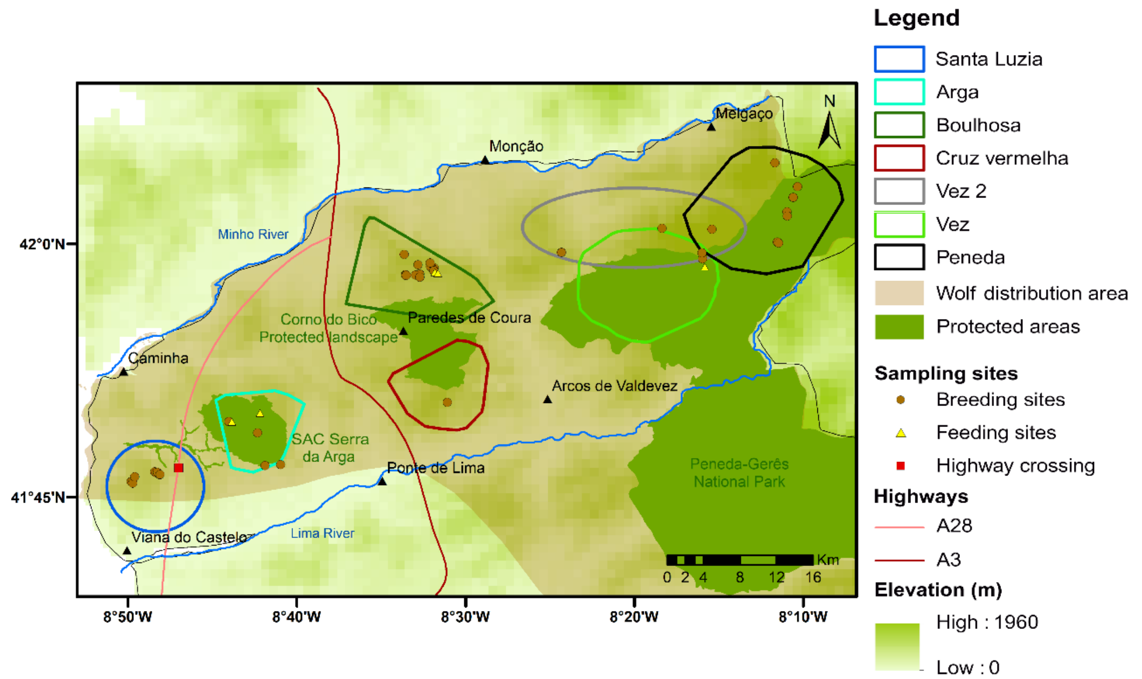

Figure 6: *Study area in Alto Minho with the representation of the territories attributed to the sampled packs including the location of the sampling sites based on camera trapping, namely breeding sites, feeding sites and a crossing structure in the highway A28. It is also indicated the location of protected areas (dark green) included in this study area.*

The region at south of Douro river is located between the Douro river, to the north, and the highway IP5, to the south, with its eastern limit in the municipalities of Penedono and Trancoso (Figure 7). Although this area is one of the last strongholds for wolves in south of Douro river, the only environmental protection status is the “SAC Rio Paiva” of the Natura 2000 network, having nearby the “SAC Serra de Montemuro” and Serra da Estrela Natural Park. The study area is characterized by a mountainous region with low slopes, with average altitudes around 700-800m and a maximum altitude of 1011m. It comprises the mountains of Leomil, Lapa, Sirigo and several small massifs located between Penedono and Trancoso, with a predominance of eruptive rocks such as granite (Serronha *et al.* 2019). The climate is typically Mediterranean, with dry-warm summers and rainy winters, with an annual cumulative rainfall of 1400 mm (IPMA, 2021). The vegetation is composed by small areas of native oak forest, scattered among large forest plantation of pine (*Pinus pinaster*) and eucalyptus (*Eucalyptus globulus*), as well as scrublands (Serronha *et al.* 2019). The human density in rural areas is 27,3 people/Km^2 (INE, 2021). These areas are frequently used by humans and the landscape is dominated by agricultural fields, human settlements, infrastructures (e.g., wind farms) and a dense network of paved and unpaved roads. Although forestry and livestock breeding are major activities in this region, the intensity of the later has decreased in number of cattle, sheep and goats over the last years (Alexandre *et al.* 2000). In this area, the wolf population also appears to have some stability in relation to the number of packs detected, even though they have a very low reproductive rate (Álvares *et al.* 2015). This study area corresponds to the territory of three wolf packs, namely, Leomil, Lapa and Trancoso (Serronha *et al.* 2019). In this region, the wolf feeds on the most common ungulates, namely wild boar and herds of goats and sheep, but more commonly uses as food source several dumpsites with animal production remains from intensive farms of domestic pigs, poultry and mainly domestic rabbits, reflecting an evident scavenging behaviour (Alexandre *et al.,* 2000; Casimiro, 2017). This wolf subpopulation survives at low densities in highly humanized areas. From a trophic point of view, wolves take advantage of the proximity to humans, but consequently suffer significant mortality from anthropogenic causes. In these ecological conditions, this wolf subpopulation is in a precarious balance, which can be easily challenged by excessive habitat disturbance or fragmentation (Álvares, 2011).

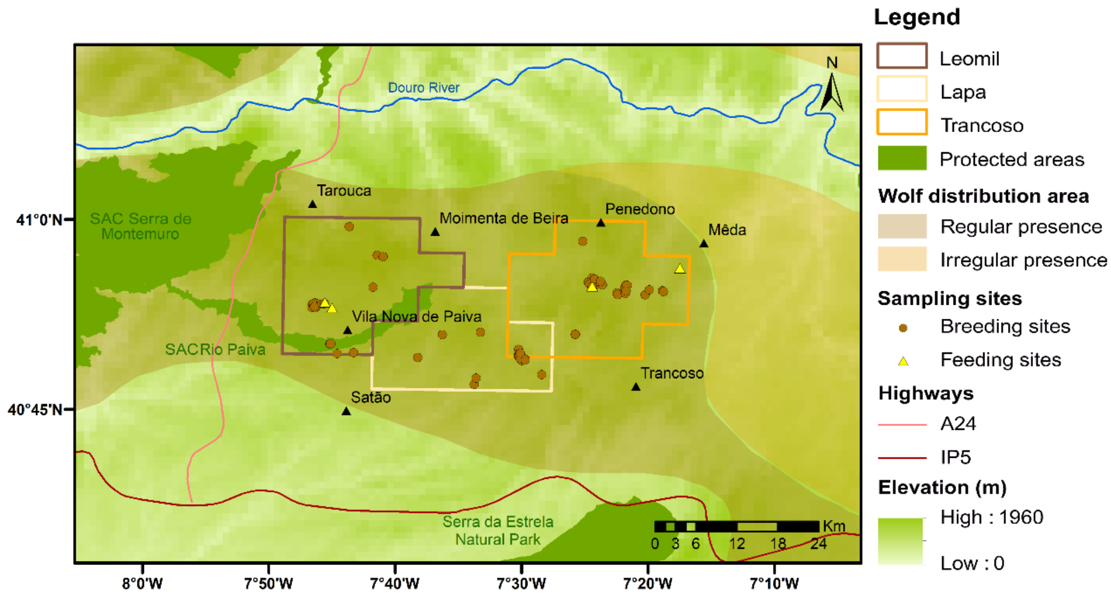

Figure 7*: Study area at south of Douro with the representation of the territories attributed to the sampled packs including the location of the sampling sites through camera trapping, namely breeding sites and feeding sites. It is also indicated the location of protected areas (dark green) included in this the study area.*

2.2. Camera trapping – data collection and analysis

Camera trapping data collected between 2015 and 2020 was already available in the scope of on-going CIBIO projects for monitoring wolf populations. The aim of this methodological procedure is to confirm the occurrence of wolf reproduction and assess the minimum number of individuals, in areas with continuous wolf monitoring in Portugal, namely Alto Minho and South of Douro (Rio-Maior *et al.* 2020; Serronha *et al.* 2020). The photographic trapping stations involved the placement of remote cameras (KeepGuard® KG-780NV and Moultrie® M-40i) with automatic firing aiming the detection of wolves in activity centres (Figure 8). These strategic locations, where the cameras were placed, include wildlife trails and unpaved roads, in areas where wolf presence is more expected, such as breeding sites (n=133), feeding sites (n=9) and road crossing structures (n=1). Breeding sites were defined as an area with a radius of 500 meters, encompassing the location where pups were detected during pup-rearing season, and camera-trapping stations were located within a buffer of 1km around each surveyed breeding site. Each camouflaged camera was attached to a tree, rock or wood post and had a motion sensor that detects not only wolf passage but also other wildlife and domestic species as well as different types of human presence. In 19% of the sampling sites located on breeding areas, bait was used in order to increase the probability of wolf detection, being placed in front of the remote cameras.

Figure 8: *Camera trapping placed on rocks in a wolf breeding site at South of Douro study area.*

Considering the sampling effort, in Alto Minho the number of night-traps per sampling site per year was on average 19.8 (with a maximum of 70 night-traps and a minimum of 2 night-traps) and in the South of Douro it was 39.2 (with a maximum of 236 night-traps and 1 night-trap minimum). The mean, maximum and minimum values of sampling effort performed at each study area, for each sampling year, are represented in Table 1, for the seven sampled packs in Alto Minho (Santa Luzia, Arga, Boulhosa, Cruz Vermelha, Vez 2, Vez and Peneda), and three sampled packs at South of Douro (Leomil, Lapa and Trancoso).

Table 1: *Sampling effort per year in number of night-traps (mean, maximum and minimum) performed at each pack breeding sites, feeding site and crossing structure, in Alto Minho and South of Douro.*

Alto Minho

Data	Pack	Year	Mean	Max	Min
Breeding sites	Boulhosa	2015	25.7	67	5
		2016	9.7	12	8
		2017	36.3	50	9
		2018	32.7	66	14
		2019	70	70	70
		2020	13.5	23	4
	Peneda	2015	17.5	27	8
		2017	25	35	15
		2018	20.3	22	19
		2019	17.5	26	9
		2020	20	20	20
	Cruz Vermelha	2016	2	2	2
	Vez	2017	35	35	35
		2019	23	23	23
		2020	10.5	11	10
	Santa Luzia	2018	13.5	15	12
		2019	45	59	38
		2020	22.5	52	5
	Arga	2020	14	15	13
Feeding sites	Boulhosa	2018	4	6	2
	Vez	2016	6	6	6
	Arga	2018	2.5	3	2
Crossing structure	Arga	2015	7	7	7

South of Douro

Data	Pack	Year	Mean	Max	Min
Breeding sites	Lapa	2015	34.2	57	2
		2016	14	14	14
		2018	43.3	109	5
		2019	63.5	189	7
		2020	27.5	34	10
	Leomil	2015	46.2	59	23
		2016	30	53	7
		2018	60.3	144	7
		2019	25.8	49	2
		2020	39.6	78	26
	Trancoso	2015	36.5	47	16
		2016	31.3	61	6
		2018	49.7	70	11
		2019	36.5	95	1
		2020	70.2	198	18
Feeding sites	Leomil	2019	60	61	59
		2020	236	236	236
	Trancoso	2019	4	4	4

For each record obtained by camera-trapping the date, hour of occurrence, number of individuals and image classification, differentiating human disturbance from wildlife species, were recorded according to the categories in Table 2. Human-related disturbance was categorized as “vehicles”, “human on foot” (that included a distinction between different human activity such as hunter or shepherd) and “domestic animals”. The classification used for wildlife included “wolf”, as the target species, as well as “mesocarnivores”, “wild ungulates” and “lagomorphs”, as potential prey/competitor species with trophic relevance for wolves.

Table 2*: Description of the categories for wildlife species and human disturbance used in the analysis of camera trapping data.*

	Category	Description
Wildlife use	Wolf	The target species, Iberian wolf (*Canis lupus signatus).*
	Mesocarnivores	Small and median sized wild carnivores, namely *red fox (Vulpes vulpes)*, badger (*Meles meles)*, wild cat (*Felis silvestris)*, common genet (*Genetta genetta)*, Egyptian mongoose (*Herpestes ichneumon) and* beech marten (*Martes foina)*.
	Wild Ungulates	*Wild boar (Sus scrofa) and roe deer (Capreolus capreolus).*
	Lagomorphs	*Iberian hare (Lepus granatensis)* and European rabbit (*Oryctolagus cuniculus)*.
Human disturbance	Vehicles	Means of transportation, namely car, four-wheel drive, tractor, motorcycle, quad bike, bike, etc.
	Human on foot	Different human activity including hunter, shepherd and other human outdoor activities, such as trekking, etc.
	Domestic animals	Species of livestock namely cattle *(Bos taurus)*, horse (*Equus caballus)*, sheep (*Ovis aries)*, goat (*Capra hircus)*, etc. as well as pets namely dogs (*Canis familiaris*) and cats (*Felis catus*).

Quantification of the observed records was made by analysing the intensity of human disturbance as well as the diversity and quantity of wildlife species observed. The sampling effort was also analysed, for each month, year, pack and wolf key area (e.g., Feeding sites, Crossing structure and Breeding sites), in order to calculate the detection rates (d.r.). Those correspond to the number of detections / 100 night-traps, and only independent records obtained with a minimum of a 30 minute interval were considered for the analysis (Boitani & Powell, 2012). Initially, a general analysis was made comparing the data obtained in Alto Minho and South of Douro regarding the detection of wildlife and human-related disturbance, also comparing the general data among Feeding sites, Crossing structure and Breeding sites. Then, an analysis of the spatial and temporal variations (circadian activity variations) of the data obtained from feeding sites and crossing structure was made (whenever information was available). Feeding sites in the territories of the sampled packs consisted of prey remains left by wolves, namely Garrano horses in Arga, Boulhosa and Vez packs (Alto Minho region). In South of Douro, the feeding sites of Leomil and Trancoso correspond to dumping places from intensive farms of domestic rabbits and cattle, respectively. Finally, a more in-depth analysis of the data obtained from camera trapping at breeding sites during the breeding season was carried out. This analysis consisted in the evaluation of regional variations in camera detections between breeding sites, sampled packs and between sampling breeding sites located inside or outside protected areas. Temporal variations in camera trapping detection along the breeding season were also evaluated in daily, monthly, and annual rates.

2.3. Assessment of trends in wildlife and human-related detections considering different intervention scenarios

The Stochastic Dynamic Methodology (StDM) is a sequential hybrid modelling protocol developed to test the dynamic relationships between dependent and independent variables, such as the impacts of anthropogenic disturbances on the biodiversity components of threatened ecosystems (Santos *et al.* 2013). In this study, the dependent variable corresponds to the records obtained by camera trapping, expressed in the number of wildlife detections, namely wolf, mesocarnivores and wild ungulates, and human-related detections, such as domestic animals and human presence (on foot and vehicles). The independent variables are expressed in the proportion of area occupied by each habitat class, considering a 2 km radius buffer around each wolf breeding site. This area around wolf breeding sites was based on the documented movement range by wolf breeding females during pup-rearing season (Rio-Maior *et al.* 2018), corresponding to a priority area assumed for protection of breeding sites (Rio-Maior *et al.* 2020) (Table 3).

***Table 3**: Description of all variables considered for the StDM modelling procedure in wolf breeding sites. * Response variables that are also considered as explanatory variables for the higher trophic levels, according to the chain components criteria shown in Figure 10.*

Variables		**Units**
Explanatory variable	**Dynamic land use classes**	
	Open areas	Proportion of land use
	Shrubland	
	Oak species	
	Other hardwoods species	
	Pine species	
	Eucalyptus	
	Invasive species	
	Agriculture	
	Fixed land use classes	
	Urban areas	Proportion of land use
	Highways	
	Paved roads	
	Dirt roads	
	Quarries	
	Wind farms	Nº of wind turbines
	Altitude	Metres (m)
Response variable	**Camera trapping detections**	
	Wolves	Normalized number of wolves detected
	Mesocarnivores*	Normalized number of mesocarnivores detected
	Ungulates*	Normalized number of ungulates detected
	Domestic animals*	Normalized number of domestic animals detected
	Humans on foot and vehicles*	Normalized number of humans and vehicles detected

To avoid high multicollinearity, the independent variables were selected after a pairwise correlation analysis using Spearman's correlation coefficient. When the correlation coefficient between two variables was equal to or greater than 0.7, only the variable with the greatest ecological empiric relevance for the dependent variable estimation was selected for further analysis. Wildlife and human-related detection rates were estimated by records obtained by camera trapping, with the data collected exhibiting a dispersed distribution. Consequently, Generalized Linear Models (GLMs) were run with a negative binomial distribution and an identity link function, considering all combinations of explanatory variables for the detections obtained in camera trapping. After the best model (model with the lower AIC) was selected among candidate models, the adjusted R^2 was calculated to assess model fitting. Moreover, a Variance Inflation Factor (VIF) analysis was performed and only predictors with VIFs lower than 5 (Morinha *et al.* 2017) remained in the model. In this way the equations obtained in the GLM were able to be incorporated into the StDM model construction. All the statistical analysis was carried out using R software (version x64 4.1.1), using he R Commander package. For model simulations purposes, the scaling normalization of the response variables was applied in order to convert floating-point feature values from their natural range into a standard range, with a similar scale between 0 and 1, by using the formula according to Patro and Sahu (2015):

$$X_{changed} = \frac{X - X_{min}}{X_{max} - X_{min}}$$

where X represents each response variable, $X_{changed}$ the respective normalized value, X_{max} and X_{min}, the respective maximum and minimum values found in the original database.

2.4. Dynamic model conceptualization and implementation

Since the StDM protocol is based on the integration of empiric, mechanistic and correlative modelling approaches, the significant partial regression coefficients of the GLM best models were assumed also as relevant holistic ecological parameters. These coefficients reflect the overall influence of the environmental variables selected that are of ecological relevance to explain the occurrence of wolves in the areas of breeding sites. The StDM basic principle is given by the balance between the “gains” of favourable and the “losses” of detrimental environmental influences, which are mediated by the respective partial GLM coefficients (Santos *et al.* 2013). The wolf presence and abundance were estimated indirectly from the camera trapping detection of wolves. In the modelling process the main variables were organized in four chain component levels (Figure 9). The first component included dynamic variables related with several economic, social and environmental data, namely the area occupied by the principal land cover/uses. The second component was related with humans and vehicles detections by camera trapping. The third component was considered the wolf main prey or competitors detected, such as wild ungulates, domestic animals and mesocarnivores. The last component was represented by camera trapping wolf detections. From a bottom-up perspective, the first component (background socio-environmental scenario) interacts with the other three components, the second component (human presence) interacts with the third component (availability of prey and competitors) and with the fourth component (wolf occurrence), and the third component interacts with the fourth component.

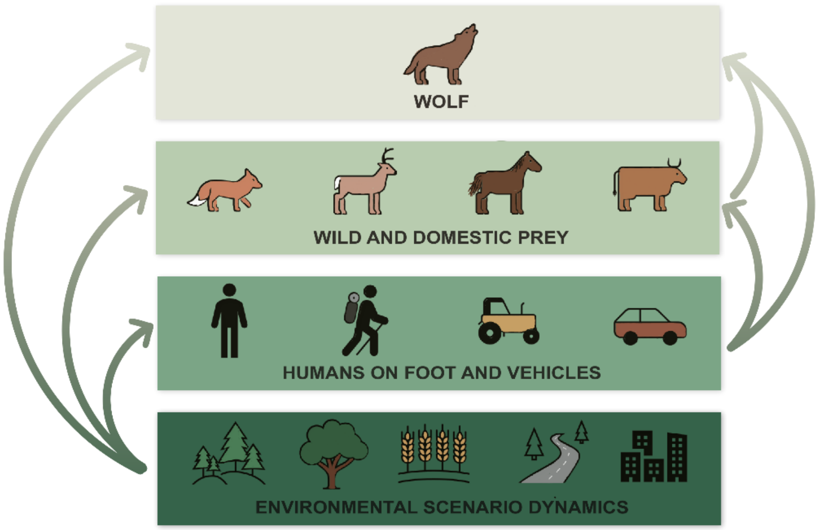

Figure 9: *Conceptual representation of the variable's organization (wolf, main prey and competitors, humans on foot and vehicles and environmental scenario dynamics) used in the modelling process.*

For this analysis the information from three sampled wolf packs was considered, taking into account the respective different ecological conditions, which reflect a gradient of human intervention between breeding sites (Figure 10): Case 1 (Peneda breeding site) has a reduced human intervention, characterized by areas of native oak forests and shrubland, located inside Peneda-Gerês National Park; Case 2 (Leomil breeding site) has an intermediate degree of human intervention, characterized by areas of pine and eucalyptus plantations as well as extensive areas of agricultural lands; Case 3 (Santa Luzia breeding site) has a high degree of human intervention, characterized by proximity to large urban areas and with a high road density, where vegetation cover is mainly dominated by invasive species (*Acacia sp.)* and forest production of eucalyptus and pine, without native oak forests.

Figure 10*: Photos from the three wolf breeding sites analysed, which reflect a gradient of human intervention: A) Case 1: Peneda breeding site; B) Case 2: Leomil breeding site; C) Case 3: Santa Luzia breeding site.*

Since fires are frequent events that can significantly shape the vegetation structure and composition with implications for wolf refuge (Lino *et al.* 2019), they were included in the dynamic model as stochastic phenomena, mediated by parameters that reproduce the fire proneness of each considered wolf breeding site, given its dominant land cover (San-Miguel-Ayanz *et al.* 2012). The post-fire succession was integrated in the land cover dynamics by using temporal rates that reproduce the number of years needed by each land cover class to reach the respective dominance (Rodrigues *et al.* 2014). Furthermore, after the calibration procedures, the average number of fires, obtained per study unit from 100 independent stochastic simulations in 10 years of simulation, was considered a reliable proxy of the regional historical trends of fire events for a period of a decade (INE, 2021) with an average value (± standard deviation) of 5.65 ± 2.55 fires from the simulations for Case 1 and Case 2 and 8.9 ± 2.56 fires from the simulations for Case 3. Finally, different scenarios were assessed, taking into account possible trends in land use inside wolf breeding sites, such as the abandonment or expansion of agriculture, and habitat management measures, namely fire mitigation measures in native oak forests and firebreak measures in shrubland areas (Table 4). A baseline scenario only influenced by post-fire succession dynamics, without land use changes and/or habitat management measures, was also considered. The overall structural-dynamic variables, combined with environmental constants regarding the positional characteristics of each study unit, allowed the simulation of trends in the use of wolves, mesocarnivores, ungulates, domestic animals and humans inside wolf breeding sites areas. The time unit chosen was the day and the simulation period was established for 10 years (expressed in days). This period was considered suitable to capture the main wildlife and human-related ongoing changes in the study area, namely those induced by the landscape and fire dynamics, as well as by the possible long term environmental management actions to mitigate the potential wolf decline in these study areas. For the development of the StDM model the software STELLA 9.0.3 was used. The original conceptual diagram, the full list of processes, parameters and equations used in the model construction are available as supplementary materials (Appendix I and II).

***Table 4**: Description of land use changes and/or habitat management measures used for the StDM modelling scenarios considered in wolf breeding sites.*

Scenarios		**Description**
Habitat management measures	Firebreak measures in shrubland areas	Mitigation of fire risk through the creation of a horizontal discontinuity in shrubland areas (fuel management ranges), decreasing the respective fire proneness.
	Fire mitigation measures in oak forests	Control of scrubland vegetable fuels in order to mitigate the risk of fire in native oak forests, decreasing the respective fire proneness.
Trends in land use	Expansion of agriculture	Increase in agricultural area, proportional to the initial agricultural area considered for each wolf breeding site.
	Abandonment of agriculture	Decrease in agricultural area, proportional to the initial agricultural area considered for each wolf breeding site.

2.5. Sensitivity analysis

In order to evaluate how changes in the main parameters of dynamic processes affected the dependent variable responses (camera trapping records) a local sensitivity analysis (SA) by one-parameter-at-a-time technique (OAT) was performed (Czitrom, 1999). For this approach, the different land cover/uses conversion parameters/rates were submitted to changes of +/− 10% and +/− 50% around the original values (Ligmann-Zielinska, 2013). The comparison between the dependent variable responses, either under the effect of those parameter changes or estimated from the respective original reference values, was expressed in percentage of each dependent variable variation, considering the simulation values obtained from the middle time of the last wolf breeding season simulated (i.e., the day 3497 of the simulation period considered). The results may be positive or negative, taking into account the trend of the selected dependent variable, representing the percentages of change in the camera trapping records between simulations with and without variation in the parameter under analysis. The percentage absolute value represents the sensitivity degree from the original reference results. In order to stabilize the model outputs for a better evaluation of the SA-OAT results, the stochasticity of the fire effects and the influence of management measures were deactivated.

3. RESULTS

3.1. Human disturbance and wildlife use in wolf key areas

3.1.1. Overall patterns

A high number of independent records of wildlife and human-related disturbances were obtained in the two study areas, namely 2009 independent records in Alto Minho and 3734 independent records in South of Douro (Table 5).

***Table 5**: Number of independent records, per year, in Alto Minho and South of Douro.*

Alto Minho		**South of Douro**	
Year	Independent records	Year	Independent records
2015	151	2015	832
2016	151	2016	219
2017	404	2017	0
2018	302	2018	959
2019	418	2019	872
2020	583	2020	852
TOTAL	**2009**	TOTAL	**3734**

In Alto Minho and South of Douro were obtained, through camera trapping, records of 10 species of wild animals (including wolves) and 14 types of sources of human disturbances, considering all key areas used by the Iberian wolf, namely breeding sites (n=133 sampling sites), feeding sites (n=9 sampling sites) and highway crossings (n=1 sampling site). Based on an overview of the data obtained in each study area (Figure 11), is evident that wolf detection rates were approximately twice as high in Alto Minho (0.0009 d.r.) compared to South of Douro River (0.0005 d.r.). Regarding wildlife records, mesocarnivores were the group with higher detection rates in both study areas (0.005 d.r. in Alto Minho; 0.004 d.r. in South of Douro), followed by wild ungulates (0.004 d.r. in Alto Minho; 0.001 d.r. in South of Douro). Among anthropogenic related disturbances, domestic animals were the group with highest detection rates, with higher values in Alto Minho (0.005 d.r. in Alto Minho; 0.004 d.r. in South of Douro), and with a similar pattern between study areas also found for vehicles (0.002 d.r. in Alto Minho; 0.001 d.r. in South of Douro) and humans on foot (0.003 d.r. in Alto Minho; 0.0005 d.r. in South of Douro).

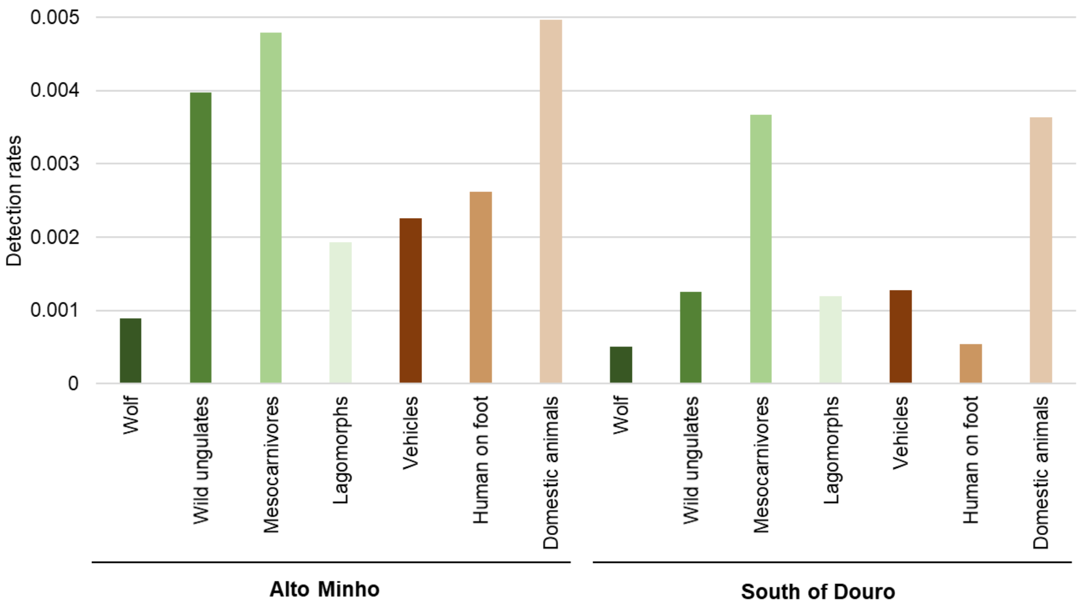

Figure 11: *Overview of detection rates (nº of detections / 100 night-traps) for each main category obtained by camera trapping in all wolf key areas in Alto Minho and South of Douro.*

A large diversity of wild mammals was detected in all sampling sites occupied by wolves, with variations in the detection rate and type of species detected in both study areas (Figure 12). All groups of wildlife, namely mesocarnivores, wild ungulates and lagomorphs, had higher detection rates in Alto Minho, being the red fox the mammal with the highest detection rate in both study areas, particularly in Alto Minho (0.004 d.r. in Alto Minho; 0.003 d.r. in South of Douro). The mesocarnivores with the second highest detection rates was the badger in South of Douro (0.00006 d.r. in Alto Minho; 0.0002 d.r. in South of Douro) and the genet in Alto Minho (0.0001 d.r. in Alto Minho; 0.00003 d.r. in South of Douro). The beech marten had higher detection rates in South of Douro (0.00008 d.r.) than in Alto Minho (0.00004 d.r.), while the wildcat was only detected in Alto Minho (0.00002 d.r. detection rates) and the mongoose only in South of Douro (0.0007 d.r. detection rates). The detection of wild ungulates inside wolf key areas in Alto Minho was approximately four times higher than in South of Douro (0.004 d.r. in Alto Minho; 0.001 d.r. in South of Douro). The species of detected ungulates includes the wild boar with detection rates three times higher in the Alto Minho (0.003 d.r. in Alto Minho; 0.001 d.r. in South of Douro), and the roe deer with detection rates almost five times higher in Alto Minho (0.0006 d.r. in Alto Minho; 0.000008 d.r. in South of Douro), being the number of records of this latter species in South of Douro highly reduced (only three records, during the last two years of sampling). Considering lagomorph species, the european rabbit had detection rates twice as high in Alto Minho (0.002 d.r.) compared to South of Douro (0.001 d.r.), while the iberian hare was only detected in South of Douro (0.0002 d.r. detection rates).

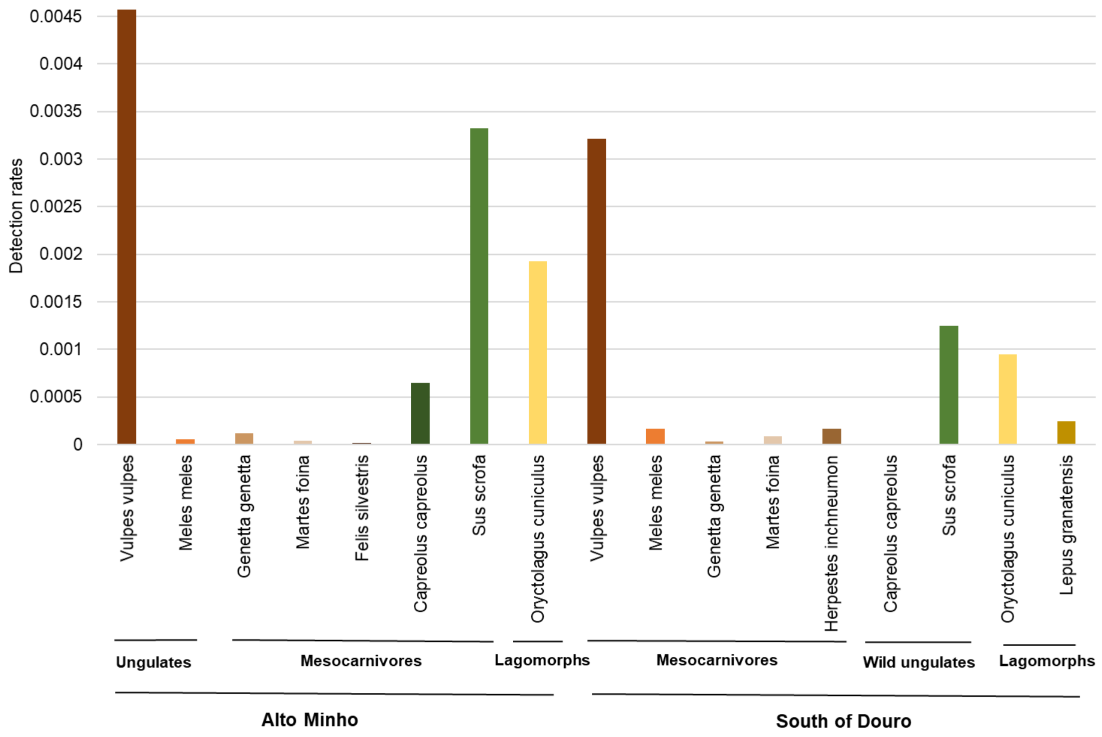

Figure 12: *Detection rates (nº of detections / 100 night-traps) for each wildlife species obtained by camera trapping considering all wolf key areas, in Alto Minho and South of Douro.*

Several human-related disturbances were also detected in all sampling sites from both study areas, with strong variations in the levels and types of disturbances between Alto Minho and South of Douro regions (Figure 13). Vehicle detection was higher in Alto Minho (0.002 d.r. in Alto Minho; 0.001 d.r. in South of Douro), comprising, mainly, passenger vehicles and four-wheel drives. The detection of humans on foot was considerably higher in Alto Minho (0.003 d.r. in Alto Minho; 0.0005 d.r. in South of Douro), highlighting the presence of people performing outdoor activities, such as trekking, with detection rates three times higher compared to South of Douro (0.001 d.r. in Alto Minho; 0.0004 d.r. in South of Douro). In relation to domestic animals, the detection of dogs was slightly higher in South of Douro (0.0009 d.r. in Alto Minho; 0.001 d.r. in South of Douro) and the presence of livestock animals was higher in Alto Minho, which include the presence of Garrano horses (*Equus caballus*) (0.002 d.r. detection rates) and cattle (*Bos taurus*) (0.002 d.r.) while in the South of Douro only sheep (*Ovis aries*) (0.002 d.r.) was detected.

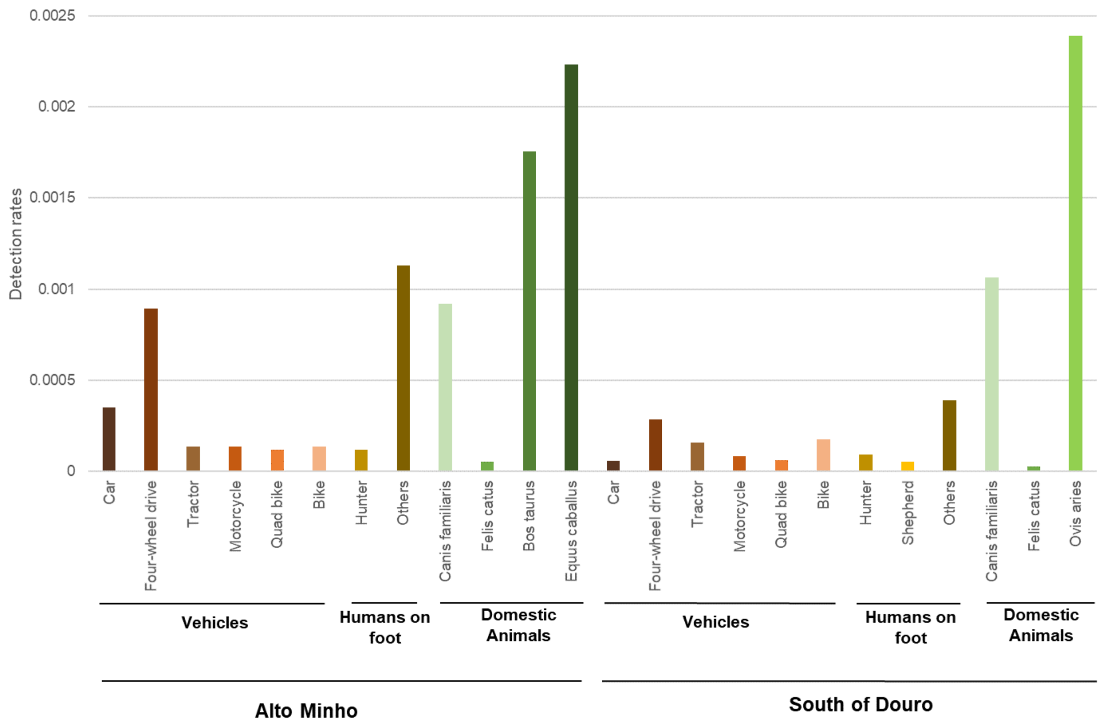

Figure 13: *Detection rates (nº of detections / 100 night-traps) for each human-related disturbances obtained by camera trapping considering all wolf key areas, in Alto Minho and South of Douro. "Others" corresponds to detections of humans on foot that do not fall under the classification of hunters or shepherd.*

Considering the different types of wolf key areas (Figure 14), breeding sites in both study areas showed similar detection rates in different categories of wildlife and human-related disturbances, highlighting the higher detection rates for mesocarnivores (0.004 d.r. in Alto Minho; 0.003 d.r. in South of Douro) and domestic animals (0.005 d.r. in Alto Minho; 0.004 d.r. in South of Douro). Different types of feeding sites were sampled in each study area, being in Alto Minho characterized by kill sites from wolf predation on livestock, while in South of Douro were related to dumping sites from intensive farming of domestic animals. Thus, the feeding sites in Alto Minho have higher detection rates of wild ungulates (0.01 d.r. in Alto Minho; 0.0003 d.r. in South of Douro), while in South of Douro the higher detection rates are mesocarnivores (0.0057 d.r. in Alto Minho; 0.007 d.r. in South of Douro). In the crossing structure of a highway sampled in Alto Minho only wolves (0.001 d.r.) and vehicles (0.01 d.r.) were detected.

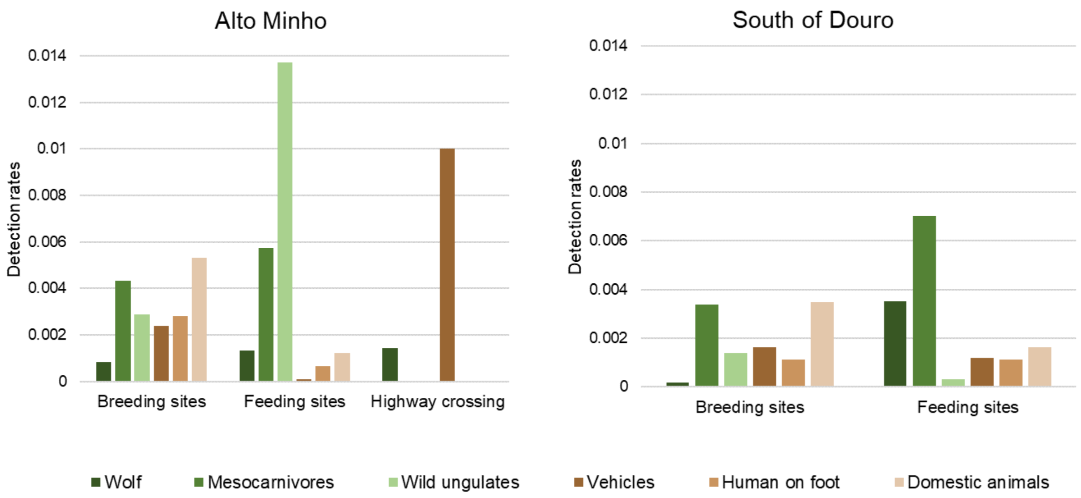

Figure 14: *Detection rates (nº of detection / 100 night-traps) for wildlife groups and human-related disturbances in each wolf key area in Alto Minho and South of Douro.*

3.1.2. Feeding sites and highway crossing structure

In the sampled feeding sites in each study area, besides wolves the only wild species detected were red fox (0.02 d.r. in Alto Minho; 0.007 d.r. in South of Douro) and wild boar (0.04 d.r. in Alto Minho; 0.0003 d.r. in South of Douro) as shown in Figure 15.

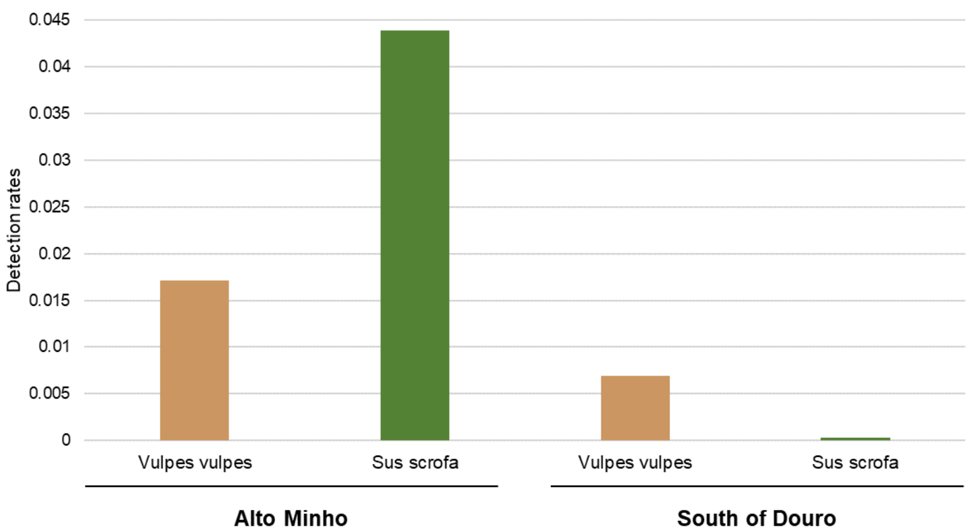

Figure 15: *Detection rates (nº of detections / 100 night-traps) for each wildlife species obtained by camera trapping in wolf feeding sites in Alto Minho and South of Douro.*

In Alto Minho the human-related disturbances detected in wolf feeding sites (Figure 16) comprised outdoor activities, such as trekking (0.0006 d.r.) and the presence of domestic animals as dogs (0.002 d.r.), cattle (0.001 d.r.) and horses (0.0003 d.r.). In wolf feeding sites from South of Douro, besides the detection of humans doing outdoor activities (0.0007 d.r.) and domestic animals as dogs (0.002 d.r.) and cats (0.00003 d.r.), vehicles were also detected, namely Four-wheel drives (0.0003 d.r.) and tractors (0.0007d.r.).

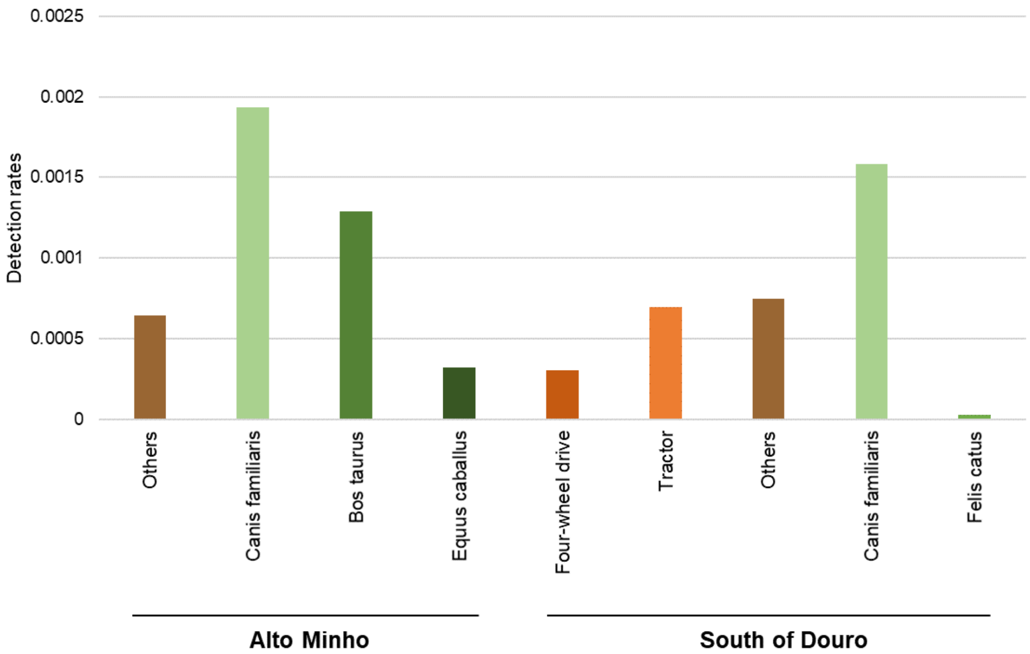

Figure 16: *Detection rates (nº of detections / 100 night-traps) for each human-related disturbances obtained by camera trapping in wolf feeding sites in Alto Minho and South of Douro. "Others" corresponds to detections of humans on foot that do not fall under the classification of hunters or shepherd.*

Regarding regional variation among sampled wolf packs (Figure 17), camera trapping data in feeding sites only detected wolf presence in Boulhosa, Vez and Leomil. Arga and Leomil showed a greater detection of mesocarnivores, while Boulhosa, Vez and Trancoso had more detection of wild ungulates. Considering human-related detections, vehicles were only observed in Leomil, humans on foot were detected in Vez, Leomil and Trancoso, and domestic animals in Boulhosa, Vez, Leomil and Trancoso.

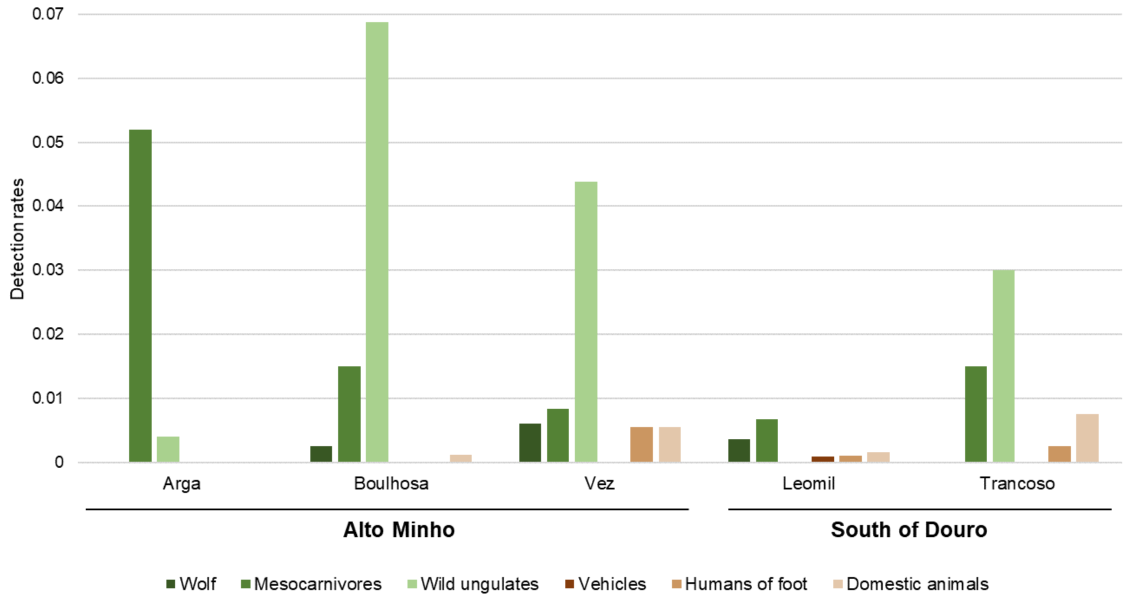

Figure 17: *Regional variation in detection rates (nº of detections / 100 night-traps) for the main categories of wildlife and human-related disturbances obtained by camera trapping in wolf feeding sites in Alto Minho and South of Douro.*

Considering the detection rates along the circadian cycle in wolf feeding sites (Figure 18), in Alto Minho, wildlife species presented two peaks of occurrence during the night period, mainly between 19h and 22h and between 2h and 4h. The detection of humans and domestic animals in Alto Minho were dispersed but mostly concentrated during daytime, showing a higher detection rate of humans between 14h and 17h and domestic animals between 7h and 8h. The detection of wildlife species in South of Douro was higher between late afternoon and early morning, from 17h to 7h. Human detection in South of Douro occurred mostly during the day, following a similar pattern with the detection of domestic animals.

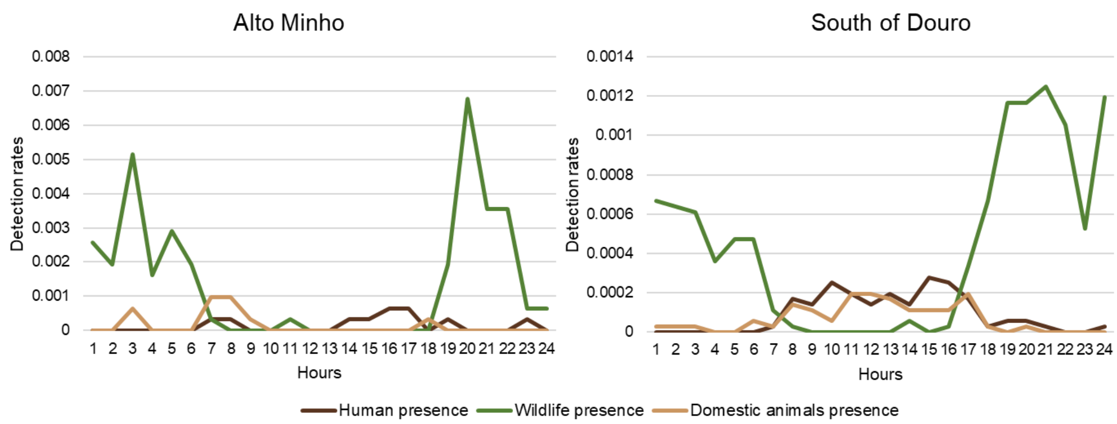

Figure 18: *Circadian variation in detection rates (nº of detections / 100 night-traps) for wildlife, human presence and domestic animals obtained by camera trapping, in wolf feeding sites in Alto Minho and South of Douro*.

Regarding seasonal variations (Figure 19), in Alto Minho wolf feeding sites were only sampled between August and October, showing a decrease in wild species detections and an increase in human detections during this period. In feeding sites from South of Douro, the detection of wild species was greater during summer and autumn months and the detection of humans and domestic animals was higher between July and January.

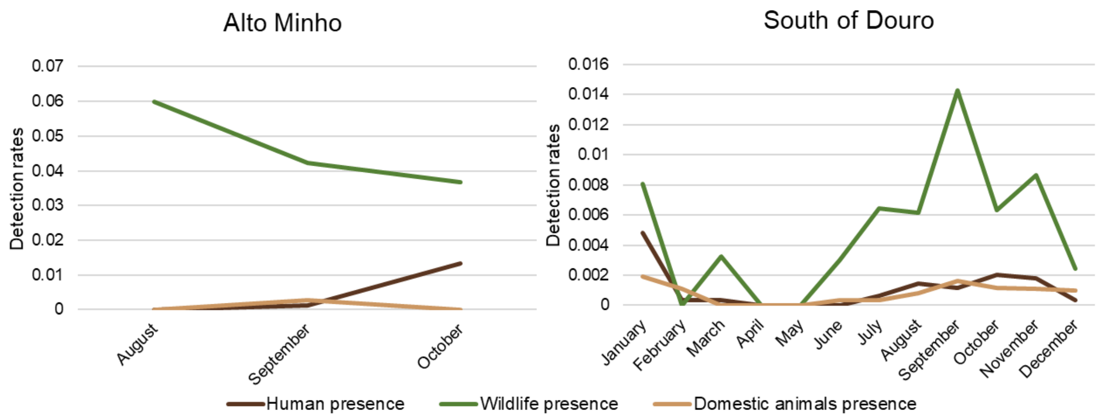

***Figure 19**: Monthly variation in detection rates (nº of detections / 100 night-traps) for wildlife, human presence and domestic animals, in wolf feeding sites in Alto Minho and South of Douro.*

The only crossing structure comprising a local road under A28 highway, within the territory of Santa Luzia pack in Alto Minho, was sampled during August and showed that wolf detections occurred at night (23h), while vehicles were detected during afternoon, between 15h and 18h (Figure 20).

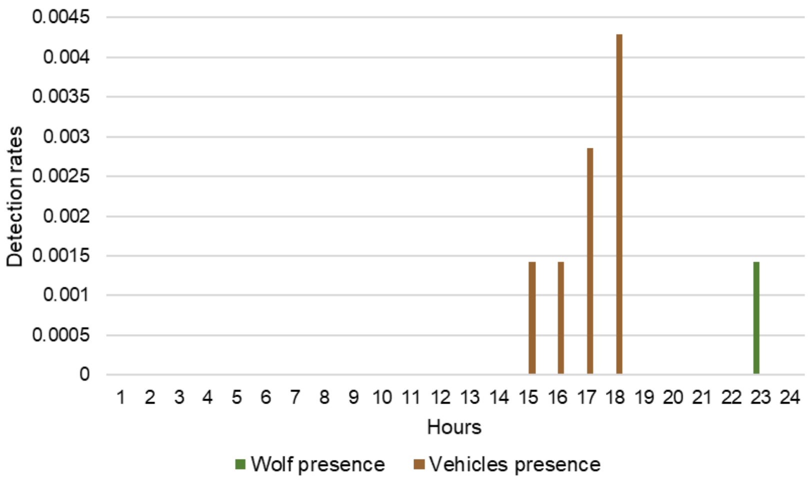

***Figure 20:** Circadian variation in detection rates (nº of detections / 100 night-traps) for wolf and vehicles obtained by camera trapping, in a highway crossing structure in Alto Minho.*

3.1.3. Breeding sites

In the areas used as breeding sites by wolves, a great diversity of wild mammals was detected during the breeding season (Figure 21). Regarding mesocarnivores, red fox had the highest detections in the two study areas (0.004 d.r in Alto Minho; 0.003 d.r in South of Douro). Badger and beech marten had more detection rates in South of Douro (Badger: 0.00006 d.r in Alto Minho; 0.0002 d.r in South of Douro; beech marten: 0.00004 d.r in Alto Minho; 0.00009 d.r in South of Douro) while genet had more detection rates in Alto Minho (0.0001 d.r in Alto Minho; 0.00005 d.r in South of Douro). The european wild cat was only detected in wolf breeding sites from Alto Minho (0.00002 d.r.) while mongoose was only found in South of Douro (0.0002 d.r.). In wolf breeding sites from Alto Minho region, higher detection rates were obtained in the two species of wild ungulates (0.002 d.r. of wild boar; 0.0006 d.r. of roe deer) compared to South of Douro region (0.001 d.r. of wild boar; 0.000007 d.r. of roe deer). Considering the lagomorphs, european rabbit had higher detection rates in Alto Minho (0.002 d.r in Alto Minho; 0.00086 d.r in South of Douro) and iberian hare was only detected in South of Douro (0.0002 d.r.).

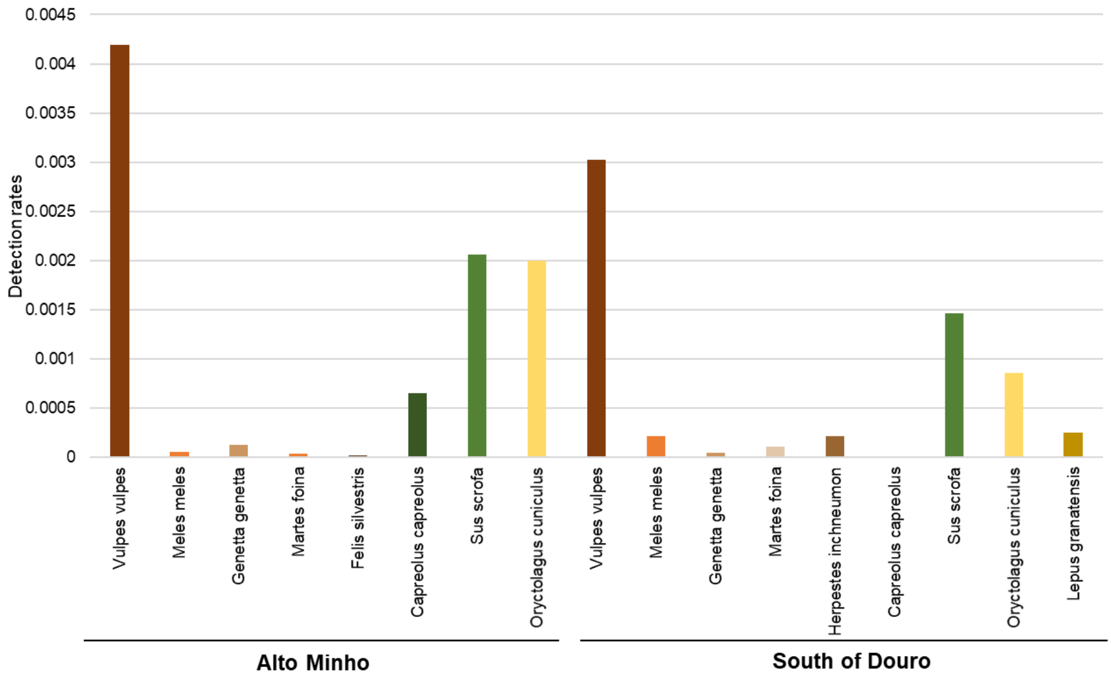

***Figure 21**: Detection rates (nº of detections / 100 night-traps) for each wildlife species obtained by camera trapping inside wolf breeding sites in Alto Minho and South of Douro.*

The human-related disturbances detected were higher in breeding sites at Alto Minho than in South of Douro (Figure 22). The detection of different classes of vehicles was greater in Alto Minho (0.004 d.r. of cars; 0.0009 d.r. of four wheel drives; 0.0001 d.r. of motorcycles; 0.0001 d.r. of quad bikes; 0.0001 d.r. of bikes), excluding the detection of bicycles, which had greater detections in South of Douro (0.00007 d.r. of cars; 0.0003 d.r. of four wheel drives; 0.0001 d.r. of motorcycles; 0.000007 d.r. of quad bikes; 0.0002 d.r. of bikes). The detection of different classes of human on foot was also greater in Alto Minho (0.0001 d.r of hunters; 0.001 d.r. of others), with only shepherds being detected in South of Douro (0.00009 d.r of hunters; 0.00004 d.r of shepherds; 0.0004 d.r. of others). Regarding domestic animals, detections of cats were higher in Alto Minho (0.00006 d.r in Alto Minho and 0.00003 d.r in South of Douro) while detection of dogs was higher in South of Douro (0.0009 d.r in Alto Minho and 0.001 d.r in South of Douro). Finally, the livestock species detected inside wolf breeding sites consisted of *Equus caballus* (0.002 d.r.) and *Bos taurus* (0.002 d.r.) in Alto Minho and *Ovis aries* (0.003 d.r.) in the South of Douro.

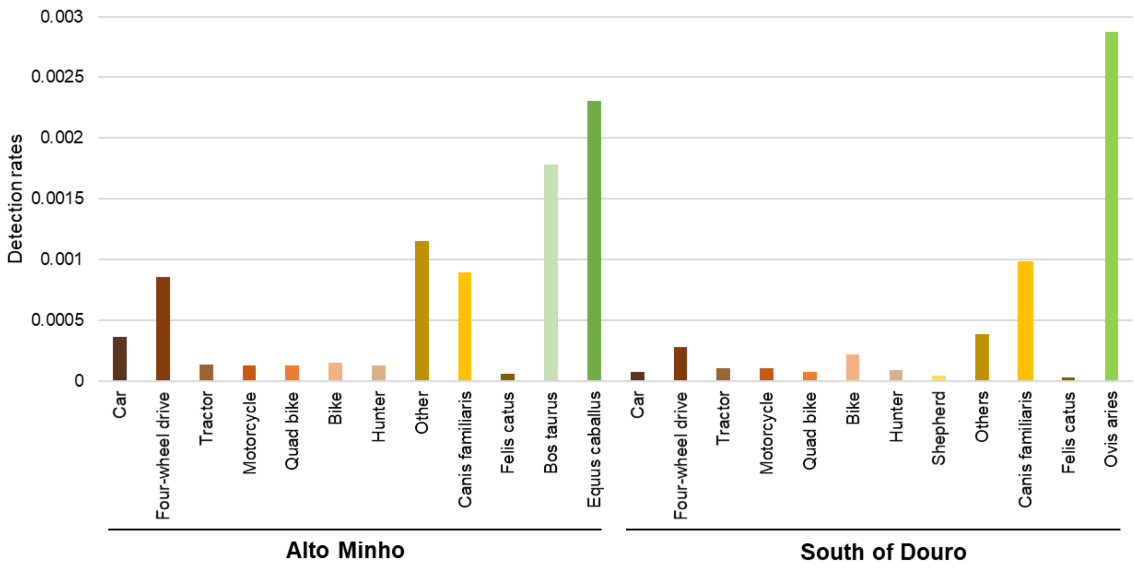

Figure 22: *Detection rates (nº of detections / 100 night-traps) for each human-related disturbances obtained by camera trapping inside wolf breeding sites in Alto Minho and South of Douro. "Others" corresponds to detections of humans on foot that do not fall under the classification of hunters or shepherd.*

There were regional variations in detections between the different breeding sites for each sampled pack (Figure 23). In Alto Minho region, the breeding site with the greatest detection of wolves was Santa Luzia. The Vez breeding site had higher detection rates of mesocarnivores, wild ungulates, vehicles and humans on foot. Then, the Cruz Vermelha breeding site had higher detection rates of domestic animals. In

South of Douro, Leomil had the breeding site with higher detection rates of wolf and mesocarnivores. Lapa breeding site had higher detection rates of wild ungulates and vehicles. Finally, Trancoso breeding site, had higher detection rates of humans on foot and domestic animals.

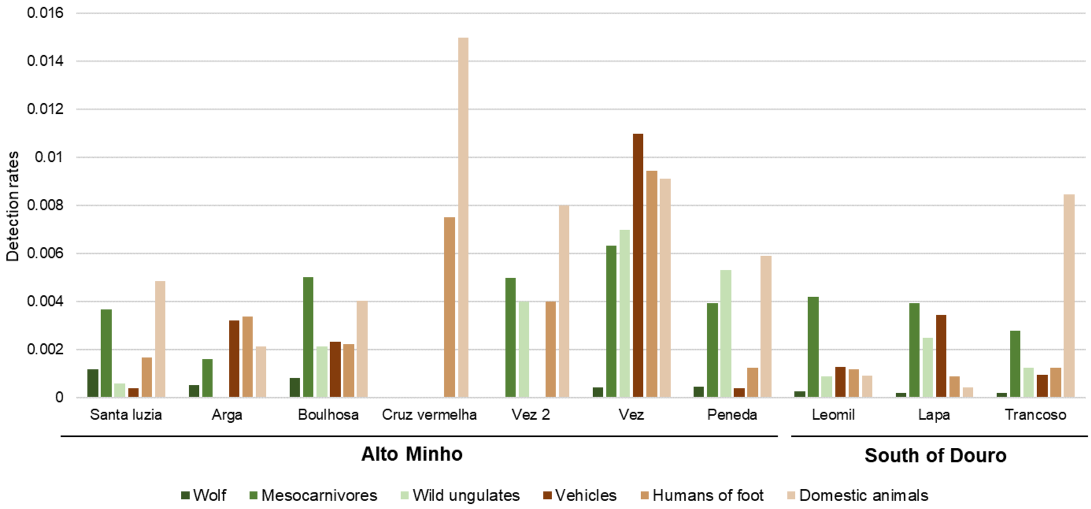

Figure 23: *Regional variation in detection rates (nº of detections / 100 night-traps) for the main categories of wildlife and human-related disturbances obtained by camera trapping, considering each pack breeding site in Alto Minho and South of Douro*.

The spatial variations between sampled breeding sites located inside and outside protected areas in Alto Minho (Figure 24), showed higher detection rates of wolves and mesocarnivores outside protected areas while higher detection rates of wild ungulates were inside protected areas. Detections of vehicles were higher outside protected areas but humans on foot and domestic animals had higher detection rates inside protected areas.

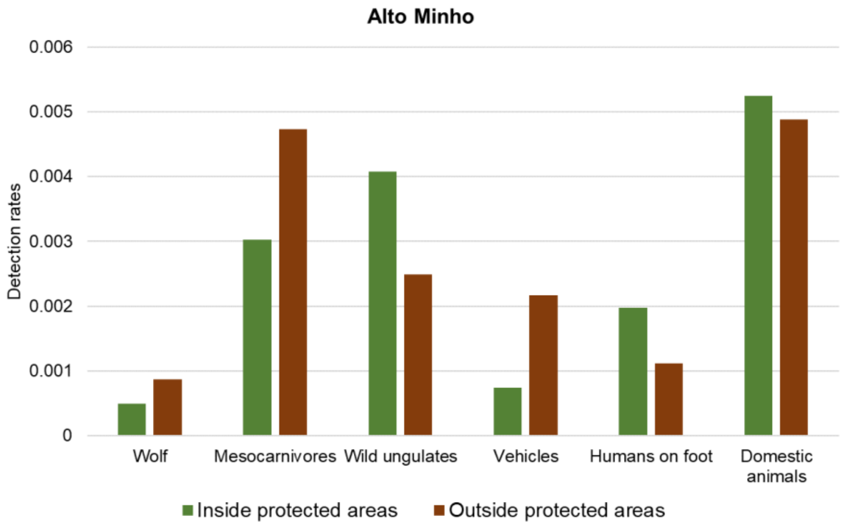

Figure 24: *Variation in detection rates (nº of detections / 100 night-traps) for the main categories of wildlife and human-related disturbances obtained by camera trapping, considering inside and outside protected areas in Alto Minho.*

Regarding the detection rates along the circadian cycle in the two study areas (Figure 25), wildlife species occurred mainly during the period between 19h/20h and 7h/8h, showing a greater detection of activity during the night and early morning. In contrast, the detection of human presence was higher during daytime, between 8h/9h and 19h/20h. The detection of domestic animals in Alto Minho occurred mostly during daytime following a similar pattern with the detection of human presence. In the case of South of Douro, there were also more detections of domestic animals during day, showing peaks of detection due to the passage of large herds of sheep (see Figure 22).

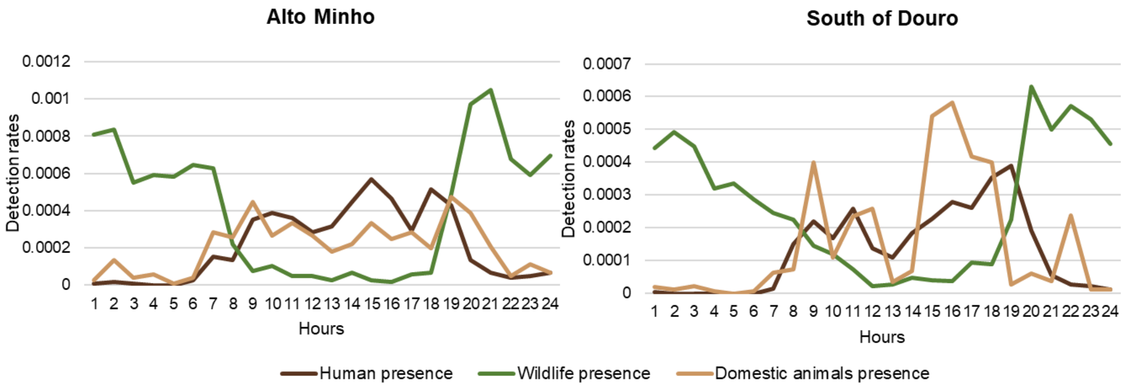

Figure 25: *Circadian variation in detection rates (nº of detections / 100 night-traps) for wildlife, human presence and domestic animals obtained by camera trapping, inside wolf breeding sites in Alto Minho and South of Douro*.

Regarding seasonal variations throughout pup rearing season (July-October) (Figure 26), in Alto Minho the detection of wildlife decreased between July and August and increased between August and October, while domestic animals showed an increase along the assessed season. The detection of human presence had also a decrease in the early pup-rearing season and an increase between August and September. In South of Douro the presence of wildlife was higher during August and September, while a sharp decrease in detection of domestic animals was registered in August. The human presence had an increase between July and August, continuing approximately constant in the remaining months.

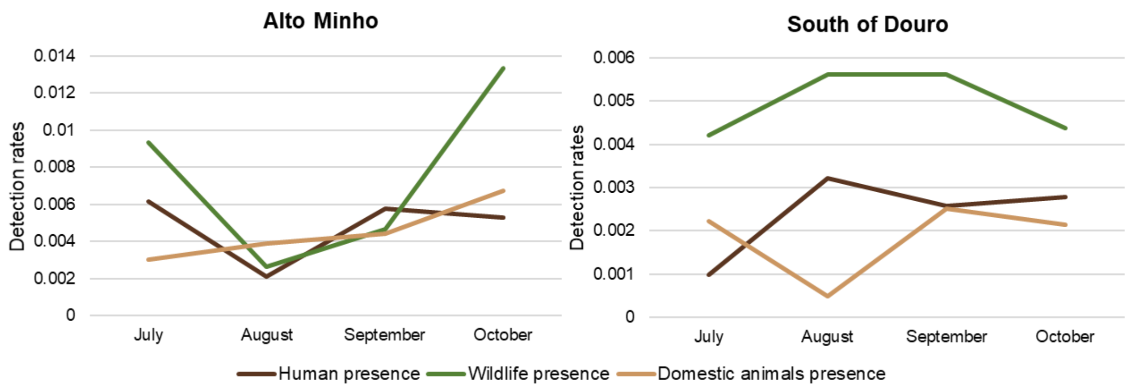

Figure 26*: Monthly variation during pup rearing season (July-October) in detection rates (nº of detections / 100 night-traps) for wildlife, human presence, and domestic animals, inside wolf breeding sites in Alto Minho and South of Douro.*

Regarding variation on detection rates inside wolf breeding sites over the sampled years (2015-2020), wildlife detections fluctuated, showing a recent decrease in both study areas. Human presence remained approximately constant in both study areas, except in the year 2020 for Alto Minho region, with an evident increase in detection rates. The yearly detection rates of domestic animals inside wolf breeding sites had wide fluctuations, showing in the last few years (since 2018) an increasing trend in Alto Minho and a sharp decrease in South of Douro (Figure 27).

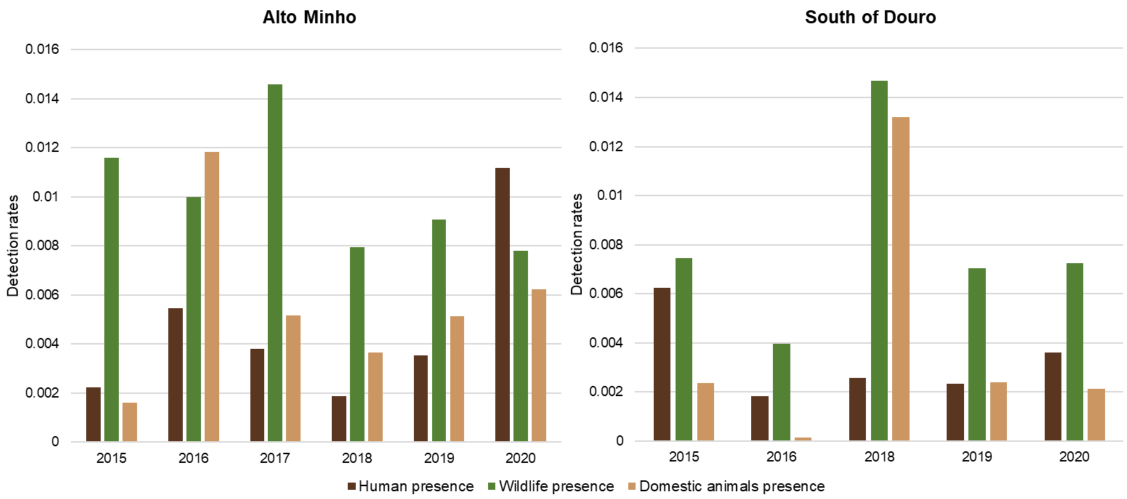

Figure 27: *Yearly variation in detection rates (nº of detections / 100 night-traps) for wildlife, human presence and domestic animals obtained by camera trapping, inside wolf breeding sites in Alto Minho and South of Douro.*

3.2. Spatiotemporal trends on the use of wolf breeding sites

3.2.1. Determinant variables in wolf breeding site use

According to the best model selection, the records obtained by camera trapping (expressed by the number of wildlife and human-related detections) were influenced by the independent variables presented in Table 6. Wolf detections inside breeding sites were positively related with mesocarnivores, humans and vehicles detections, as well as with the distance from wind farms, dirt roads, agriculture, oak forests, and eucalyptus plantations, while negatively related with domestic animals detections, paved roads, and pine plantations. Although the equation of mesocarnivores has the lowest explanatory power, which may result from its opportunistic/generalist behaviour, with a transversal use across all the gradient of land use/cover in the study areas, this group was positively associated with paved roads, agriculture and shrubland, while negatively related with altitude, urban areas, wind farms and pine species. The wild ungulates were positively associated with wind farms, agriculture, oak species, pine species and open areas, while negatively related with humans and vehicles, altitude, urban areas, invasive vegetation and shrublands. The domestic animals were positively related with other hardwood species and invasive species and negatively with altitude, urban areas, and dirt roads. Finally, humans and vehicles were positively related with altitude, quarries, highways, paved roads, dirt roads, oak forests, and invasive vegetation, and negatively with other hardwoods species, pine species and eucalyptus (Table 6).

Table 6 *– Beta value (β) and confidence interval (CI) of explanatory variables from selected GLMs for the detections obtained by camera trapping. sample size (N); adjusted coefficient of determination (R^2). "n.a."- not applicable since the independent variable was not selected.*

	Wolf (N=1028)		**Mesocarnivores** (N=1973)		**Wild ungulates** (N=727)		**Domestic animals** (N=753)		**Humans on foot and vehicles** (N=807)	
	R^2=0.26		R^2=0.02		R^2=0.22		R^2=0.25		R^2=0.25	
Explanatory variables	β	CI	β	CI	β	CI	β	CI	β	CI
Open areas	n.a.	n.a.	n.a.	n.a.	6,01E-07	8,18E-08	n.a.	n.a.	n.a.	n.a.
Shrubland	n.a.	n.a.	4,73E-08	1,49E-08	-9,44E-08	3,73E-08	n.a.	n.a.	n.a.	n.a.
Oak species	4,24E-07	1,25E-07	n.a.	n.a.	2,24E-07	7,73E-08	n.a.	n.a.	1,57E-07	7,37E-08
Other hardwood species	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	1,37E-06	3,87E-08	-8,41E-07	1,48E-07
Pine species	-1,69E-07	7,44E-08	-4,78E-10	2,13E-10	6,22E-10	3,81E-10	n.a.	n.a.	-3,97E-09	3,53E-09
Eucalyptus	7,92E-07	1,39E-07	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	-9,24E-07	1,70E-07
Invasive species	n.a.	n.a.	n.a.	n.a.	-4,89E-06	1,47E-06	2,75E-07	1,69E-07	5,10E-06	6,60E-07
Agriculture	3,54E-07	1,25E-07	1,06E-07	3,31E-08	5,76E-07	8,49E-08	n.a.	n.a.	n.a.	n.a.
Urban areas	n.a.	n.a.	-2,63E-07	7,37E-08	-9,52E-07	4,42E-07	-1,88E-06	1,76E-07	n.a.	n.a.
Highways	n.a.	n.a.	0.00E+00	0	n.a.	n.a.	n.a.	n.a.	2,13E-04	5,62E-05
Paved roads	-1,29E-04	3,20E-05	2,16E-05	8,02E-06	n.a.	n.a.	n.a.	n.a.	4,21E-05	1,77E-05
Dirt roads	2,00E-05	1,27E-05	0	0	n.a.	n.a.	-1,27E-04	3,11E-06	3,22E-05	8,46E-06
Quarries	n.a.	n.a.	0	0	n.a.	n.a.	0	0	2,87E-06	5,70E-07
Wind farms	6,61E-02	3,26E-02	-1,21E-02	7,55E-03	1,27E-01	1,53E-02	0	0	0	0
Altitude	n.a.	n.a.	-6,41E-04	2,02E-04	-2,97E-03	5,90E-04	-2,73E-03	2,28E-04	2,52E-03	5,08E-04
Mesocarnivores	2,30E-01	8,94E-02	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Ungulates	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Domestic animals	-3,62E-02	3,72E-02	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Humans and vehicles	1,89E-01	5,84E-02	n.a.	n.a.	-1,73E-01	5,81E-02	n.a.	n.a.	n.a.	n.a.

3.2.2. Trends on wolf breeding site use considering post-fire succession dynamics

For demonstration purposes, model simulations of detection trends were performed for wolves, mesocarnivores, wild ungulates, domestic animals, and humans in the surveyed breeding sites from 3 packs occurring at different ecological settings: Leomil, Santa Luzia and Peneda. Overall, since each single simulation seemed to be strongly influenced by the stochastic pattern of fire events, the average local use was projected for all dependent variables obtained through camera trapping, from 100 independent simulations, in order to capture more consistent trends for each study wolf breeding site (figures 28, 29, 30, 31 and 32).

Under the baseline scenario (only influenced by post-fire succession dynamics, without land use change scenarios and/or habitat management measures), the Leomil breeding site, despite having a smaller wolf´s use in the beginning of the simulation, is the only case where an increase in the detection rates is predicted (increase of 7% in 10 years). In Peneda and Santa Luzia, is expected a decrease in wolf use of the breeding sites (decrease of 2% and 10% in 10 years, respectively). In the case of Santa Luzia, although having higher initial predicted detection values of wolf use, it is where the biggest relative decrease is estimated (Figure 28).

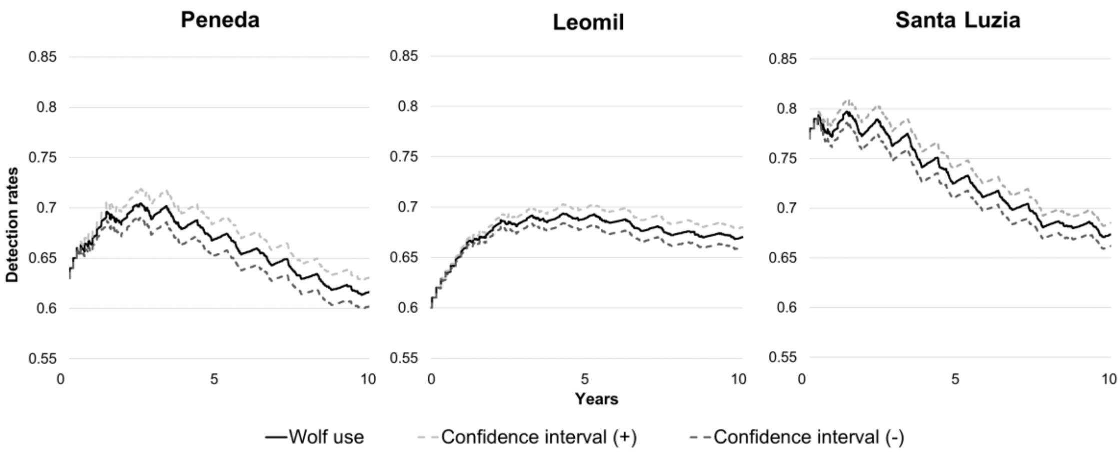

Figure 28*: Average model simulations of wolf use trends, with the respective 95% confidence limits, from 100 independent simulations, for the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years (without land use change scenarios and habitat management measures).*

Regarding mesocarnivore use (Figure 29), the initial predictions for the Peneda breeding site display less use for these species, also showing a greater decrease over the years (decrease of 24% in 10 years). The Leomil and Santa Luzia breeding sites correspond to the places with better initial predictions for mesocarnivore use, however a decrease in both sites is also expected (decrease of 20% in 10 years in both sites).

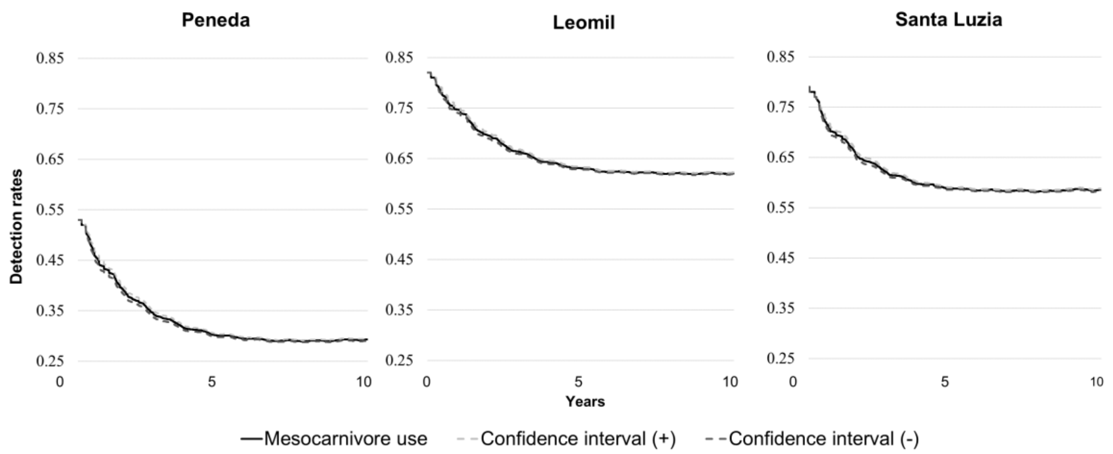

Figure 29*: Average model simulations of mesocarnivore use trends, with the respective 95% confidence limits, from 100 independent simulations, for the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years (without land use change scenarios and habitat management measures).*

After a steep initial decrease of wild ungulates' detection at the three sites in the first 3-4 years, an increase is predicted at all sites until the end of the simulation period. Wild ungulates have higher early predictions in the Leomil breeding site, however the ungulate use of this site, at the end of the simulation period, is still about 14% lower than the actual use. In the Peneda breeding site, a lower value of use of wild ungulates is also expected (decrease of 13% in 10 years). In Santa Luzia, despite presenting smaller predictions values, it is the only case where a higher wild ungulate use is expected, compared with the initial use (increase of 7% in 10 years) (Figure 30).

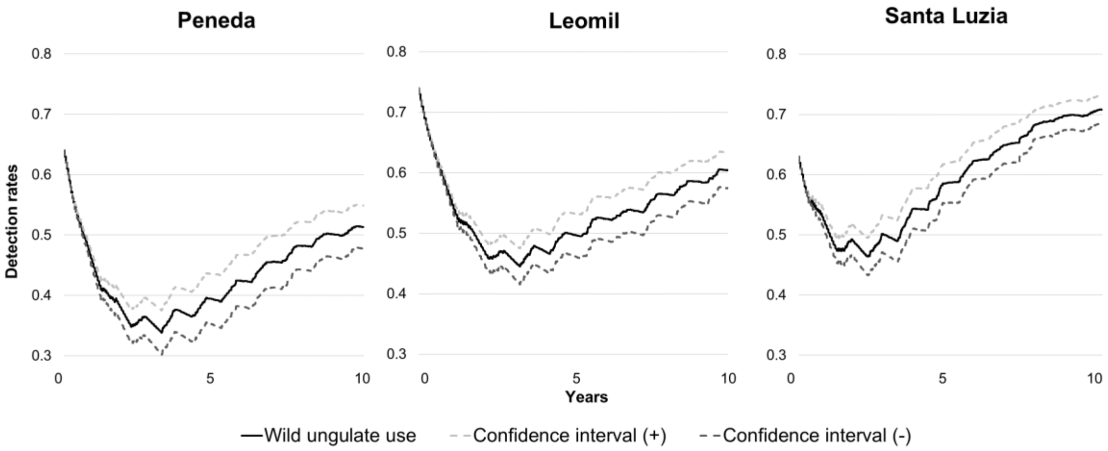

Figure 30*: Average model simulations of wild ungulate use trends, with the respective 95% confidence limits, from 100 independent simulations, for the 3 case studies corresponding to the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years (without land use change scenarios and habitat management measures).*

The use of domestic animals is similar at the three sites, with a small increase in the first years and a smooth decline until the end of the simulation period. However, the Peneda breeding site is the only area where an increase in domestic animal use is predicted (increase of 3% in 10 years). In Leomil, it is expected a lower use of domestic animals at the end of the simulation period, comparing with the use at the beginning (decrease of 1% in 10 years). Finally, Santa Luzia, despite the small increase in the initial years, in the remaining predicted timespan the trends fluctuate around initial occurrence values, without substantial changes (Figure 31).

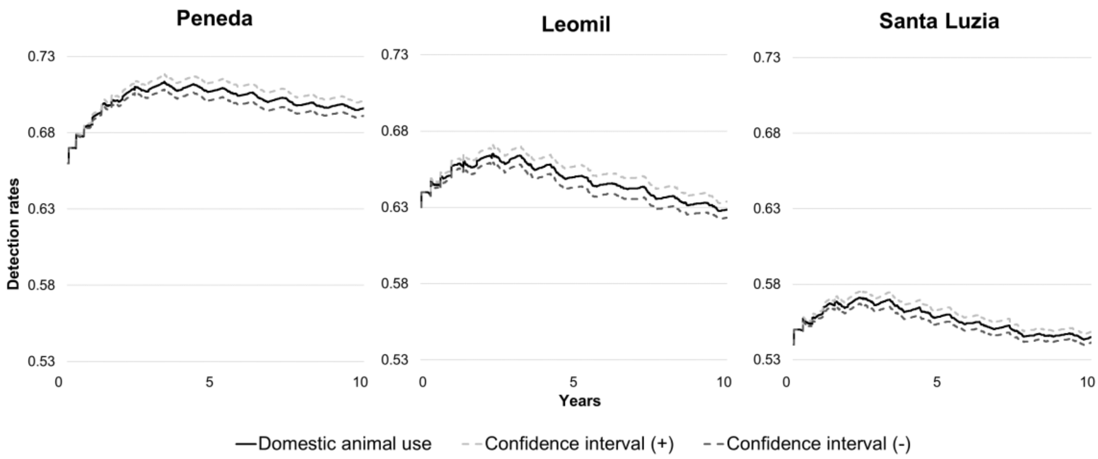

Figure 31: *Average model simulations of domestic animal use trends, with the respective 95% confidence limits, from 100 independent simulations, for the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years (without land use change scenarios and habitat management measures).*

Finally, the use of humans on foot and vehicles is expected to increase sharply in the first years of the simulation with a subsequent smooth decline in all the three studied breeding sites. Leomil is the area where the greatest variation is expected (increase of 24% in Peneda, 30% in Leomil and 22% in Santa Luzia, throughout a simulation period of 10 years) (Figure 32).

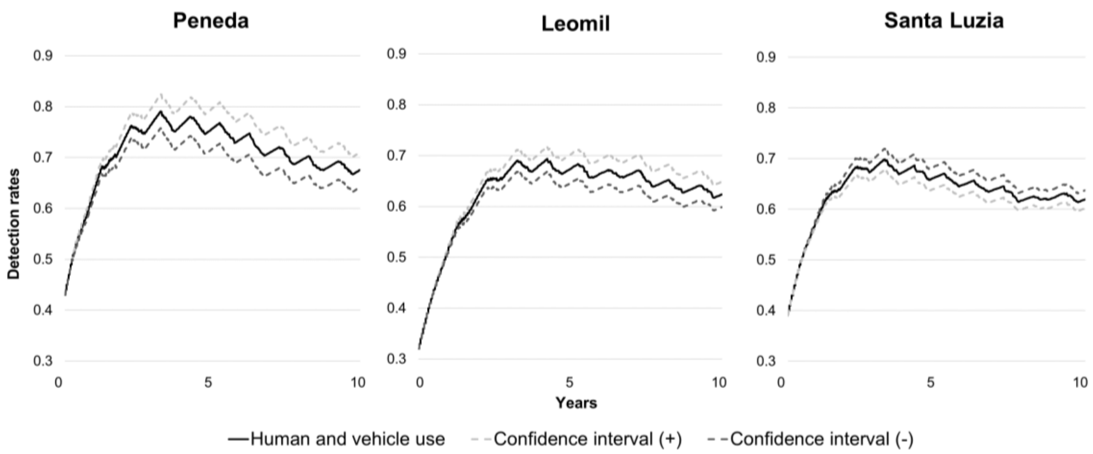

Figure 32: *Average model simulations of humans and vehicle use trends, with the respective 95% confidence limits, from 100 independent simulations, for the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years (without land use change scenarios and habitat management measures).*

Considering the integration of all use records per breeding site, in Peneda and Leomil the increase in human presence coincides with the increase of domestic animal and wolf use, and with a decrease in mesocarnivore and wild ungulate use. In contrast, the Santa Luzia breeding site reveals that the increase in human presence coincides with the stabilization of domestic animal use and the decrease of wolf, mesocarnivore and wild ungulate uses (figure 33).

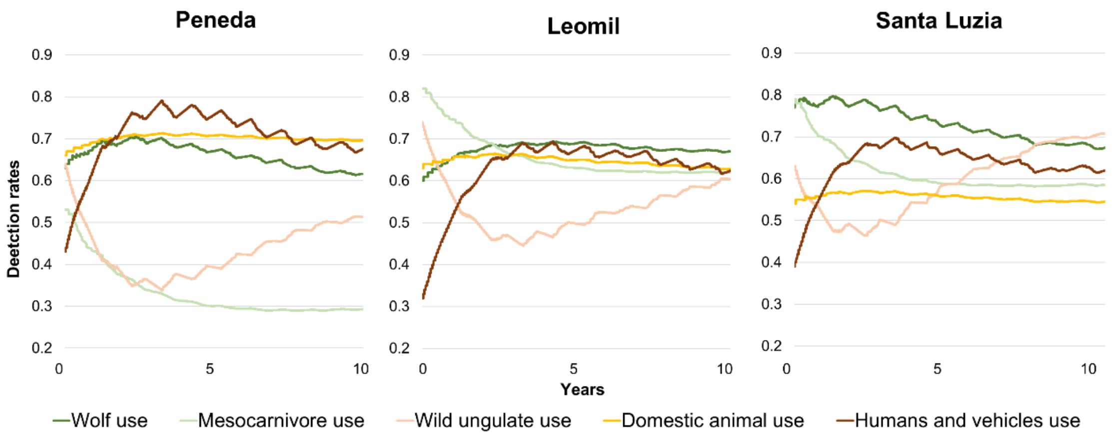

***Figure 33**: Comparison between average model simulations of wolf use, mesocarnivore use, wild ungulate use, domestic animal use and humans and vehicles use trends, with the respective 95% confidence limits, from 100 independent simulations, for the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years (without land use change scenarios and habitat management measures).*

All the background simulations of land use dynamics associated with the response variable trends shown above are available in Appendix III, IV and V.

3.2.3. Trends on wolf breeding site use under simulated changes of land use and habitat management

Considering possible contrasting land use scenarios, mainly induced by changes on agricultural activity, and the simulated effects of measures for habitat management to mitigate fire risk in the three packs studied (Appendix III), different combinations of environmental influences exhibited distinct consequences in the use trends, depending on each specific breeding site's context. Regarding wolf use (Figure 34), in the Peneda breeding site the fire mitigation measures in areas of oak forests and firebreak measures in shrubland appear to be adequate habitat management options to help mitigate the expected overall decline in use of this breeding site by wolves. In the Leomil breeding site, the wolf seems to benefit mainly from the expansion of agricultural activities and fire mitigation measures in shrublands. In both cases, the abandonment of agriculture appears to intensify the wolf´s decline. In the Santa Luzia breeding site, the implementation of mitigation measures for fires in oak forests and firebreak measures in shrubland also seems to benefit the wolf´s use scenario. In this last case agriculture expansion showed a small decrease of wolf use when comparing with the baseline scenario.

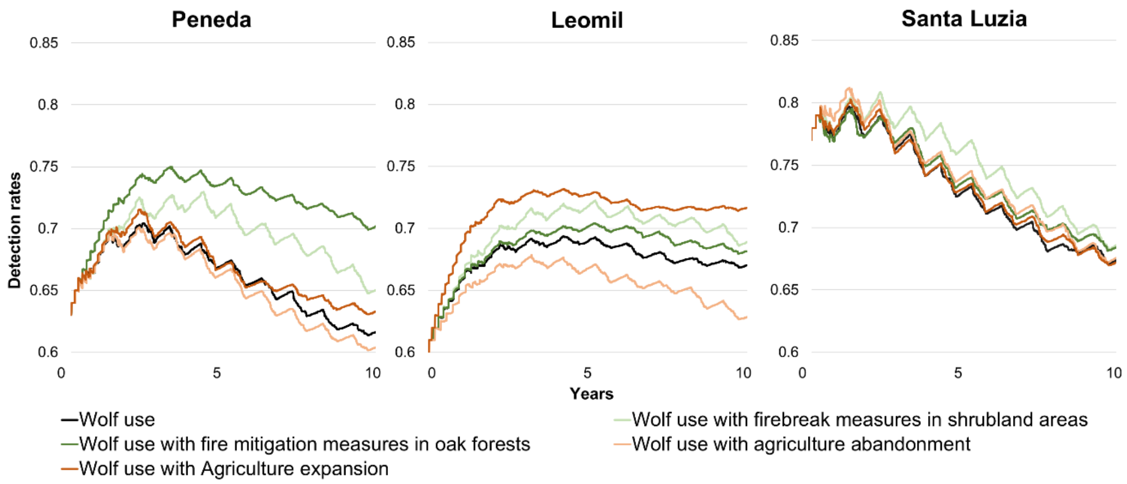

Figure 34: *Average model simulations of wolf use trends, considering possible scenarios of agricultural land use change and habitat management implementation, in the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years. "Wolf use" corresponds to the baseline scenario.*

The simulations for the mesocarnivore use (Figure 35) in the Peneda and Santa Luzia breeding sites show a declining trend in both cases. However, this trend becomes less sharp when management of shrubland with firebreak measures are applied. In the Leomil breeding site, the expansion of agriculture suggests reverting the decreasing trends of mesocarnivore use, being the only scenario where it tends to increase. The abandonment of agriculture seems to have adverse effects on these species in the 3 cases studied, accentuating its decline even further (especially in the Leomil breeding site).

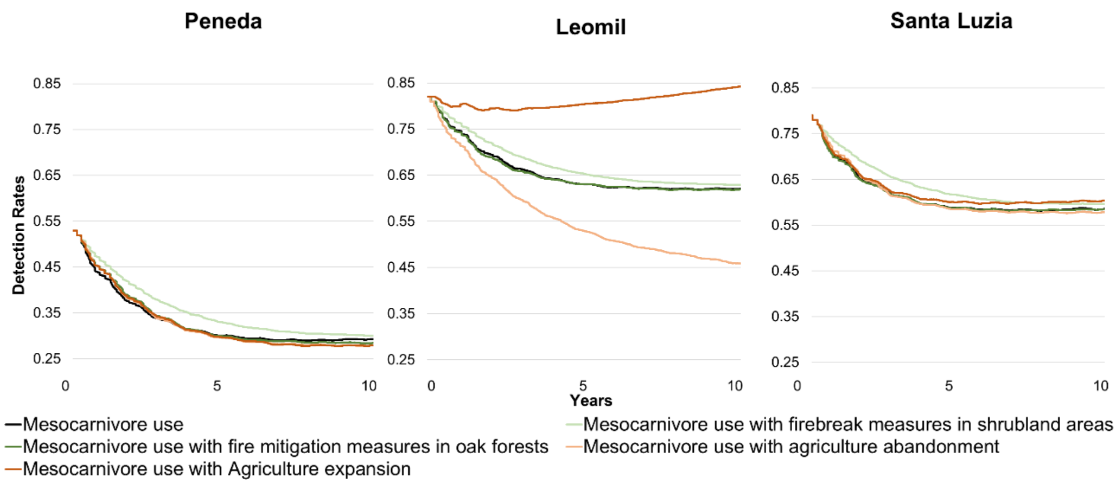

Figure 35: *Average model simulations of mesocarnivore use trends, considering possible scenarios of agricultural land use change and habitat management implementation, in the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years. "Mesocarnivore use" corresponds to the baseline scenario.*

The simulations for wild ungulate use (Figure 36) show that in the Peneda breeding site the baseline scenario, without alterations in agricultural trends and habitat management, seems to be the most favourable. In contrast, a higher decrease in wild ungulate use is predicted with application of firebreak measures in shrubland. At the Leomil breeding site, the expansion of agriculture shows a substantial benefit for wild ungulates, promoting an increase in the use of this group in the next 10 years. Finally, in the Santa Luzia breeding site the expansion of agriculture and the mitigation of fires in areas of oak forests favour the use by wild ungulates. In these last two packs (Leomil and Santa Luzia), the abandonment of agriculture and the implementation of firebreak measures in shrublands seemed to be harmful scenarios leading to an inferior use by wild ungulates than with the baseline scenario.

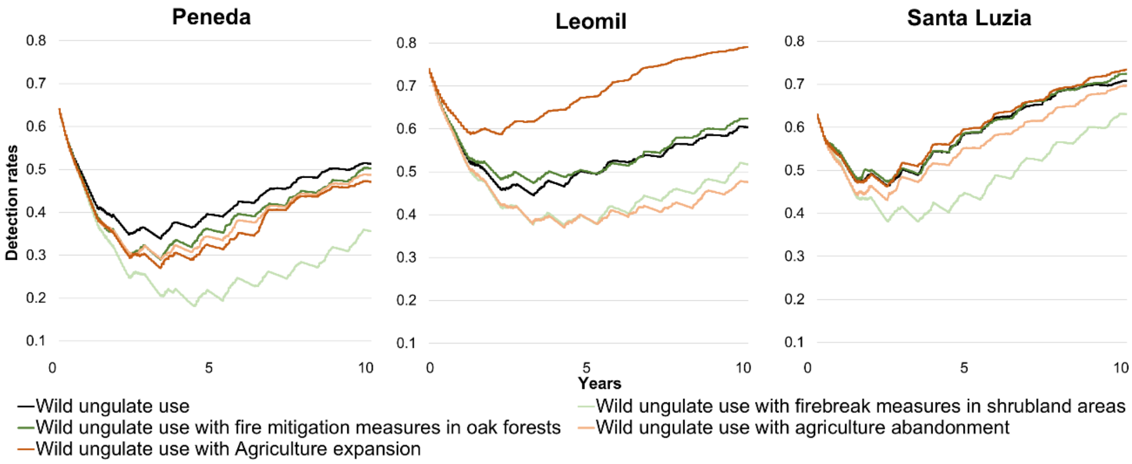

***Figure 36**: Average model simulations of wild ungulate use trends, considering possible scenarios of agricultural land use change and habitat management implementation, in the 3 case studies corresponding to the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years. "Wild ungulate use" corresponds to the baseline scenario.*

The domestic animal trends seem to benefit from the implementation of firebreak measures in shrubland and abandonment of agriculture in the three packs studied, although continuing to show a decreasing trend in the long-term throughout the period of simulation. The expansion of agriculture appears to be the most negative scenario for domestic animals, showing a small decrease of use in the final years in the Peneda and Santa Luzia breeding sites and a substantial reduction in the Leomil breeding site, beginning earlier in time (Figure 37).

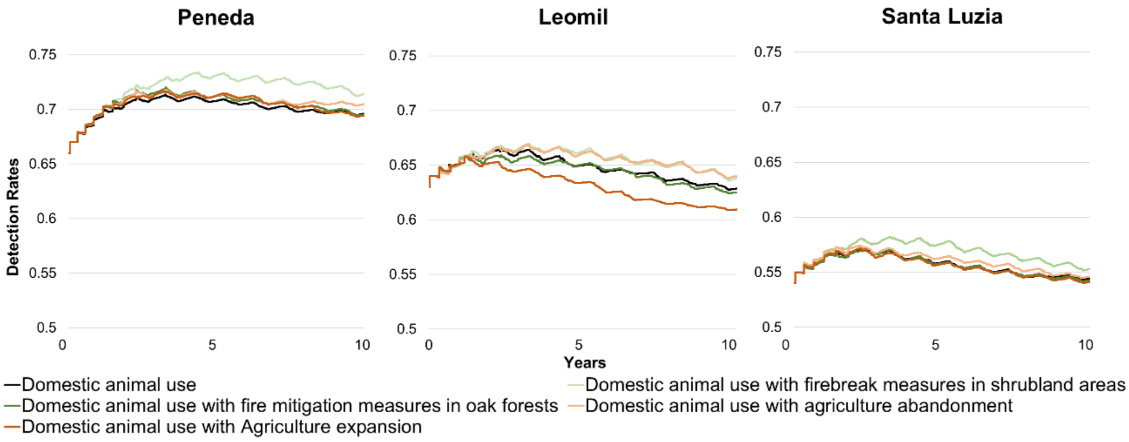

Figure 37: *Average model simulations of domestic animal use trends, considering possible scenarios of agricultural land use change and habitat management implementation, in the breeding sites of Peneda, Leomil and Santa Luzia over 10 years. "Domestic animal use" corresponds to the baseline scenario.*

Finally, all the simulated scenarios in land use and habitat management benefit human and vehicle use in the Peneda breeding site, comparing with the reference situation (baseline scenario). However, implementation of firebreak in shrublands seems to be the scenario leading to a bigger increase in human use. At the Leomil and Santa Luzia breeding sites the use of firebreak measures in shrublands and the abandonment of agriculture seem to promote a higher human use. However, despite the sharp increase of human use in the initial years, all scenarios show a decline in human and vehicle use in the remaining years of the simulation period (Figure 38).

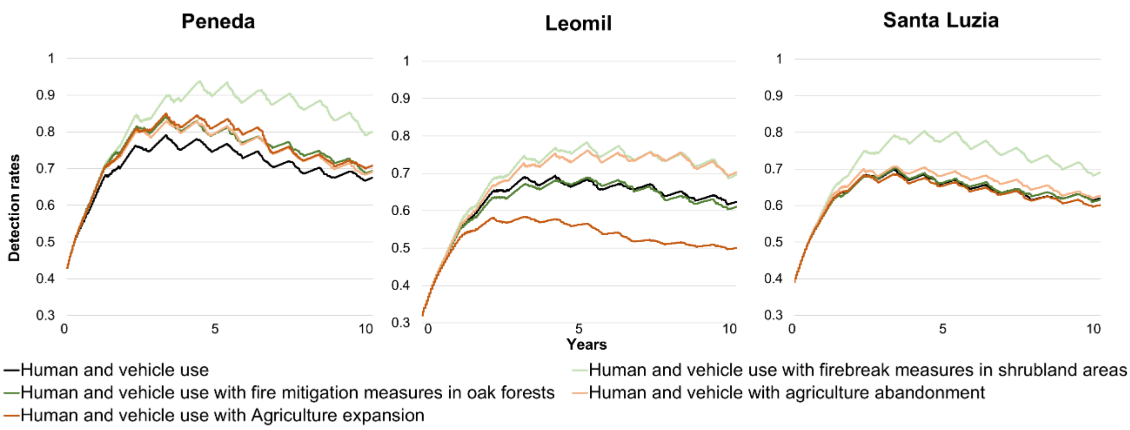

Figure 38: *Average model simulations of human and vehicle use trends, considering possible scenarios of agricultural land use change and habitat management implementation, in the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years. "Humans and vehicle use" corresponds to the baseline scenario.*

3.2.4. Sensitivity analysis of model simulations

The sensitivity analysis (Appendix VI) showed that the variation of land use parameters does not appear to exhibit a strong influence on the variation of most response variables considered, based on camera trapping detections and expressed in wolf breeding site use (for wolf, mesocarnivores, wild ungulates, domestic animals and humans and vehicles), in any of the three cases studied (Peneda, Leomil and Santa Luzia breeding sites). Overall, the response variables most influenced by the variation of land use parameters are the human presence (on foot and vehicles) and wild ungulates use. These two response variables have greater variations associated with oak species, other hardwood species, and, especially, invasive species changes, with major consequences for the prediction of wild ungulate use and human presence, regardless of the scenario considered.

4. DISCUSSION

4.1. Patterns of wildlife use and human disturbance in wolf areas

Our findings based on camera trapping revealed that the wolf key areas in different regions of Portugal show a wide diversity of wildlife despite the high human presence and activity, which can be expected given the human-dominated landscape that characterizes wolf range in Europe (Owens, 2012; Santos, *et al.,* 2007; Rio-Maior *et al*., 2019). The highest detection rates registered for wild mammals (wolf, mesocarnivores, ungulates and lagomorphs) in Alto Minho should be related to the higher habitat availability and better refuge conditions, consisting of more forested and mountainous areas (Rio-Maior *et al.,* 2019, 2020). In addition, this study region also includes several areas of high ecological value that are part of the Natura 2000 network, including the Special Area of Conservation of Peneda-Gerês, which is also the single National Park in Portugal (Rio Maior *et al.* 2020). However, human disturbance was also higher in the Alto Minho region due, most likely, to the high human density and greater proximity to several large urban centers, which mostly occur along the Minho and Lima river valleys. This area also suffers from high tourist pressure related to ecotourism/nature tourism, due to the beautiful landscapes and well preserved habitats (INE, 2021). Despite these higher levels of human disturbance detected by camera trapping, higher wolf detection rates have been also recorded in this region, in accordance with previous knowledge reporting an increasing wolf population with high densities (Nakamura *et al.,* 2021). This is related to a high availability of wild ungulates and livestock in Alto Minho, as reflected by the higher detection rates obtained in this study, which represent the main prey for wolves (Álvares, 2011; Torres & Fonseca, 2016; Pimenta *et al.,* 2018). Furthermore, wolf predation becomes easier in this region due to the free-ranging husbandry regime that Garrano horses and cattle occur, with poor vigilance and protection (Álvares, 2011; Pimenta *et al.,* 2018). In the case of South of Douro, despite lower levels of human disturbance, reduced levels of wild prey were also observed, with roe deer being virtually absent. In addition, the reduced detection rate of livestock (except in Trancoso breeding site) combined with a decreasing trend in the number of livestock in this region over the last years, suggests a reduction of food sources in this region, which may explain the low detection rates for wolves as a result of the precarious status of this subpopulation with low breeding rates (Alexandre *et al.* 2000).

Regarding wolf feeding sites in the two study areas, the only wildlife detections were of wolf, fox and wild boar. Since the feeding sites in Alto Minho consisted of the remains of preys predated by wolves, this showed that scavenger species such as foxes and wild boars, can benefit from the presence of this top predator, using killed carcasses as food source, despite the risk of being preyed by wolves (Licht *et al.,* 2010; Ripple & Beschta, 2012; Owens, 2012; Martins *et al.,* 2020; Rossa *et al.,* 2021). However, in South of Douro, the sampled dumping sites from intensive farms of domestic animals, mostly rabbits and poultry, are the few food sources available in this region for wolves, as a result of a reduced density of ungulates, either wild or domestic (Alexandre *et al.* 2000). The feeding sites had reduced detection rates of human-related disturbances and showed a circadian segregation with wolves and other wildlife, revealing that wild animals use these sites to feed when human presence is lower, mostly at night and early morning. Considering the crossing structure sampled in the A28 highway, a temporal segregation of wolf use with human activity was detected, revealing that a structure originally built for rural vehicle usage allows wolf movement and connectivity between packs on either side of the highway. Thus, this structure, in addition to benefiting humans, can also have an important role in allowing dispersion movements of wolves, being able to mitigate the barrier effect usually caused by highways (Santos, *et al.,* 2007; Boitani & Powell, 2012; Martinig & Bélanger-Smith, 2016). This structure may also be related to one of the factors that allowed the natural recolonization of a pack in the Santa Luzia mountain in 2018, allowing the connection with other neighbouring breeding packs such as Arga pack (Nakamura, *et al.,* 2019).

The sampled breeding sites showed that besides being used by wolves, they are also places that provide refuge for a great diversity of wildlife, as detected in other European regions (Owens, 2012). However, these areas are also used by people for various purposes, such as leisure, agriculture, and hunting, and sometimes are also grazing areas for livestock (Eggermann *et al.* 2011). Wild ungulates, that are preferential prey for wolves, have higher detection rates in the breeding sites that are within protected areas in Alto Minho, possibly due to a better quality of habitat inside protected areas (Barja, 2009; Rio Maior *et al.* 2020). Despite this, wolf detection was lower within protected areas of Alto Minho, possibly due to greater breeding instability presented by the two sampled packs (Arga and Peneda packs) (Nakamura *et al.* 2021). In South of Douro, the Leomil pack displays higher detection rates of wolves and mesocarnivores, probably, due to a higher concentration of intensive production farms of domestic animals (namely rabbits, poultry and pigs), that allow for a constant food source (Serronha *et al.* 2020). Regarding variations in detection along the circadian cycle, a higher incidence of wild animals was observed during the period of lower human presence, namely at night and early morning. This seems to be a behavioural adaptation to avoid disturbances and risk of human-caused mortality, which can allow wildlife, such as wolves, to coexist with humans in those disturbed areas (Llaneza *et al.*, 2012; Zimmermann *et al.,* 2014; Sazatornil *et al.,* 2016). Considering the variations in wildlife use and human disturbances over the months during wolf pups rearing season, there was, in most cases, an increase in detection rates of wildlife and domestic animals, probably due to the overlapping of rearing seasons of these various species, which mainly corresponds to summer months (Barja, 2009). Variations over the sampled years showed a decreasing trend in wildlife detection, which may be related to land use changes associated with socio-economic trends in Portugal, causing habitat fragmentation and limiting suitable breeding areas and habitat connectivity (Santos, *et al*., 2007; Lino et al., 2019). Example of that is the increasing number of roads and other infrastructures and the widespread forest plantation with commercial interest, mostly eucalypt and pine monocultures, leading to drastically decreased of native forest (Santos, *et al.,* 2007; Álvares *et al.,* 2015; Lino *et al.,* 2019). A substantial increase of human disturbance registered in 2020 in Alto Minho is likely due to the emergence of the Covid-19 Pandemic, which led to an increase of outdoor/touristic activities in natural areas supporting the hypothesis initially considered (Soga *et al*., 2021). This increase in human presence in these natural areas could have negative consequences in the range of wild species distribution, as they avoid places with higher human presence. (Sazatornil *et al*., 2016; Corradini *et al.,* 2021).

4.2. Trends in wolf breeding site use

Considering wildlife species (wolf, mesocarnivores, and wild ungulates), the expected decrease in detections in most studied packs may be related to the increase in the occurrence and intensity of fires that are predicted to occur in the regions under study (Carmo, 2021). This has been leading to the disappearance of forest and shrubland areas, being the vegetation trends in these mountain areas, characterized by an increase of open areas with undergrowth vegetation (Lino *et al*., 2019; Carmo, 2021). As a result, wildlife has fewer refuge areas and is more exposed to anthropogenic threats (Lino *et al.,* 2019). On the contrary, the increase in human presence foreseen in all study areas might occur due to the increase in road network and other accessibilities (such as dirt roads) to areas previously inaccessible to people or vehicles, allowing the use of these natural areas for diverse human activities (increasing the risk of vehicle collision with wildlife species) (Whittington *et al.* 2005; Bell *et al.,* 2007; Ferrão da Costa *et al.* 2018).

Considering each group particularly, wolf use of the breeding site in Leomil proved to be the only site with a positive trend forecast, showing that intermediate human intervention could be more beneficial for this species when compared to sites with low or high human intervention levels, as Peneda and Santa Luzia, respectively. These results are in agreement with the “Intermediate Disturbance Hypothesis” already proven in various ecological contexts (Malavasi *et al.,* 2009; Gonçalves *et al.,* 2012). This level of intermediate human interference allows the existence of some refuge areas that wolves take advantage for higher proximity to humans, specifically for food resources such as domestic animals (mainly livestock) or human waste (Álvares *et al.,* 2015). Regarding the species of generalist mesocarnivores, which show a preference for areas with higher human intervention, this group seems to be able to predate on domestic species, like poultry or rabbits, being also capable to benefit from agricultural areas, namely orchard areas and human waste (Verdade, *et al.* 2011; Martins, *et al.,* 2020). Wild ungulates also show a preference for areas with intermediate human disturbance, such as areas with agriculture where they can feed and open areas with pastures, combined with some patches of native oak forest (Ciach & Fröhlich, 2019). Overall, wildlife seems to benefit from a heterogeneous landscape, in a compromise between natural patches with areas where human activities are developed, such as domestic animal production and traditional agriculture (Torras & Saura, 2008; Gonçalves *et a.,* 2012). In Alto Minho region, the production of livestock has been decreasing over the last few years, contrasting with the prediction for domestic animals use in the breeding sites sampled (INE, 2021). In South of Douro, the model simulations for domestic animal use coincides with the decreasing of livestock production that has been witnessed in this region during the past decade (Alexandre *et al*. 2000; INE, 2021). The increase in human presence, as previously mentioned, in addition to the easier access, may also be related to a higher demand for outdoor activities, namely trekking activities, or by four-wheel-drive vehicles, motorcycles, and quad bikes, as here documented by camera trapping data (Bell *et al,* 2007; Ferrão da Costa *et al.* 2018; Corradini *et al.,* 2021).

Considering the possible interaction between the different groups analysed, the expected increase in human use seem to affect species of mesocarnivores and wild ungulates, and also the wolf use in a more humanized context (Santa Luzia breeding site). This might happen due to the wild species’ adaptative behaviours to avoid areas with high human use, even if they present adequate ecological conditions, as to prevent risks associated with human interaction (Whittington *et al.,* 2005; Owens, 2012; Corradini *et al.,* 2021).

With regard to the different land use scenarios simulated in wolf breeding sites, it is possible to observe that in each ecological context, different habitat management priorities are needed in order to benefit wildlife use. Firebreak measures in shrublands and fire-mitigation measures in oak forests were shown to benefit and/or not negatively influence the wild species use, showing that this management in forestry areas, besides preventing economic damage for human populations, has positive impacts on these wild mammals (Carmo, 2021). The wild ungulate species' use was the only exception, predicting a decrease in their use with the shrubland management measures. This may be related to the wild ungulates' preference for open pasture areas linked to the margins of forest patches, rather than areas dominated by shrublands (Ciach & Fröhlich, 2019). The presence of agricultural areas also proved to be an important factor for the use of wolf breeding areas by wildlife. Thus, the expansion of agriculture, which corresponds mostly to small parcels of traditional agriculture in the context of mountainous areas, may provide food for several species of mesocarnivores and wild ungulates, which in turn can be beneficial to a top predator like the wolf (Verdade*, et al.* 2011; Ciach & Fröhlich, 2019). On the other hand, scenarios of agricultural abandonment, sometimes impulsively associated with rewilding processes, can lead to homogeneous and fire-prone systems that are not necessarily suitable habitats (MacDonald *et al.* 2000). The two categories of human-related uses (humans on foot/vehicles and domestic animals) showed a positive association and were not affected by fire mitigation management in shrublands and oak forests, possibly due to the type of humans-related detections in camera trapping that consist, mainly, of humans practicing trekking or hunting activities (occasionally accompanied by dogs). These outdoor activities related to leisure and hunting benefit from the abandonment of agriculture, possibly because the reduction of the agricultural area may allow an increase of adequate habitat for game species together with higher attraction for natural areas to perform leisure activities (Bell *et al.,* 2007). The reduction in agriculture land may also allow an increase of pasture area for livestock, leading to a higher use of domestic animals.

According to our results, the conservation/management of wolf breeding sites is mandatory to mitigate the impending local extinction risk of the last small and fragmented populations in Portugal. In this context, our modelling framework is easily adaptable and applicable to other type of contexts and can support the design of optimized conservation strategies by increasing the knowledge of endangered population responses to the land use and climate changes, quantifying some of the main negative effects and testing local-specific management actions for mitigation and conservation planning (Bastos *et al.* 2018).

4.3. Conservation and management implications

Suitable forest management in wolf breeding areas was shown to be a priority conservation measure for the recovery of this large carnivore, especially considering the trends in climate changes and social-economic factors that have been intensifying the occurrence and effects of fires on the ecosystems more vulnerable to the effects of climate change (Carmo, 2021). Fire disturbance, besides affecting refuge conditions and the few remaining patches of autochthonous forests (Lino *et al.,* 2019), also prevent habitat recovery for wild prey, which is a crucial management goal in order to reduce current levels of livestock depredations and consequent conflict with humans (Pimenta *et al.* 2018). Therefore, it is imperative to implement habitat management measures in order to promote suitable conditions for wolf breeding sites and to recover wild prey populations, such as roe deer, particularly on South of Douro region, where this wild prey is highly reduced (Torres & Fonseca, 2016; Ferrão da Costa *et al.* 2018). Thus, firebreak measures in shrublands and clearing measures in oak forests proved to be effective conservation measures for the wolf and their wild prey. It should also be ensured that this forest management in wolf breeding sites does not coincide with the wolf breeding season (Álvares *et al*., 2011). Thus, when breeding sites occur in more humanized contexts, it is imperative to preserve and/or create refuge zones around breeding sites, especially by promoting native forests, in order to help wolves to persist in human grounds, also with a clear influence on prey diversity and abundance, which can be particularly critical for wolf's population viability (Owens, 2012).

Bearing in mind that many of these habitats are altered due to a long-term human intervention, mountain agricultural areas are documented to be an alternative source of food for wildlife, especially when their natural habitat is deteriorated (MacDonald *et al.* 2000; Verdade, *et al.* 2011). Thus, some form of incentive for human populations to continue agricultural activities with traditional techniques in these ecological contexts would be a benefit for wildlife, namely wolves and their wild prey. In addition, it must also be ensure the adoption of legal measures that allow the carcasses of domestic animals to be deposited in open spaces or dumps (and not being collected due to sanitary regulations), enabling their use as an important trophic resource for several mesocarnivores and some wolf packs, as shown in the South of Douro (Álvares *et al.,* 2015). In addition to habitat management strategies, the development of studies, on wolf seasonal diet in specific habitats, regions and populations, complemented with the characterization of prey diversity and abundance is of great importance to understand the habitat suitability for this carnivore. The data obtained by camera trapping, proved to be an asset for the above-mentioned studies, especially considering wild mammal species.

Despite the preference of wildlife for areas with intermediate human intervention, often due to alternative sources of food, it is necessary to manage and regulate human activities inside wolf breeding sites. To prevent potential negative impacts of human presence on wolf reproduction, the known breeding sites should be protected, considering a buffer at least 2 km from breeding locations, avoiding the development of new infrastructures (e.g., wind farms, roads, and quarries) in this area (Álvares *et al.,* 2011; Ferrão da Costa *et al*. 2018; Rio-Maior *et al.,* 2019). If the construction activities occur in these areas, they should be restricted during the wolf breeding period (defined as May–August) and should not be carried out when wolf breeding females' activity is maximal, namely during night. (Rio-Maior *et al*., 2018) This concern is especially relevant for the small and endangered wolf population at South of Douro, considered being on the verge of extinction (Álvares *et al.,* 2015). Finally, the development of Highway crossings for rural use in humanized landscapes, proved to be sufficient to allow the connection between packs, with no need to build specific fauna passages. Therefore, these crossing structures should be built and managed for a co-use by facilitating the movement of both humans and wildlife across roads (Ree & Grift, 2015).

The modelling framework presented in this study is particularly suitable for management recommendations in the scope of conservation programs, namely by anticipating, with scientific credibility, future ecological consequences associated with land use changes for endangered terrestrial species, such as the Iberian wolf populations that are peripheral. In this perspective, we highlight the interplay between model-based research and time series of data from long-term ecological monitoring, allowing the precise development of increasingly accurate models, with introduction of other drivers, interactions and interferences with precise applicability conditions, which will make the methodology more appealing, instructive and credible to decision-makers and environmental managers. All these actions should be promoted in order to allow wolf persistence in human grounds, a topic with strong relevance given the increasing worldwide scenario of human encroachment.

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6. APPENDIXES

APPENDIX I- Conceptual Diagrams of the Model

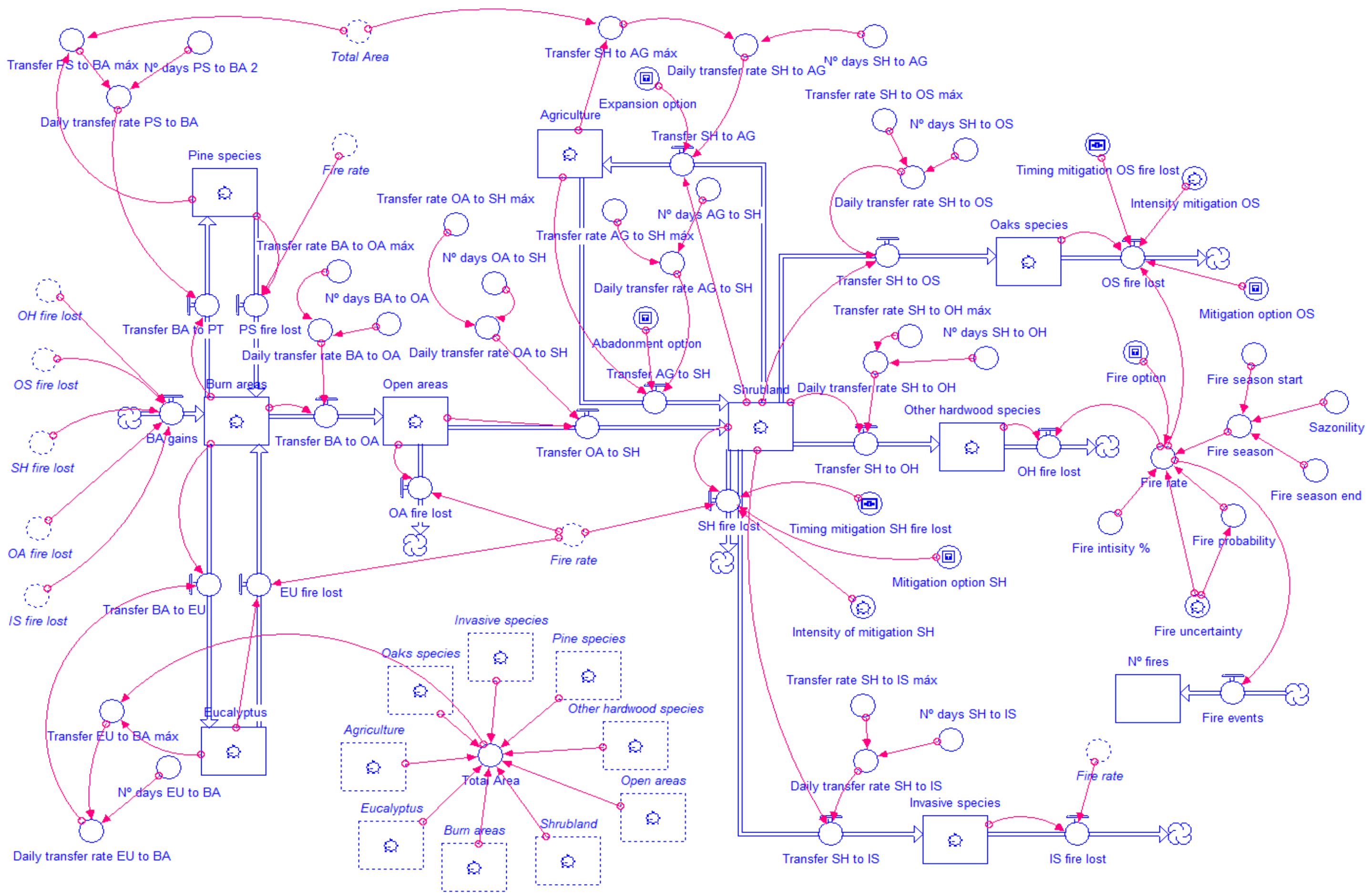

Figure S1: *Conceptual diagram of the Model used to recreate the post-fire succession in breeding sites vegetation. AB-Burn areas; OA-Open areas; SH-Shrubland; AG-Agriculture; OS-Oak species; OH- Other hardwood species; IS-Invasive species; PS-Pine species; EU-Eucalyptus.*

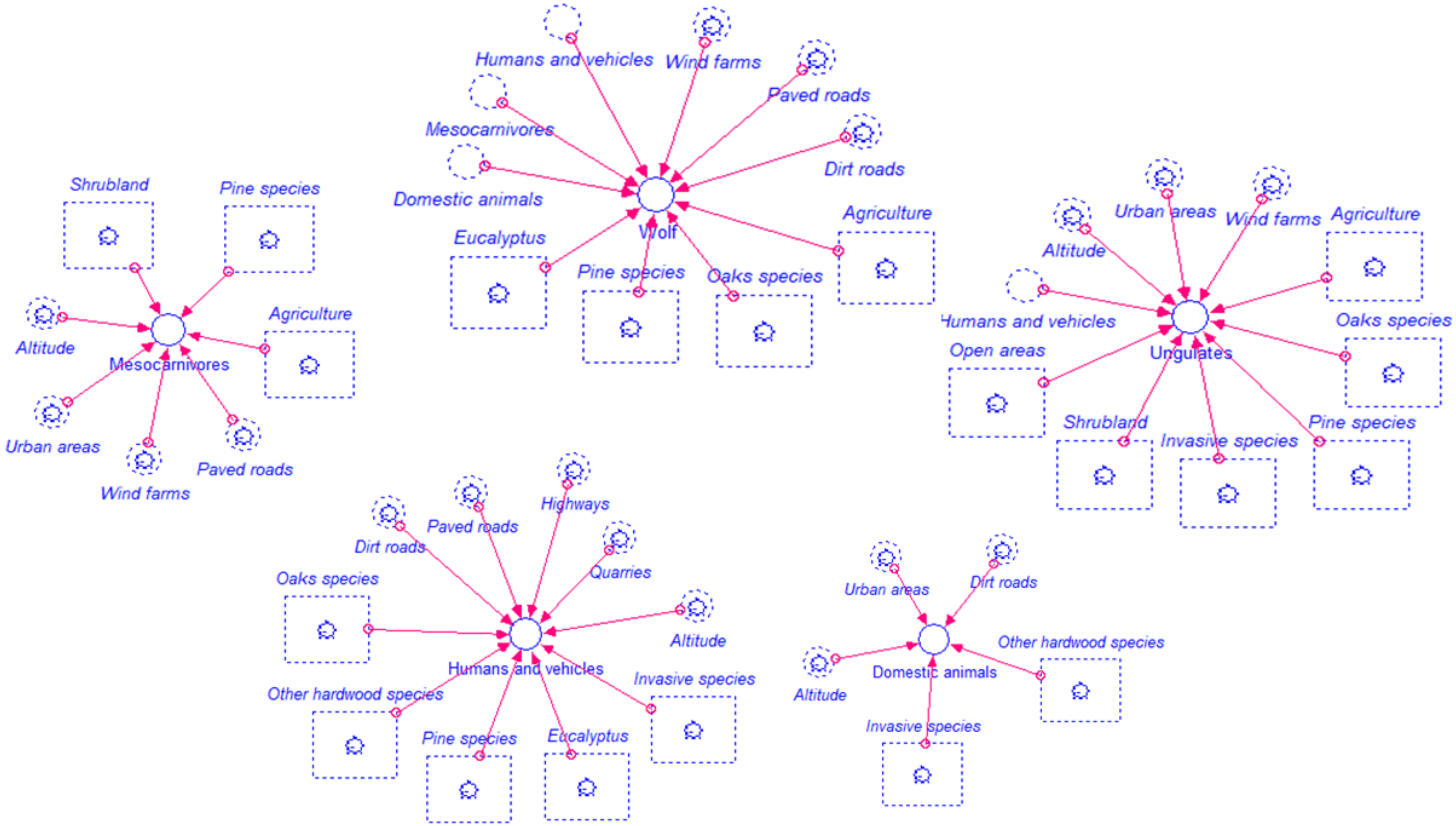

***Figure S2**: Conceptual Diagrams of the land use dynamic influencing the response variables.*

APPENDIX II- Mathematic equations included in the model.

Agriculture(t) = Agriculture(t - dt) + (Transfer_SH_to_AG - Transfer_AG_to_SH) * dt

INIT Agriculture = 0

INFLOWS:

Transfer_SH_to_AG = IF Expansion_option=1 THEN Daily_transfer_rate_SH_to_AG*Shrubland ELSE 0

OUTFLOWS:

Transfer_AG_to_SH = IF Abadonment_option=1 THEN Daily_transfer_rate_AG_to_SH*Agriculture ELSE 0

Burn_areas(t) = Burn_areas(t - dt) + (BA_gains + EU_fire_lost + PS_fire_lost - Transfer_BA_to_OA - Transfer_BA_to_EU - Transfer_BA_to_PT) * dt

INIT Burn_areas = 0

INFLOWS:

BA_gains = IS_fire_lost+SH_fire_lost+OS_fire_lost+OH_fire_lost+OA_fire_lost

EU_fire_lost = Fire_rate*Eucalyptus

PS_fire_lost = Fire_rate*Pine_species

OUTFLOWS:

Transfer_BA_to_OA = Daily_transfer_rate_BA_to_OA*Burn_areas

Transfer_BA_to_EU = Daily_transfer_rate_EU_to_BA*Burn_areas

Transfer_BA_to_PT = Daily_transfer_rate_PS_to_BA*Burn_areas

Eucalyptus(t) = Eucalyptus(t - dt) + (Transfer_BA_to_EU - EU_fire_lost) * dt

INIT Eucalyptus = 0

INFLOWS:

Transfer_BA_to_EU = Daily_transfer_rate_EU_to_BA*Burn_areas

OUTFLOWS:

EU_fire_lost = Fire_rate*Eucalyptus

Invasive_species(t) = Invasive_species(t - dt) + (Transfer_SH_to_IS - IS_fire_lost) * dt

INIT Invasive_species = 0

INFLOWS:

Transfer_SH_to_IS = Daily_transfer_rate_SH_to_IS*Shrubland

OUTFLOWS:

IS_fire_lost = Fire_rate*Invasive_species

N¼_fires(t) = N¼_fires(t - dt) + (Fire_events) * dt

INIT N¼_fires = 0

INFLOWS:

Fire_events = IF Fire_rate >0 THEN 1 ELSE 0

Oaks_species(t) = Oaks_species(t - dt) + (Transfer_SH_to_OS - OS_fire_lost) * dt

INIT Oaks_species = 0

INFLOWS:

Transfer_SH_to_OS = Daily_transfer_rate_SH_to_OS*Shrubland

OUTFLOWS:

OS_fire_lost = IF Mitigation_option_OS=1 AND TIME>= Timing_mitigation_OS_fire_lost THEN (1-Intensity_mitigation_OS)*Fire_rate*Oaks_species ELSE Fire_rate*Oaks_species

Open_areas(t) = Open_areas(t - dt) + (Transfer_BA_to_OA - Transfer_OA_to_SH - OA_fire_lost) * dt

INIT Open_areas = 0

INFLOWS:

Transfer_BA_to_OA = Daily_transfer_rate_BA_to_OA*Burn_areas

OUTFLOWS:

Transfer_OA_to_SH = Daily_transfer_rate_OA_to_SH*Open_areas

OA_fire_lost = Fire_rate*Open_areas

Other_hardwood_species(t) = Other_hardwood_species(t - dt) + (Transfer_SH_to_OH - OH_fire_lost) * dt

INIT Other_hardwood_species = 0

INFLOWS:

Transfer_SH_to_OH = Daily_transfer_rate_SH_to_OH*Shrubland

OUTFLOWS:

OH_fire_lost = Fire_rate*Other_hardwood_species

Pine_species(t) = Pine_species(t - dt) + (Transfer_BA_to_PT - PS_fire_lost) * dt

INIT Pine_species = 0

INFLOWS:

Transfer_BA_to_PT = Daily_transfer_rate_PS_to_BA*Burn_areas

OUTFLOWS:

PS_fire_lost = Fire_rate*Pine_species

Shrubland(t) = Shrubland(t - dt) + (Transfer_OA_to_SH + Transfer_AG_to_SH - Transfer_SH_to_OS - Transfer_SH_to_IS - SH_fire_lost - Transfer_SH_to_OH - Transfer_SH_to_AG) * dt

INIT Shrubland = 0

INFLOWS:

Transfer_OA_to_SH = Daily_transfer_rate_OA_to_SH*Open_areas

Transfer_AG_to_SH = IF Abadonment_option=1 THEN Daily_transfer_rate_AG_to_SH*Agriculture ELSE 0

OUTFLOWS:

Transfer_SH_to_OS = Daily_transfer_rate_SH_to_OS*Shrubland

Transfer_SH_to_IS = Daily_transfer_rate_SH_to_IS*Shrubland

SH_fire_lost = IF Mitigation_option_SH=1 AND TIME>= Timing_mitigation_SH_fire_lost THEN (1-Intensity_of_mitigation_SH)*Fire_rate*Shrubland ELSE Fire_rate*Shrubland

Transfer_SH_to_OH = Daily_transfer_rate_SH_to_OH*Shrubland

Transfer_SH_to_AG = IF Expansion_option=1 THEN Daily_transfer_rate_SH_to_AG*Shrubland ELSE 0

Abadonment_option = 0

Altitude = 0

Daily_transfer_rate_AG_to_SH = (1+Transfer_rate_AG_to_SH_máx)^(1/N¼_days_AG_to_SH)-1

Daily_transfer_rate_BA_to_OA = (1+Transfer_rate_BA_to_OA_máx)^(1/N¼_days_BA_to_OA)-1

Daily_transfer_rate_EU_to_BA = (1+Transfer_EU_to_BA_máx)^(1/N¼_days_EU_to_BA)-1

Daily_transfer_rate_OA_to_SH = (1+Transfer_rate_OA_to_SH_máx)^(1/N¼_days_OA_to_SH)-1

Daily_transfer_rate_PS_to_BA = (1+Transfer_PS_to_BA_máx)^(1/N¼_days_PS_to_BA_2)-1

Daily_transfer_rate_SH_to_AG = (1+Transfer_SH_to_AG_máx)^(1/N¼_days_SH_to_AG)-1

Daily_transfer_rate_SH_to_IS = (1+Transfer_rate_SH_to_IS_máx)^(1/N¼_days_SH_to_IS)-1

Daily_transfer_rate_SH_to_OH = (1+Transfer_rate_SH_to_OH_máx)^(1/N¼_days_SH_to_OH)-1

Daily_transfer_rate_SH_to_OS = (1+Transfer_rate_SH_to_OS_máx)^(1/N¼_days_SH_to_OS)-1

Dirt_roads = 0

Domestic_animals = 6.04745810434-0.00272529152*Altitude-0.00000187555*Urban_areas-0.00012674000*Dirt_roads+0.00000137230*Other_hardwood_species+0.00000027536*Invasive_species

Domestic_animals_Final = IF Domestic_animals_Max-Domestic_animals_Min=0 OR Domestic_animals=0 THEN 0 ELSE (Domestic_animals-Domestic_animals_Min)/(Domestic_animals_Max-Domestic_animals_Min)

Domestic_animals_Max = 10

Domestic_animals_Min = -13.8791

Expansion_option = 0

Fire_intisity_% = RANDOM(0,0.5)

Fire_option = 0

Fire_probability = ROUND(RANDOM(1,Fire_uncertainty))

Fire_rate = IF Fire_option=1 AND Fire_probability=Fire_uncertainty AND Fire_season=1 THEN Fire_intisity_% ELSE 0

Fire_season = IF Sazonility >=Fire_season_start AND Sazonility<=Fire_season_end THEN 1 ELSE 0

Fire_season_end = 270

Fire_season_start = 90

Fire_uncertainty = 4

Highways = 0

Humans_and_vehicles = (-3.285616516625+0.002518403467*Altitude+0.000002865990*Quarries+0.000212977212*Highways+0.000042149962*Paved_roads+0.000032227826*Dirt_roads+0.000000157273*Oaks_species-0.000000840988*Other_hardwood_species-0.000000003965*Pine_species-0.000000924360*Eucalyptus+0.000005096259*Invasive_species)

Humans_and_vehicles_Final = IF Humans_and_vehicles_Max-Humans_and_vehicles_Min=0 OR Humans_and_vehicles=0 THEN 0 ELSE (Humans_and_vehicles-Humans_and_vehicles_Min)/(Humans_and_vehicles_Max-Humans_and_vehicles_Min)

Humans_and_vehicles_Max = 10

Humans_and_vehicles_Min = -6.73035

Intensity_mitigation_OS = 0

Intensity_of_mitigation_SH = 0

Mesocarnivores = -3.516e-01-6.411e-04*Altitude-2.627e-07*Urban_areas-1.207e-02*Wind_farms+2.163e-05*Paved_roads+1.063e-07*Agriculture-4.778e-10*Pine_species+4.726e-08*Shrubland

Mesocarnivores_Final = IF Mesocarnivores_Max-Mesocarnivores_Min=0 OR Mesocarnivores=0 THEN 0 ELSE (Mesocarnivores-Mesocarnivores_Min)/(Mesocarnivores_Max-Mesocarnivores_Min)

Mesocarnivores_Max = 0.054736

Mesocarnivores_Min = -1.26212

Mitigation_option_OS = 0

Mitigation_option_SH = 0

N¼_days_AG_to_SH = 4*365

N¼_days_BA_to_OA = 2*365

N¼_days_EU_to_BA = 365*3

N¼_days_OA_to_SH = (10-2)*365

N¼_days_PS_to_BA_2 = 365*3

N¼_days_SH_to_AG = 365

N¼_days_SH_to_IS = 5*365

N¼_days_SH_to_OH = 10*365

N¼_days_SH_to_OS = 10*365

Paved_roads = 0

Quarries = 0

Sazonility = COUNTER(0,365)

Timing_mitigation_OS_fire_lost = 0

Timing_mitigation_SH_fire_lost = 0

Total_Area = Agriculture+Burn_areas+Eucalyptus+Invasive_species+Oaks_species+Open_areas+Other_hardwood_species+Pine_species+Shrubland

Transfer_EU_to_BA_m‡x = Eucalyptus/Total_Area

Transfer_PS_to_BA_m‡x = Pine_species/Total_Area

Transfer_rate_AG_to_SH_m‡x = 1

Transfer_rate_BA_to_OA_m‡x = 1

Transfer_rate_OA_to_SH_m‡x = 1

Transfer_rate_SH_to_IS_m‡x = 1

Transfer_rate_SH_to_OH_m‡x = 1

Transfer_rate_SH_to_OS_m‡x = 1

Transfer_SH_to_AG_m‡x = Agriculture/Total_Area

Ungulates = 7.118e-01-1.729e-01*Humans_and_vehicles-2.966e-03*Altitude-9.516e-07*Urban_areas+1.273e-01*Wind_farms+5.757e-07*Agriculture+2.240e-07*Oaks_species+6.215e-10*Pine_species-4.892e-06*Invasive_species-9.443e-08*Shrubland+6.009e-07*Open_areas

Ungulates_Final = IF Ungulates_Max-Ungulates_Min=0 OR Ungulates=0 THEN 0 ELSE (Ungulates-Ungulates_Min)/(Ungulates_Max-Ungulates_Min)

Ungulates_Max = 5

Ungulates_Min = -15

Urban_areas = 0

Wind_farms = 0

Wolf = -3.1974122013-0.0361625599*Domestic_animals+0.2304640891*Mesocarnivores+0.1891601681*Humans_and_vehicles+0.0660815955*Wind_farms-0.0001294036*Paved_roads+0.0000199525*Dirt_roads+0.0000003540*Agriculture+0.0000004240*Oaks_species-0.0000001691*Pine_species+0.0000007919*Eucalyptus

Wolf_Final = IF Wolf_Max-Wolf_Min=0 OR Wolf=0 THEN 0 ELSE (Wolf-Wolf_Min)/(Wolf_Max-Wolf_Min)

Wolf_Max = 2.470387

Wolf_Min = -10.225

APPENDIX III- Vegetation trends

Vegetation trends considering different scenarios

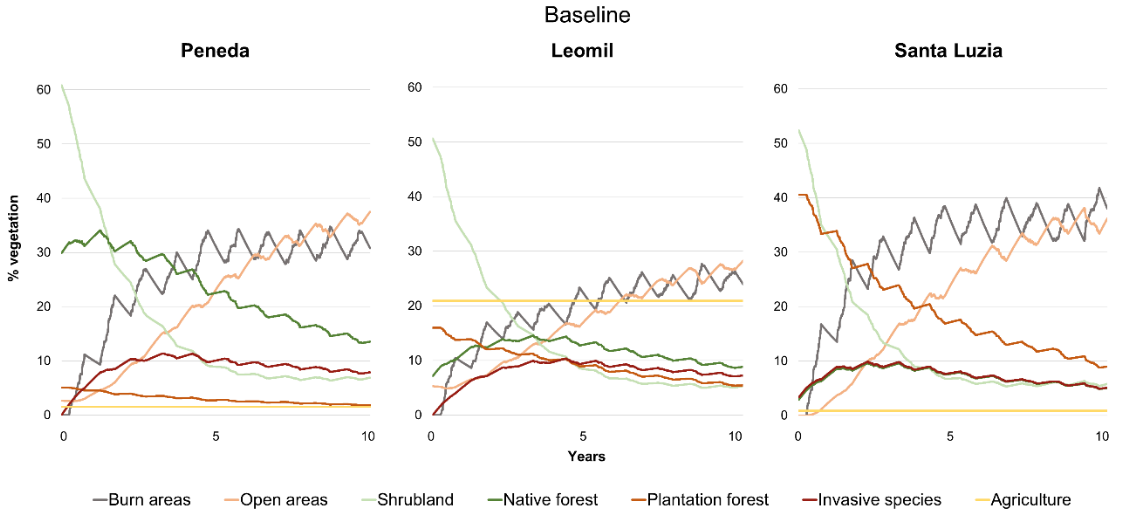

Figure S3: *Average model simulations of vegetation trends, considering the baseline scenario, in the 3 case studies corresponding to the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years.*

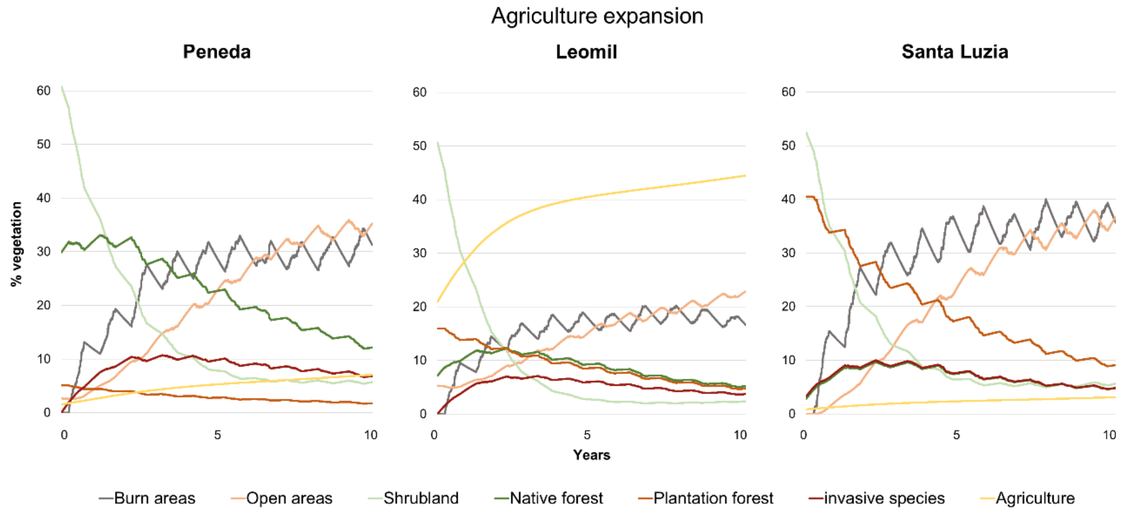

Figure S4: *Average model simulations of vegetation trends, considering the agriculture expansion scenario, in the 3 case studies corresponding to the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years.*

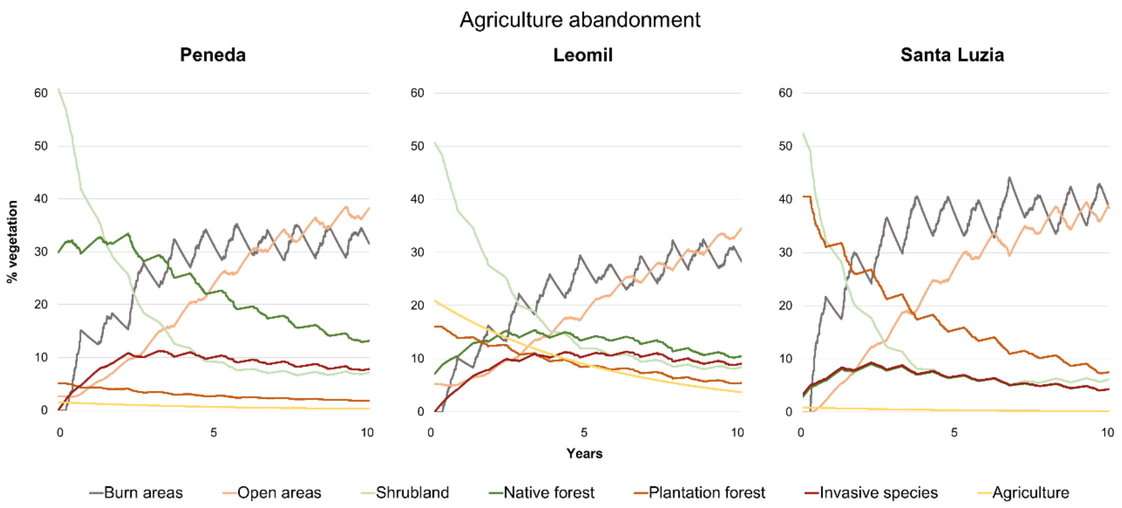

Figure S5: *Average model simulations of vegetation trends, considering the agriculture abandonment scenario, in the 3 case studies corresponding to the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years.*

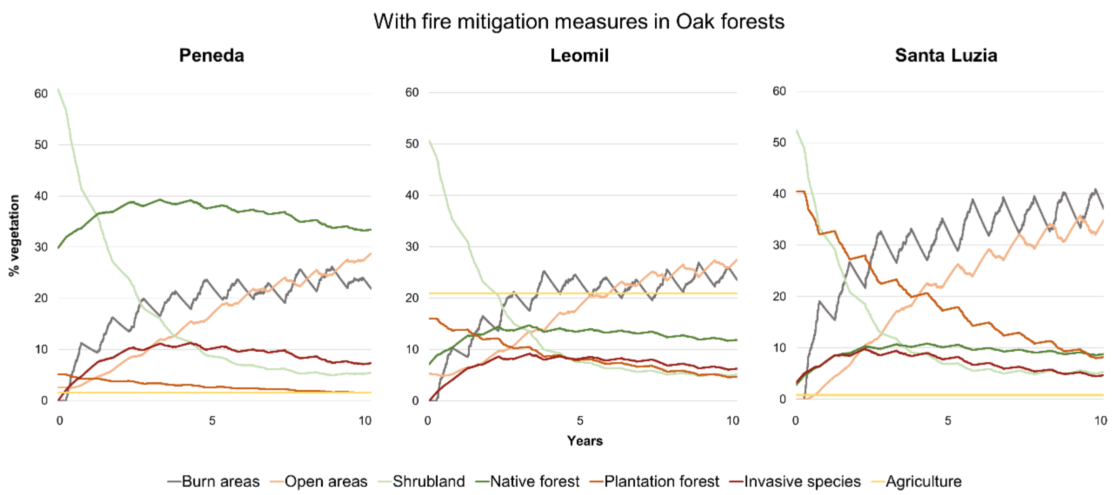

Figure S6: *Average model simulations of vegetation trends, considering the mitigation measures in Oak forests scenario, in the 3 case studies corresponding to the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years.*

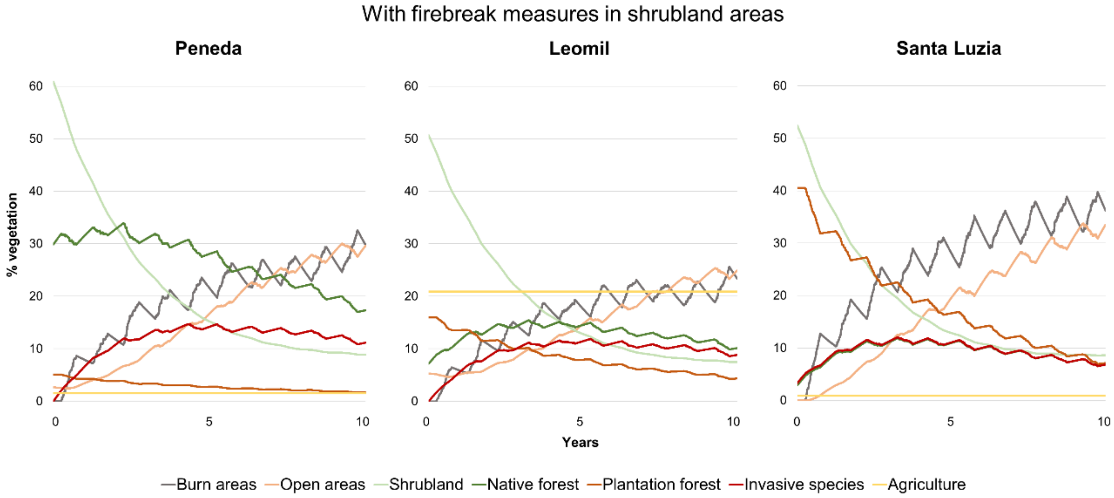

Figure S7: *Average model simulations of vegetation trends, considering the firebreak measures in shrubland areas scenario, in the 3 case studies corresponding to the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years.*

Trends considering agriculture expansion context

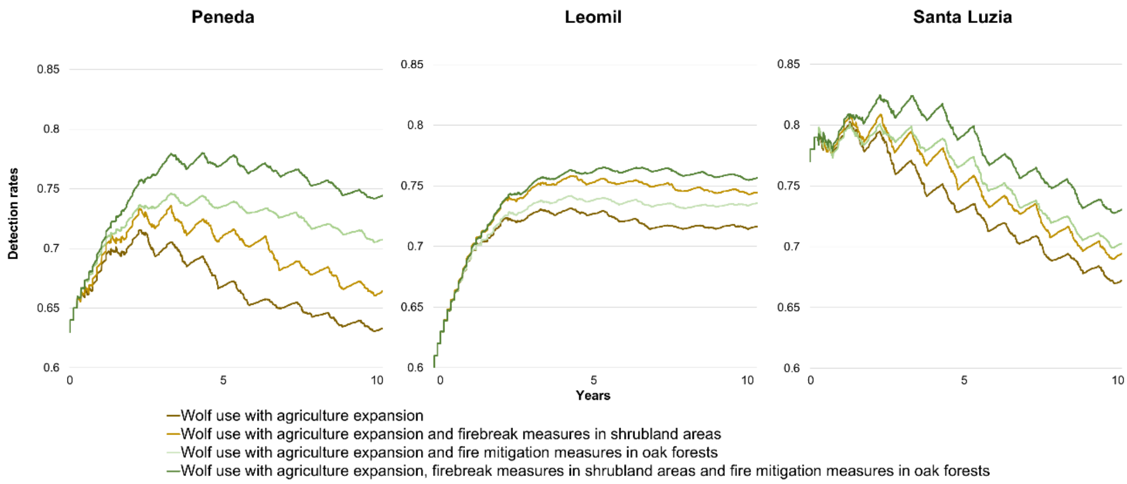

Figure S8*: Average model simulations of wolf use trends, considering combination of different scenarios of agricultural land use change and habitat management implementation, in the 3 case studies corresponding to the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years.*

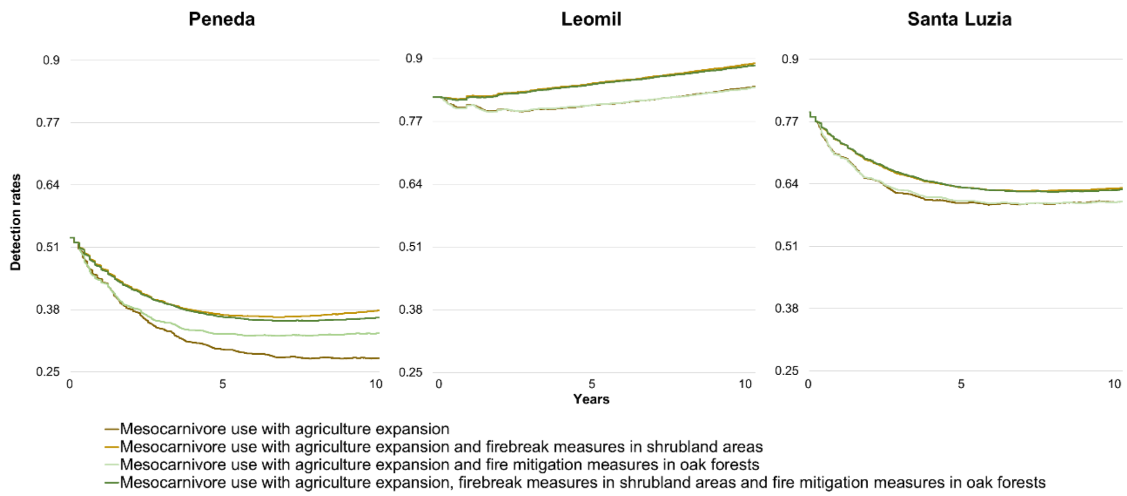

Figure S9*: Average model simulations of mesocarnivore use trends, considering combination of different scenarios of agricultural land use change and habitat management implementation, in the 3 case studies corresponding to the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years.*

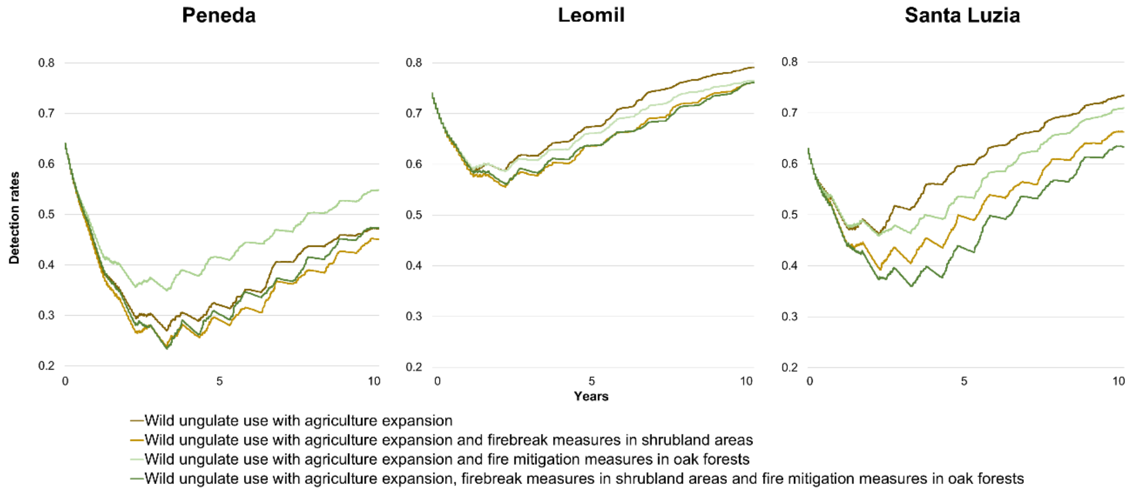

Figure S10: *Average model simulations of wild ungulate use trends, considering combination of different scenarios of agricultural land use change and habitat management implementation, in the 3 case studies corresponding to the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years.*

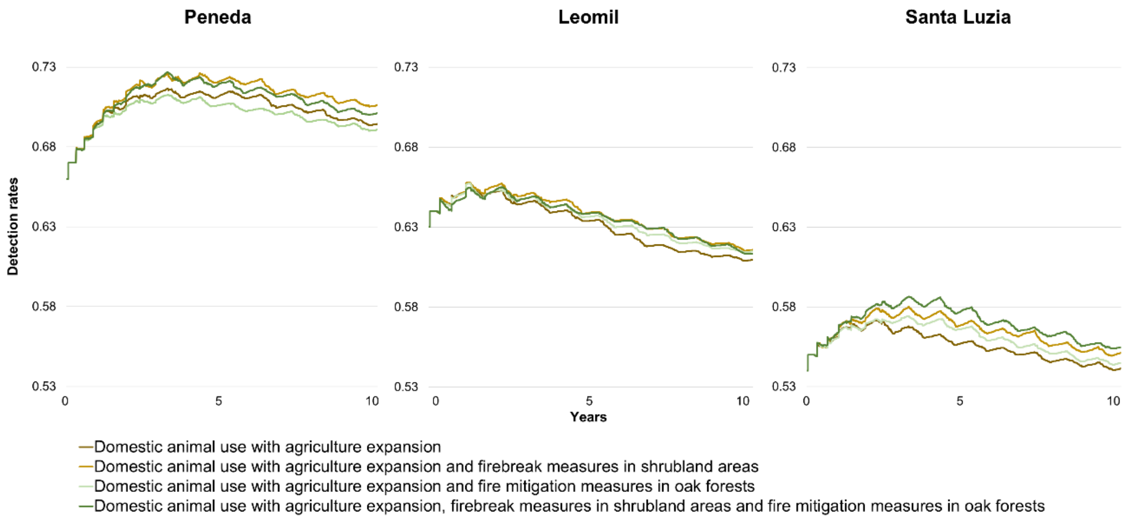

Figure S11: *Average model simulations of domestic animal use trends, considering combination of different scenarios of agricultural land use change and habitat management implementation, in the 3 case studies corresponding to the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years.*

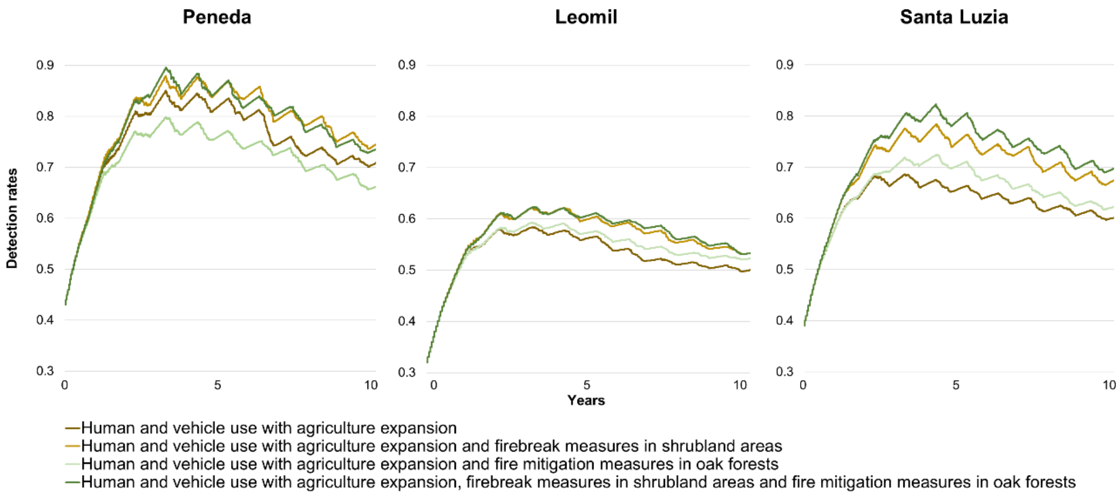

Figure S12: *Average model simulations of human and vehicle use trends, considering combination of different scenarios of agricultural land use change and habitat management implementation, in the 3 case studies corresponding to the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years.*

APPENDIX V- Agriculture abandonment scenario

Trends considering agriculture abandonment context

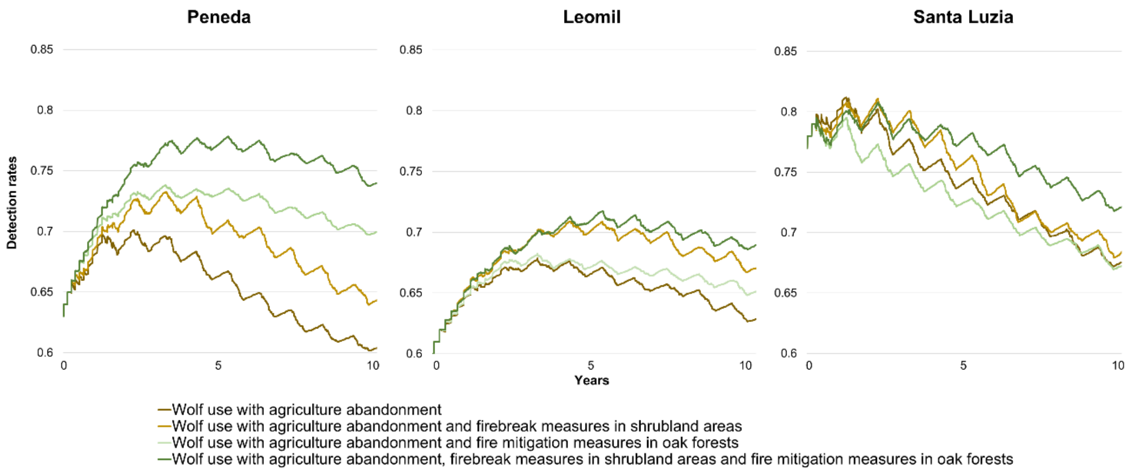

***Figure S13**: Average model simulations of wolf use trends, considering combination of different scenarios of agricultural land use change and habitat management implementation, in the 3 case studies corresponding to the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years.*

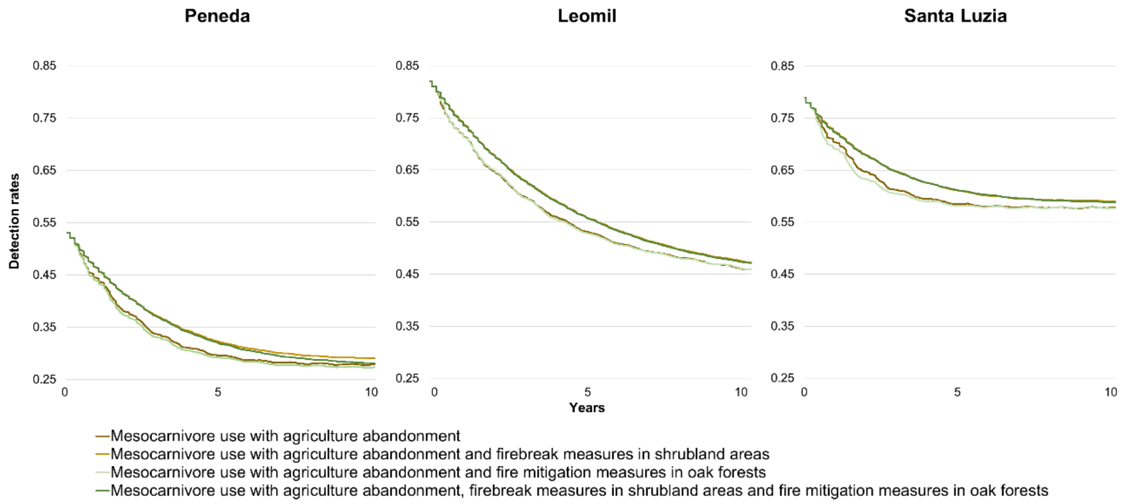

***Figure S14**: Average model simulations of mesocarnivore use trends, considering combination of different scenarios of agricultural land use change and habitat management implementation, in the 3 case studies corresponding to the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years.*

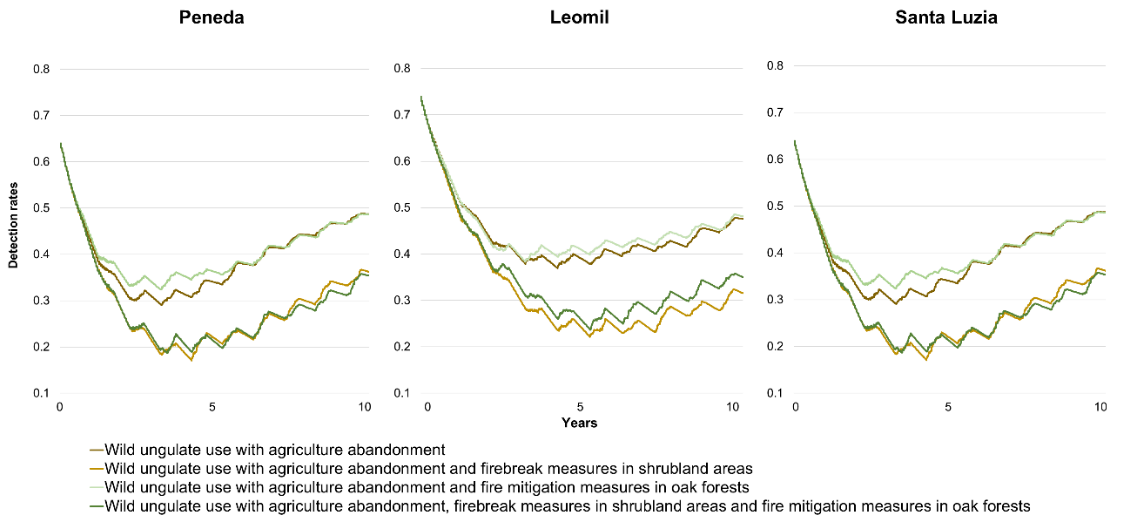

Figure S15: *Average model simulations of wild ungulate use trends, considering combination of different scenarios of agricultural land use change and habitat management implementation, in the 3 case studies corresponding to the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years.*

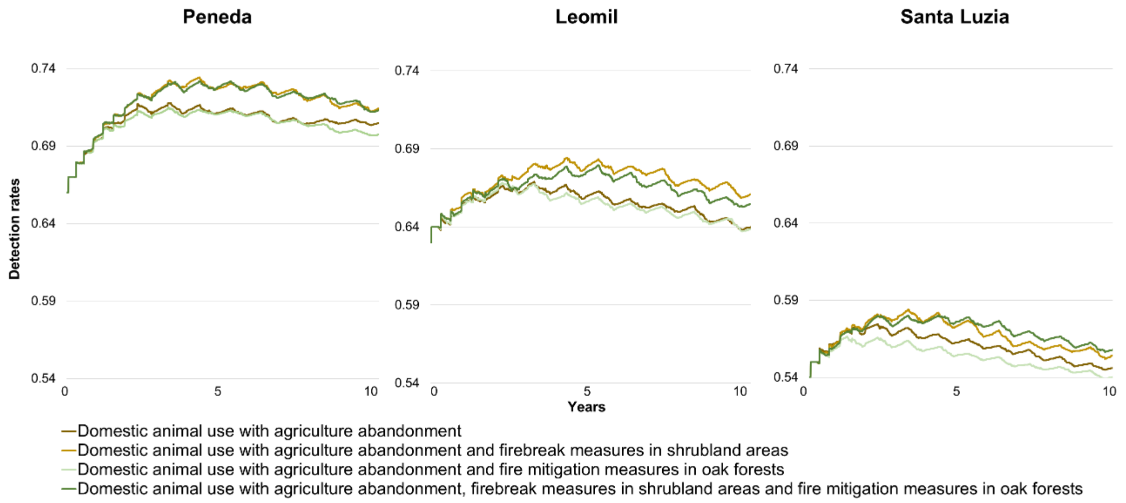

Figure S16: *Average model simulations of domestic animal use trends, considering combination of different scenarios of agricultural land use change and habitat management implementation, in the 3 case studies corresponding to the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years.*

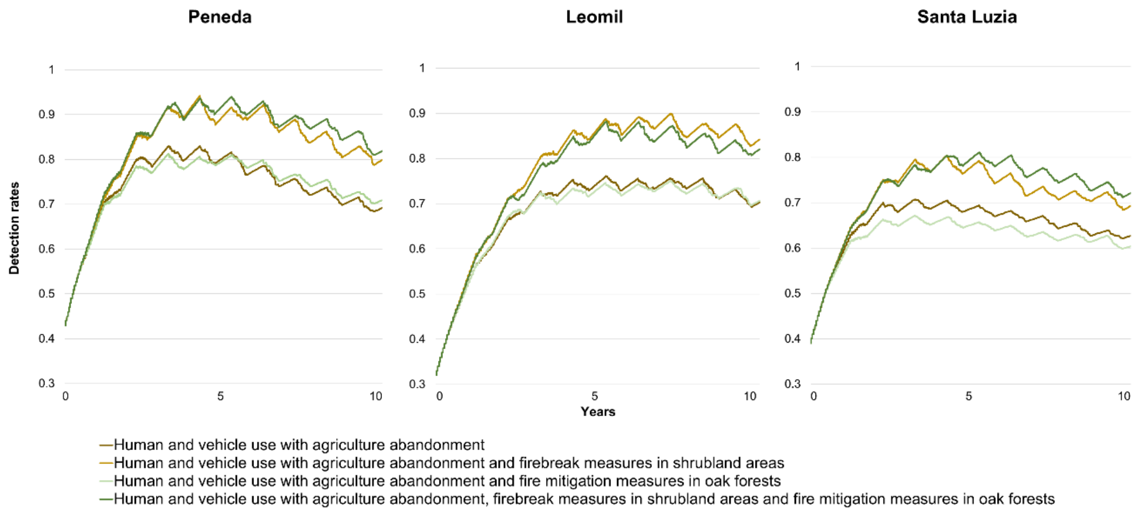

***Figure S17**: Average model simulations of human and vehicle use trends, considering combination of different scenarios of agricultural land use change and habitat management implementation, in the 3 case studies corresponding to the breeding sites of Peneda (Case 1), Leomil (Case 2) and Santa Luzia (Case 3) over 10 years.*

APPENDIX VI- Sensitivity analysis

***Table S1:** Sensitivity analysis (one-parameter-at-a-time) carried out for the wolf, mesocarnivores, ungulates, domestic animals and human on foot and humans, given +/− 10% and +/−50% variation in demographic parameters, for each scenario considered.*

	Peneda breeding site																			
	Wolf				Mesocarnivores				Ungulates				Domestic animals				Humans			
Parameter	-50%	-10%	10%	50%	-50%	-10%	10%	50%	-50%	-10%	10%	50%	-50%	-10%	10%	50%	-50%	-10%	10%	50%
Open areas	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Shrubland	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Oak species	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-0,3	0,0	0,1	0,3	0,0	0,0	0,0	0,0	0,1	0,0	0,0	-0,1
Other hardwoods species	0,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-0,3	0,0	0,1	0,3	0,0	0,0	0,0	0,0	0,1	0,0	0,0	-0,1
Invasive species	-0,1	0,0	0,0	0,1	0,1	0,0	0,0	0,0	1,0	0,2	-0,1	-0,6	0,0	0,0	0,0	0,0	-0,3	-0,1	0,0	0,2
Agriculture	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

	Leomil breeding site																			
	Wolf				Mesocarnivores				Ungulates				Domestic animals				Humans			
Parameter	-50%	-10%	10%	50%	-50%	-10%	10%	50%	-50%	-10%	10%	50%	-50%	-10%	10%	50%	-50%	-10%	10%	50%
Open areas	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Shrubland	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Oak species	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-0,2	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,1	0,0	0,0	-0,1
Other hardwoods species	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-0,2	0,0	0,0	0,1	-0,1	0,0	0,0	0,0	0,1	0,0	0,0	-0,1
Invasive species	-0,1	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,5	0,1	-0,1	-0,4	0,0	0,0	0,0	0,0	-0,3	0,0	0,1	0,2
Agriculture	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

	Santa Luzia breeding site																			
	Wolf				Mesocarnivores				Ungulates				Domestic animals				Humans			
Parameter	-50%	-10%	10%	50%	-50%	-10%	10%	50%	-50%	-10%	10%	50%	-50%	-10%	10%	50%	-50%	-10%	10%	50%
Open areas	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Shrubland	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Oak species	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-0,2	0,0	0,0	0,2	0,0	0,0	0,0	0,0	0,1	0,0	0,0	-0,1
Other hardwoods species	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-0,2	0,0	0,0	0,2	0,0	0,0	0,0	0,0	0,1	0,0	0,0	-0,1
Invasive species	-0,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,6	0,1	-0,1	-0,4	0,0	0,0	0,0	0,0	-0,3	0,0	0,1	0,2
Agriculture	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0