Universidade de Aveiro
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João Ricardo Lavoura Puga

Efeitos imediatos e a curto prazo dos incêndios na fauna de ecossistemas florestais

Immediate and short-term fire effects on animal communities in forest ecosystems

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia, realizada sob a orientação científica do Doutor Nelson Abrantes, Investigador Auxiliar do Departamento de Ambiente e Ordenamento da Universidade de Aveiro e coorientação do Doutor Jan Jacob Keizer, Investigador Principal com Habilitação do Departamento de Ambiente e Ordenamento da Universidade de Aveiro e do Doutor Francisco Moreira, Investigador Principal do Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade do Porto.

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“Look deep into nature and you will understand everything better”

Albert Einstein

o júri

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palavras-chave

Incêndios florestais, fauna, macroinvertebrados do solo, mamíferos, pinhais, eucaliptais, carvalhais, *mulching*.

resumo

Nas últimas décadas, os incêndios florestais tornaram-se um importante problema social, económico e ambiental em todo o mundo, devido à sua maior frequência, severidade e extensão de áreas ardidas. Portugal está longe de ser uma exceção a este respeito, tendo contribuído significativamente para as estatísticas da área ardida da UE com mais de 300.000 ha queimados anualmente durante os últimos anos. Os mega incêndios de Junho e Outubro de 2017 com as suas consequências dramáticas em termos de mortes humanas merecem uma menção especial no presente contexto, pois forneceram as áreas de estudo para a maior parte do trabalho apresentado nesta tese. A mitigação dos efeitos das alterações climáticas ao nível do risco de incêndio e na restauração de ecossistemas degradados, incluindo os resultantes de incêndios recorrentes, são agora preocupações de grande relevância na agenda das políticas e organizações internacionais. Os incêndios e os efeitos adversos a eles associados têm recebido considerável atenção da comunidade científica em regiões propensas a incêndios florestais. Contudo, os efeitos adversos dos incêndios sobre a fauna terrestre são ainda pouco conhecidos. Esta lacuna de conhecimento foi abordada nesta tese, mas dada a curta duração da bolsa de doutoramento, o principal foco foi na avaliação dos efeitos imediatos e a curto prazo dos incêndios florestais. Pela mesma razão, o estudo foi limitado a apenas dois grupos distintos da fauna terrestre, nomeadamente, macroinvertebrados terrestres e mamíferos de médio porte, cuja seleção se deve à sua importância ecológica e aparente resiliência aos efeitos adversos dos incêndios florestais em áreas onde este é recorrente. Os macroinvertebrados terrestres foram o objeto de estudo de três dos quatro estudos incluídos nesta tese (Capítulos 2 a 4), dois dos quais foram realizados na área ardida de Pedrogão Grande, de Junho a Julho de 2017. O estudo dos mamíferos de médio porte foi realizado na área de pinhal das Dunas de Quiaios (Capítulo 5), que ardeu durante Outubro de 2017.

Os dois estudos na área de Pedrogão Grande abordaram especificamente o papel das pedras na salvaguarda de macroinvertebrados terrestres a elas associados dos efeitos imediatos dos incêndios e/ou na recolonização imediatamente a seguir ao incêndio. Para este fim, todos os macroinvertebrados encontrados sob as pedras foram quantificados e recolhidos para identificação ao nível da família, durante as primeiras 2 semanas após o incêndio. A amostragem foi feita em cinco povoamentos florestais queimados e cinco não queimados dominados por carvalhos (Capítulo 2), bem como em dez plantações florestais queimadas e dez não queimadas, igualmente divididas entre pinheiro-bravo e eucalipto (Capítulo 3).

resumo

Os resultados mostraram diferenças significativas entre os locais queimados e não queimados para os três tipos de floresta (carvalhal, pinhal e eucaliptal), não só em termos da abundância, mas também da riqueza e diversidade da comunidade de macroinvertebrados encontrada debaixo das pedras. Estas diferenças revelaram uma elevada mortalidade promovida pelo incêndio, possivelmente combinada com uma reduzida recolonização pós-incêndio. Ao mesmo tempo, a comunidade de macroinvertebrados encontrada debaixo das pedras, tão pouco tempo após o incêndio, sugere que estas desempenharam um papel importante na sobrevivência e/ou fase inicial da recolonização. A mortalidade encontrada aparenta estar ligada às dimensões das pedras, tendo sido encontradas correlações positivas significativas entre maiores valores de abundância e riqueza de macroinvertebrados com pedras de áreas maiores e enterradas no solo a maiores profundidades. O incêndio também parece ter afetado a função da comunidade de macroinvertebrados dos três tipos de floresta ao nível da dieta, aumentando a abundância relativa de predadores e omnívoros na comunidade. De um modo geral, os resultados entre tipos de floresta mostraram uma maior diversidade de macroinvertebrados terrestres nas florestas de carvalhos antes e depois do incêndio, e mortalidade após o incêndio mais elevada nas plantações, particularmente nas não nativas.

O terceiro estudo relativo a macroinvertebrados terrestres (Capítulo 4) abordou os efeitos a curto prazo da gestão pós-fogo, mais especificamente na utilização de uma medida com o objetivo de reduzir o risco de erosão do solo após incêndios florestais, denominada de *mulching*. O estudo foi realizado a par com um outro estudo no âmbito do projecto RECARE (iniciado após um incêndio florestal em Agosto de 2015 nas imediações de Miranda do Corvo) que se focou na eficácia do *mulching* (neste caso resíduos de eucalipto) na proteção do solo da erosão testando duas taxas de aplicação diferentes (2,6 e 8,0 Kg ha^{-1}). Mais especificamente, foram utilizadas pitfalls para a amostragem de macroinvertebrados em duas plantações de eucalipto que tinham sido anteriormente instrumentadas com três parcelas de erosão, de 2m por 8m, replicadas por tratamento (ou seja, as duas diferentes taxas de aplicação de *mulching* e a ausência de *mulching*). A amostragem de macroinvertebrados foi realizada sete vezes durante o primeiro ano após o incêndio, começando imediatamente após a aplicação do *mulching* (efetuada entre Setembro e Outubro de 2015). Os resultados em ambos os locais de estudo revelaram apenas pequenas diferenças na abundância, riqueza, diversidade e equitabilidade da comunidade de macroinvertebrados, não só entre os três tratamentos, mas também ao longo do tempo após a aplicação do *mulching*. No final do estudo concluiu-se que a aplicação de *mulching*, usando resíduos de eucaliptos, como medida de redução da erosão no solo após um incêndio, não parece suscitar preocupações sobre possíveis efeitos adversos a curto prazo sobre a comunidade de macroinvertebrados do solo em eucaliptais. Os resultados obtidos estão em conformidade com um estudo anterior cujo foco foram os efeitos do *mulching* sobre a mesma comunidade, mas a médio-prazo.

resumo

O estudo sobre mamíferos abordou os efeitos imediatos e a curto prazo dos incêndios florestais num total de 13 espécies pré-selecionadas com base em informações prévias sobre a sua presença na área de estudo. Para este efeito, a ocorrência de vários indicadores da presença de cada espécie foi monitorizada em duas ocasiões, ou seja, durante o primeiro e no segundo Inverno a seguir ao incêndio. Foram monitorizadas 18 quadrículas de 2 km por 2 km, na área de pinhal das Dunas de Quiaios, nove na área que ardeu em Outubro de 2017 e as restantes em áreas não ardidas em redor da mesma. Dentro de cada quadrícula, a ocorrência dos indicadores de presença selecionados foi registada a cada 100 m, para dez pontos de controlo ao longo dos trilhos florestais. Os resultados revelaram um número menor de espécies e uma frequência de presença de indícios mais baixa nas quadrículas queimadas do que nas não queimadas, mas estas diferenças só foram estatisticamente significativas durante o primeiro Inverno após o incêndio. Ao nível da espécie, para cinco das 11 espécies que foram encontradas, a ocorrência de indícios da sua presença foi significativamente menos frequente nas quadrículas queimadas do que nas não queimadas, especialmente durante o primeiro Inverno após o incêndio. Essas cinco espécies incluem animais de dietas e comportamentos distintos, nomeadamente três carnívoros, um omnívoro e um herbívoro. Somente para o coelho-bravo a frequência de indícios de presença encontrados foi significativamente maior nas quadrículas queimadas do que nas não queimadas, e apenas durante o primeiro Inverno após o incêndio.

No geral, os resultados revelaram que a resiliência aos efeitos adversos do fogo, imediatamente após e a curto prazo, bem como o impacte de medidas de mitigação pós-fogo é variável entre *taxa* distintos. Deste modo, diferentes espécies suscitam diferentes preocupações para a sua conservação antes e após um incêndio e, consequentemente, implicam medidas distintas para mitigar os efeitos negativos dos incêndios, tanto ao nível da espécie como da comunidade. A importância das pedras na superfície do solo para as comunidades de macroinvertebrados terrestres, tanto antes como depois do incêndio, deverá ser levada em conta aquando da realização de operações de mobilização do solo em grande escala. Isto é especialmente relevante durante o estabelecimento de novas plantações de eucaliptos. Além disso, o licenciamento da caça após os incêndios exige especial cautela, sendo por isso a monitorização das populações de animais selvagens após os incêndios e durante as épocas de caça subsequentes, recomendada. Uma das mais importantes conclusões desta tese é alertar para a necessidade de mais estudos sobre os efeitos dos incêndios na fauna, alargando o leque de grupos e espécies a estudar, e incluindo os efeitos a médio e longo prazo, particularmente em áreas propensas a incêndios e para espécies cujo estado de conservação já representa uma preocupação anterior à problemática associada aos incêndios.

keywords

Wildfires, fauna, ground-dwelling macroinvertebrates, mammals, pine plantations, eucalypt plantations, oak forests, mulching.

abstract

Over the past decades, wildfires have become an important social, economic, and environmental problem across the globe, due to their increased frequency, severity, and spatial extent. Portugal is far from being an exception in this respect, having contributed significantly to the burnt area statistics of the EU and having more than 300.000 ha burnt yearly during the past years. The mega fires of June and October 2017 with their dramatic consequences in terms of human fatalities deserve special mention in the present context, as they provided the study areas for the bulk of the work presented here. Mitigation of the foreseen effects of further climate change on fire hazard regimes and restoration of degraded ecosystems, including from recurrent wildfires are now important concerns on the agenda of international policies and organizations. Wildfire regimes and their impacts have received considerable research attention in fire-prone regions. Some impacts, however, are still poorly known. This includes the impacts on terrestrial fauna. This knowledge gap was addressed in this PhD study but given its short duration of four years, the focus was on assessing the immediate and short-term effects of wildfires. For the same reason, the PhD study was limited to two distinct terrestrial fauna groups, i.e. ground-dwelling macroinvertebrates and medium-sized mammals due to their ecological importance and potential resilience to adverse wildfire effects. The former group was the study object of three of the four studies included in this thesis (Chapters 2 to 4), two of which were carried out in the June-2017 burnt area of Pedrogão and its immediate surroundings. By contrast, the latter group was studied in and around one of the coastal areas that burnt during October 2017, that of the Costal Woodlands of the Quiaios Dunes (Chapter 5).

The two studies in and around the Pedrogão burnt area specifically addressed the role of surface stones in the immediate wildfire effects on ground-dwelling macroinvertebrates, as habitat of refuge during the fire and/or recolonization immediately afterwards. To this end, macroinvertebrates present under stones were quantified and collected for identification at the family level during the first 2 weeks after the wildfire. The sampling was done at five burnt and five unburnt forest stands dominated by oaks (Chapter 2) as well as at ten burnt and ten unburnt forest plantations, equally divided over maritime pine and eucalypt (Chapter 3). The results showed significant differences between the burnt and unburnt sites for all three forest types, not only in terms of the abundance but also of the richness and diversity of the macroinvertebrate communities under stones. These differences suggested a high mortality by the wildfire, possibly combined with a reduced post-fire recolonization. This mortality seemed linked to the dimensions of the stones. At the same time, however, the stones revealed relevant macroinvertebrate communities so soon after the wildfire, suggesting that they played an important role in survival and/or the early stages of recolonization. Significant positive correlations between macroinvertebrate abundance and richness significantly increased with increasing stone area and depth. The wildfire also seemed to have affected the functional composition of the macroinvertebrate communities of the three forest types, by increasing the relative abundance of predator and omnivore *taxa*. Overall results between forest types showed higher ground-dwelling macroinvertebrate diversity in oak forests before the wildfire, and higher post-fire mortality rates in plantations, particularly in non-native forests.

The third macroinvertebrate study (Chapter 4) addressed the short-term effects of post-fire land management or, more specifically, a so-called emergency stabilization measure or, in other words, a soil conservation measure aiming at reducing the risk of soil erosion after wildfires. To this end, use was made of a field study into the effectiveness of mulching with eucalypt logging residues at two contrasting application rates (2.6 and 8.0 Kg ha^{-1}) that was set up in the framework of the RECARE project after an August-2015 wildfire. More specifically, pitfall traps were used to sample ground-dwelling macroinvertebrates at two eucalypt plantations that had each been instrumented with three replicate erosion plots of 2m by 8m per treatment (i.e. the two mulching rates and doing-nothing). The macroinvertebrate sampling was done on a total of seven occasions during the first post-fire year, starting immediately after mulching in September and October 2015. The results at both study sites revealed only minor differences in macroinvertebrate abundance, richness, diversity, and equitability, not only between the three treatments but also with time-since-mulching. Therefore, mulching with eucalypt logging residues in eucalypt plantations to reduce soil erosion risk did not seem to raise concerns on possible adverse short-term effects on the ground-dwelling macroinvertebrate community. This finding was in line with the mid-term effects observed in a prior study.

The study on mammals addressed the immediate and short-term effects of wildfires on a total of 13 medium-sized species that were pre-selected based on prior information on their (expected) presence in the study area. To this end, the occurrence of various indicators of the species' presence was surveyed at two occasions, i.e. during the first as well as the second winter following the wildfire. This was done for a total of 18 grid cells of 2 km by 2 km that were outlined in and around the coastal dune area burnt during October 2017 such that they were equally divided between burnt and unburnt conditions. Within each grid cell, the occurrence of the indicators was recorded for ten checkpoints at 100 m distances along forest tracks. The results revealed fewer species and a lower overall species frequency in the burnt than unburnt grid cells, but these differences were only statistically significant during the first post-fire winter. At the level of the individual species, five out of the 11 species that were encountered were significantly less frequent in the burnt than unburnt grid cells, especially during the first post-fire winter. This included carnivore, omnivore and 1 herbivore species. By contrast, only one species, the European rabbit was significantly more frequent in the burnt than unburnt grid cells, but this fire effect was short-lived, being limited to the first post-fire winter.

Overall, the present results revealed that *taxa* differed markedly in their resilience to the immediate and short-term impacts of wildfires and, in the case of the ground-dwelling macroinvertebrates also in their resilience to post-fire mulching. Thereby, different species raise different concerns for their conservation following wildfires and, hence, imply distinct measures to mitigate these possible wildfire effects. More specifically, the importance of surface stones for macroinvertebrate communities both before and after fire would suggest against large-scale soil mobilization operations. This is especially relevant for the establishment of new eucalypt plantations. Also, hunting licensing following wildfires would seem to require special caution and monitoring of population dynamics following wildfires and hunting seasons. Perhaps the most important conclusion of this thesis is that further research on wildfire effects on fauna is needed, especially with respect to mid-and long-term effects and on species in fire-prone areas whose conservation status is already a concern without wildfires.

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Chapter 1

1 General introduction

1.1 Wildfires in the world

Fire is an Earth system process that started millions of years ago since terrestrial vegetation started to develop, and Earth's atmosphere became oxygenated enough for combustion to occur through natural ignition sources (Scott & Glasspool, 2006; Bowman *et al*., 2009). Fire has been critical for the atmospheric chemistry alterations throughout time and a driving force on the diversity of terrestrial vegetation (Marlon *et al*., 2009). It has been instrumental in human history, as for millennia, humans used fire to transform the landscape and improve soil productivity in agriculture. Initially, at relatively small scales, often enhancing biodiversity in the affected areas (Bowman *et al*., 2009; Fuhlendorf *et al*., 2010), but as human society and technology evolved, the wildfire's size, occurrence, and frequency increase worldwide, so do the extent of their negative impacts. Nowadays, fire is a dynamic ecological force with evolutionary consequences shaped by human actions (McLauchlan *et al*., 2020). Wildfire events are now a combination of land management, human activity, cultural traditions, and climate and weather conditions (San-Miguel-Ayanz *et al*., 2017).

In recent years, large wildfires have become more common worldwide, propagating over extended periods and affecting large areas worldwide (Stephens *et al*., 2014; Jones *et al*., 2016). These mega-fires tend to concentrate in Western North America, Australia, and the Mediterranean Basin where the combination of fire-prone ecosystems, changing climate conditions, and human-induced alterations throughout time favours the occurrence of these events (Costa *et al*., 2020), and consume large areas of natural forests in South America, Africa, and Asia (Krawchuk *et al*., 2009), but recently expanded further to higher latitudes of the Arctic Circle affecting boreal forests and peatlands (McCarthy *et al*., 2021; Natole Jr. *et al*., 2021). Large wildfires cause severe alterations in ecosystems, particularly in those dominated by non-native vegetation, historically open forests with high tree density encroachment, heavily altered by humans or lacking biological and landscape heterogeneity (Keane *et al*. 2008; Pausas *et al*. 2008; Stephens *et al*., 2014).

Climate change promotes additional threats to current fire management as the length of the wildfire season grows with each passing year, with favourable weather conditions that affect fire ignition and propagation (San-Miguel-Ayanz *et al*., 2017). The situation worsens as human-induced alterations over the centuries have culminated in large areas of fire-dependent ecosystems worldwide that now contain primarily fire-tolerant and fire-adapted species, most of the times non-native, often at the expense of fire-sensitive species (Bowman *et al*., 2009). This transformation into fire-dependent ecosystems worldwide is a phenomenon that is still expanding because as fire acts as a key driver on ecosystems (Pastro *et al*., 2014), so plant communities also respond to wildfires, altering fire regimes, through a self-reinforcing cycle that selects traits and species to survive within a given fire regime (Rogers *et al*., 2015). The seasonal repetition of these ecological and climate events, over time, has broadened the negative impact of wildfires worldwide, leading to an overall loss of biodiversity (CBD, 2001).

1.2 Wildfires in Portugal

Portugal is one of the most affected countries by wildfires worldwide, with a high occurrence every year (Doerr & Santín, 2013; Rego & Silva, 2014; San-Miguel-Ayanz *et al*., 2021) (Figure 1.1), including mega-fires as the dramatic events that occurred in 2017 with more than one hundred losses of human lives and 500 000 ha of forest. People in Portugal have used fire since prehistory to promote agriculture and pastoral activities (Pausas *et al*. 2008), using fire as a tool to alter the landscape. This repeated fire use led to a growing number of homogeneous and extensive areas, which increased the fire hazard over time. In Portugal, as in other Mediterranean regions, socio-economic changes led to the progressive abandonment of rural zones (Vaz, 2009; Moreira *et al*., 2011), leading to profound landscape transformations. First into shrublands and abandoned agricultural areas promoted by the migration of people from rural areas to cities, and later into the transformation of those same areas into extensive forest plantations promoted by government policies and industry (Vaz, 2009; Moreira *et al*., 2011). Portugal is now occupied by 36% of forests, from which more than 70% are plantations formed by maritime pine (*Pinus pinaster*), blue gum eucalypt (*Eucalyptus globulus*), or cork oak (*Quercus suber*) (IFN6, 2015), used for the exploration of timber wood, paper pulp, and cork. Maritime pine is a fire-prone tree that promotes highly flammable plantation forests with high combustion loads (Godinho-Ferreira *et al*. 2006). Since wildfires became more common and destructive each coming year, eucalypts started replacing pines, which are economically more appealing (Godinho-Ferreira *et al*. 2006; Silva, 2007). Due to its economic importance cork oak still occupies vast regions of Portugal while other oak tree species, despite also being less fire-prone due to natural adaptations (Moreira *et al*., 2011), are scattered around the country in small pockets of vegetation totalling less than 3% of the total forested area (ICNF, 2020). After centuries of change, most of the forest in Portugal today corresponds to forest plantations with fire-prone characteristics (IFN6, 2015; San-Miguel-Ayanz *et al*., 2021).

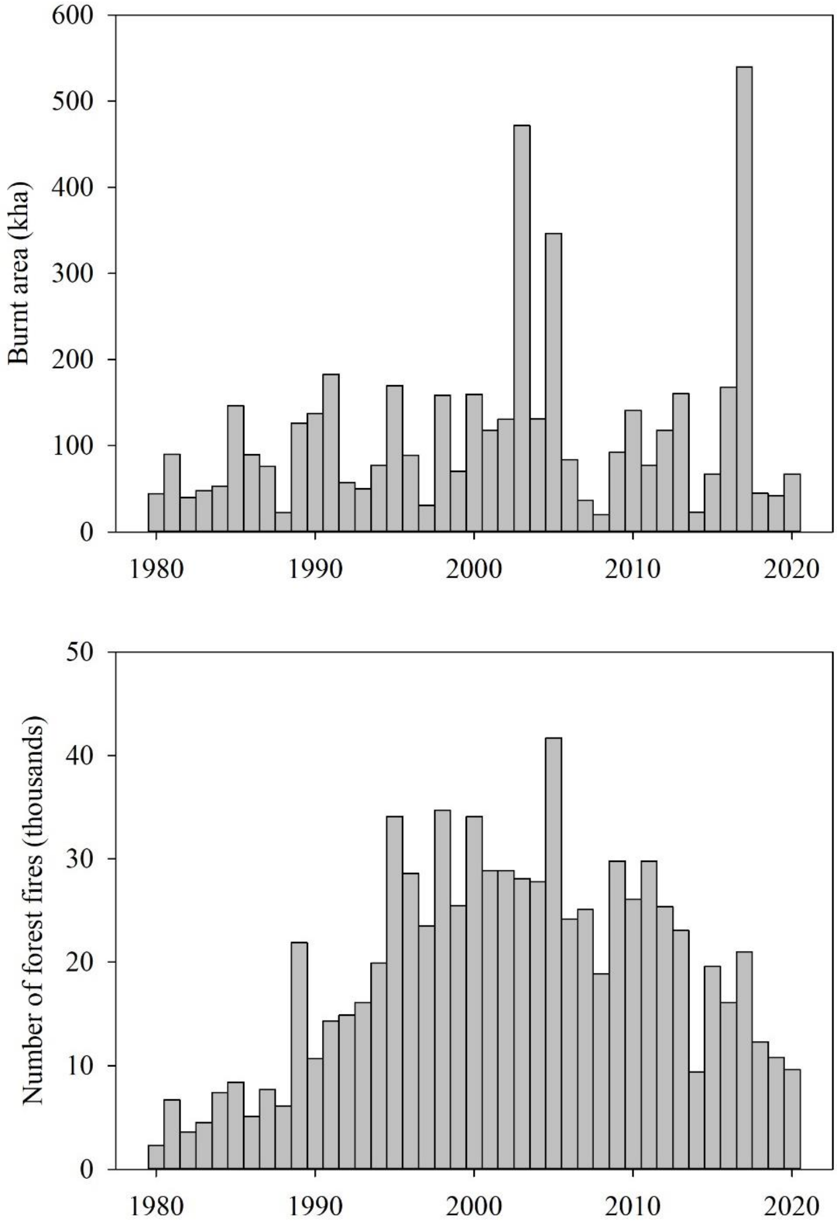

Figure 1.1 Burnt areas and number of fires in Portugal 1980-2020. (San-Miguel-Ayanz *et al*. 2021)

1.3 Wildfire effects on biodiversity

Wildfires can negatively affect both plants and animals directly or indirectly. The direct effects of fire on plants are mainly the death, by total or partial fire consumption, or later post-fire mortality due to injuries caused by the fire (Brown & Smith, 2000; Moreira *et al*., 2010). The rate of mortality and the injury level is often related to a combination of factors that include fire regime, site features, and the characteristics of the plant itself (Gill *et al*., 2002; Moreira *et al*., 2011). Post-fire survival is influenced by additional factors such as preand-post-fire land and forest management activities, drought, erosion, disease, or pests (Halofsky *et al*., 2020; Albery *et al*., 2021).

Many plants have developed strategies that protect them from fire. Bark thickness, vegetative insulation, above-ground resprouting, below-ground roots, and underground stems are the most common, often preventing death during a wildfire (Brown & Smith, 2000; Moreira *et al*., 2011). Another common plant strategy is focused on dispersion and rapid occupation of the burnt area after the wildfire through fire-resistant seeds even if the original plant dies (Brown & Smith, 2000; Moreira *et al*., 2010). As post-fire conditions vary, so can the vegetation, even temporarily, as many plants are stimulated to reproduce following a fire while others depend on it to germinate. Some of the most common plant post-fire responses include increased productivity and flowering, fire-stimulated seed release and dispersal, and improved seedling germination and establishment (Brown & Smith, 2000). In Portugal, the trees that now represent most of the country's forests have at least one of these traits. Oaks (*Quercus* spp.) are post-fire resprouters that can have higher bark thickness, giving extra protection against fire, while maritime pine's strategy against wildfires is seedling recruitment, with seed emerging after Autumn's first rains (Moreira *et al*., 2010). Eucalypts are also post-fire resprouters (Catry *et al*., 2013), given their origin from regions where wildfires are seasonal.

Contrary to plants, when we consider the full extent of studies related to animal ecology and wildfires, the information is limited for most groups (Clarke, 2008; Chia *et al*., 2016; Pausas, 2019), particularly regarding the immediate post-fire effects. The direct effects of fire on animals are identical to plants, including death or injury due to fire (Smith, 2000; Moreira *et al*., 2010). However, as an immediate reaction to fire, dispersal is also commonly assumed as a direct effect because it can rapidly cause changes in the composition of fauna at a site (Nimmo *et al*., 2018). Animal's ability to move enabled the development of responses to avoid wildfire's direct effects, such as seeking refuge on unburned patches, moving out of the area entirely, or burrowing into the soil to escape the heat (Smith, 2000). These responses to wildfires vary for each animal group and between *taxa* and can widely differ from regions where wildfires are seasonal and other areas where only lately wildfires started to become more common each year (Pausas & Parr, 2018). Hence, how these and other effects occur and to what extent, the *taxa* involved, the interaction between *taxa* and the landscape, and how each species responds to these after-fire processes, are largely unknown (Clarke, 2008).

Indirect fire effects on both plants and animals are related to physical alterations in the ecosystem. As fire alters the landscape, changes in the vegetation structure and composition reflect on the availability of resources for animals. Alterations in food quantity and quality as well as in cover and microhabitat characteristics often lead to changes in the structure and composition of animal communities (Moreira *et al*., 2010; Hradsky *et al*., 2017; Certini *et al*., 2021). These changes can affect few species or the whole community and can be temporary or permanent (Pastro *et al*., 2014). Some of these changes can have positive effects in the immediate for some animals, mainly benefitting predators and scavengers through an increase of prey and corpse abundance in the burnt and adjacent areas and granivores that have easier access to seed banks (Smith, 2000).

Because of their ecological relevance and fast response to alterations in the ecosystem, invertebrates have high relevance in ecosystems and are commonly used as bioindicators (Abrantes, 2018; Borges *et al*., 2021). Vertebrates, in addition to their ecological relevance, usually have longer lifespans, higher mobility, and higher adaptability to changes in the environment, giving different spatial-temporal responses crucial to complement other information regarding each ecosystem. Nevertheless, for both cases, knowledge is still scarce given both groups' biodiversity (Titley *et al*., 2017), and when related to wildfires, seldom contradictory (Zaitsev *et al*., 2016; Geary *et al*., 2019; Pausas, 2019).

Ground-dwelling macroinvertebrates are a highly diverse part of soil biota associated with the upper layers of soil and more exposed to wildfire effects and is, therefore, one of the most studied groups of invertebrates regarding fire effects. This group includes several *taxa*, from which ants, spiders, and beetles are the most cited across fire-related studies. Ants are often described as the first colonizers after-disturbance, rapidly exploiting available space and resources after a wildfire (Andersen *et al*. 2009; Antunes *et al*. 2009; Bess *et al*. 2002), but not without a decrease in their diversity (Vasconcelos *et al*., 2017). Among beetles, both abundance and diversity tend to decrease after a wildfire (Cook & Holt, 2006), but some *taxa* such as rove beetles and carabids can benefit from the fire as post-fire habitats become more suitable for hunting, as well as saproxylic beetles that feed on dead and decaying wood (Wikars and Schimmel 2001; Buddle *et al*. 2005; Elia *et al*. 2012; Fredriksson *et al*., 2020; Mason *et al*., 2021). Spider abundance and diversity also tends to decrease after a wildfire (Wikars & Schimmel, 2001; Niwa & Peck, 2002; Verble-Pearson & Yanoviak 2014)), particularly among plant-related *taxa* (Niwa & Peck, 2002), only returning to pre-fire values after several years (Buddle *et al*. 2000). For ground-dwelling macroinvertebrate groups such as centipedes, isopods, snails, among others, their abundance and diversity also decrease after the fire and tend to maintain lower values than before the wildfire for several years (Sgardelis *et al*. 1995; Kiss & Magnin, 2004; Moretti *et* *al*., 2004). Overall, for most ground-dwelling macroinvertebrate groups, information regarding post-fire survivability states that it is probably related to their mobility and ability to find refuge (Abbot & Maitre, 2010; Ross *et al*., 2017), but factual data to support these assumptions is very scarce (Pausas, 2019).

Information regarding fire effects on mammals is very limited, but some studies have reported relevant findings across distinct mammal groups. Small mammals can respond differently to wildfires regarding diet, feeding behavior, and habitat selection (Vieira & Briani, 2012; Griffiths & Brook, 2014) and to wildfire characteristics and post-fire conditions in the burnt area (Amacher *et al*., 2008; Fontaine & Kennedy, 2012), benefiting some species (DellaSalla & Hanson, 2015) and limiting other (Torre & Díaz, 2004; Griffiths & Brook, 2014). Forested habitat species such as squirrels tend to abandon burnt areas, avoiding fire-damaged forests during initial succession (Fisher & Wilkinson, 2005; Leonard & Koprowski, 2010), while others such as deer species can have their activity limited in burnt areas because of lower tree cover protection (Borkowski, 2004). Smaller herbivores such as hares tend to increase their abundance in larger burnt areas (Hutchen & Hodges, 2019) and in the years following the fire (Sokos *et al*., 2016). Medium and large carnivores in Californian chaparral were not negatively affected by high severity fires (Borchert, 2012) and can recover to pre-fire situations after some years (Turschak *et al*. 2010) if the affected area is within an extensive natural area and far from large urban centers (Crooks and Soule, 1999; Mantgem *et al*., 2015). While in the Mediterranean basin, large mammal communities can still be maintained, in the long-term, in previously burnt pine forest areas (Soyumert *et al*., 2019).

1.4 Post-fire mitigation measures and their effects on biodiversity

Forest management activities such as harvesting, logging, and clearing are standard to restore burnt areas for production but usually promote negative effects on wildlife (Moreira *et al*., 2010; Mauri & Pons, 2019). However, depending on the wildfire effects and the current environmental policies, several post-fire mitigation measures can be applied on the affected area to prevent further degradation. Soil erosion prevention, wildlife preservation and conservation, plant cover and habitat regeneration, are the most relevant purposes of post-fire mitigation measures (Mauri & Pons, 2019). Overall, in each of these fields, many studies show how efficient they can be in preventing short-to-long-term wildfire effects but, information about how they affect wildlife communities is scarce (Beyers, 2003; Robichaud *et al*., 2009). Mulching is a soil erosion prevention measure commonly used in burnt areas that consists in applying a layer of residue (organic or inorganic) that protects the soil surface from erosion and has a high-efficiency rate at reducing runoff and promoting the development of ground cover (Girona-García *et al*., 2021). While the long-term mulching effects on soil invertebrates have already been addressed by Puga *et al*. (2016), information about mulching's short-term effects is still missing.

1.5 Objectives

The overall aim of this thesis is to enhance knowledge on the short-term effects of wildfire on faunal communities, with focus on ground dwelling invertebrates and mammals, in native and non-native forest ecosystems. The following specific research objectives were identified:

1) Assess the role of ground stones in the safeguard of ground-dwelling macroinvertebrate community from wildfire events in different forest types;

2) Compare the immediate and short-term effects of wildfires on ground-dwelling macroinvertebrates communities in native oak forests and planted forests of maritime pine and blue gum eucalypt;

3) Evaluate the short-term effects of the post-fire management technique -mulching- on the recolonization patterns of ground-dwelling macroinvertebrates in burnt areas;

4) Assess the short-term effects of wildfires on the area-use patterns of mammal communities in Mediterranean coastal pine forests.

Overall, we hypothesized that:

a) Natural occurring structures as stones can safeguard animals from direct wildfire effects;

b) More tolerant and adaptable *taxa* can survive wildfires and influence how the immediate post-fire recolonization of burnt areas occurs;

c) Wildfire effects on animal communities are more marked on planted than on natural forests;

d) Mammal community use of burnt areas decreases after the wildfire;

e) Mulching as a post-fire mitigation measure has minimum negative effects on ground-dwelling macroinvertebrate communities.

1.6 Thesis rationale and structure

In this thesis, we initially explore the role of stones in safeguarding macroinvertebrate biodiversity from immediate wildfire effects using a new methodology approach and compare how these stone-related communities of different types of forests and land managements responded to the same wildfire by analysing community structure, function, and their relationship with abiotic factors. With this approach, we differentiated macroinvertebrate responses to wildfires, listed the most tolerant and affected *taxa*, and identified how their post-fire recolonization process of burnt areas starts in native, non-native, natural, and production forests. Being production forests more exposed to human pressure, we used this information to assess how mulching, as a post-fire mitigation measure for soil erosion, potentially affects the same community during the initial phase of the recolonization of burnt areas. Finally, we adapted methodologies to improve the information collection regarding terrestrial vertebrates, in this case, mammals, and we were able to identify short-term post-fire area-use patterns for several *taxa* in coastal maritime pine forests. We focused our study in the central region of Portugal in the aftermath of 2017's wildfires using for the macroinvertebrate study the extensive burnt area around Pedrogão Grande and for the mammals study the burnt area near Quiaios, along the coast. The 2017's fires affected vast areas of maritime pine, eucalyptus, and oak forests, creating a rare occasion to conduct these studies within the same region, with high comparability between the same forest types and over extensive areas. The mulching study was also in central Portugal, but the area had burned in 2015, so fieldwork started and concluded before 2017.

This thesis is divided in 6 Chapters: Chapter 1 provides the contextualization and background information of the research topics, defines the objectives and hypothesis and the thesis' structure. The following four chapters (Chapters 2-5) include the series of scientific papers to be submitted to international peer-reviewed journals. Chapters 2 and 3 addresses the objectives 1 and 2 and add new information about the macroinvertebrate diversity in some of the most common forest types in Portugal. Chapter 4 responds to objective 3 by examining the potential negative effects of mulching, following up the information obtained in the previous chapters. Chapter 5 adds novel information related to short-time fire effects on mammals, addressing objective 4. Chapter 6 provides an integrated overview of the most important results from the earlier chapters and adds suggestions for conservation and management and identifies future research directions.

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Chapter 2

Submitted to *Forest Ecology and Management* in 12th December of 2021 as “Immediate impacts of wildfires on ground-dwelling macroinvertebrates under stones in Mediterranean oak forests” by Puga, J. R. L., Moreira, F., Keizer, J. Abrantes, N.

2 Immediate impacts of wildfires on ground-dwelling macroinvertebrate communities under stones in Mediterranean oak forests

2.1 Introduction

Native oak forests play an important role in promoting biodiversity, providing a valuable habitat for many plants and animals (Silva, 2007; Calviño-Cancela *et al*., 2012). However, over the centuries, human activities such as agriculture, wood harvesting, grazing, and urban expansion, have led to their decline. Across Europe, very few native forests are left (Depauw *et al*., 2019), and the situation in Portugal is not an exception. Oak forests, which had been the dominant native forest in Portugal for millennia, have been extensively reduced due to human-induced changes throughout centuries (Silva, 2007). This is particularly true for deciduous oak tree forests (*Quercus faginea* and *Quercus pyrenaica*), which are currently scattered in small patches across the country and represent just 3% of the national forest area (IFN6, 2015).

Albeit wildfires are an important natural phenomenon that has occurred for millennia, and has shaped native ecosystems, wildfire regime has been intensifying and now constitutes a challenging problem worldwide (Pausas *et al*., 2008; Moreira *et al*., 2011). In the last decades, Portugal has become one of the countries in the world that is most affected by wildfires, with an annual average burnt area of 140.000 ha (Doerr & Santín, 2013; Rego & Silva, 2014; San-Miguel-Ayanz *et al*., 2017), while future climate predictions point to a worsening situation in this region of the world (Flannigan *et al*., 2013).

As natural forests globally support more than half of the known terrestrial animal species (Brockerhoff *et al*., 2008; Yekwayo *et al*., 2016), it becomes fundamental to understand the effects of fires on these ecosystems.

Invertebrates have been pointed as a good indicators of soil disturbance, because of their ecological relevance in many natural processes such as nutrient cycling, organic matter decomposition, and mineralization, as well as their role in food web dynamics, (Bedano *et al*., 2016; Pedley *et al*., 2016; Swart *et al*., 2017; Abrantes, 2018).

Nevertheless, the fire impacts are poorly known for most invertebrate groups (Swengel, 2000; Doblas-Miranda *et al*., 2009; Saunders *et al*., 2021). Most studies on invertebrate fire ecology are focused on broader *taxon*omic or functional groups (Moretti *et al*., 2006; Caut *et al*., 2013), or focus only on specific *taxa* (York, 1998; Kiss *et al*., 2004; Teasdale *et al*., 2013). These approaches can sometimes simplify or broaden the information collected community-wise, often ignoring other ecological interactions such as specific microhabitats' community responses to post-fire on a given area. Additionally, many studies often use single sampling methods that rely on capture broader community spectrums, leading to biased interpretations of the results (Swengel, 2000; Abensperg-Traun & Steven, 2006).

Direct mortality, community shifts, species displacement, food scarcity after the fire, and habitat loss are among the most usual adverse effects of wildfire on faunal communities (Sgardelis *et al*., 1995; Moreira, 2010; Kim & Holt, 2012, New, 2014), often leading to a decrease in biodiversity. However, in that regard contradictory findings have been reported (Zaitsev *et al*., 2016). Some studies have shown that many groups of ground-dwelling invertebrates are negatively affected by fire from immediately afterwards up to several years after fire (Trucchi *et al*., 2009; García-Domínguez *et al*., 2010; Elia *et al*., 2012; Verble-Pearson & Yanoviak, 2014). By contrast, other studies have reported that ground-dwelling invertebrates (ranging from order to species level) are not affected by fire or to recover fast (Siemann *et al*., 1997) and even benefit from fire (Moretti *et al*., 2006; Jacobs *et al*., 2015).

Some groups of invertebrates start recolonizing burnt areas days after the fire, depending on their resilience to fire and behavior, as well as characteristics of the fire itself (Gongalsky *et al*., 2012; Kim & Holt, 2012). It is widely accepted that post-fire recolonization of the invertebrate community starts from nearby unburnt areas and unburnt pockets of forest within the burnt areas or pockets with low fire severity (Zaitsev *et al*., 2014). However, habitats such as burnt trees can often safeguard some species from the direct impacts of fire, for example saproxylic beetles (Ulyshen *et al*., 2010). Hence, burnt trees and other natural structures such as stones or crevices, may be of great relevance to post-fire recolonization processes. Nevertheless, information related to the ecological role of natural structures and micro-habitats is scarce, particularly during natural phenomenon such as wildfires (Abbot & Maitre, 2010; Ross *et al*., 2017).

In this study the immediate effects of wildfires on the composition of ground-dwelling community in Mediterranean oak forests are investigated, focusing on the role of stones as safeguard microhabitats. The proposed hypotheses are: i) stones safeguard their associated community from direct fire effects; ii) the feeding behaviour and habitat preference of the ground-dwelling macroinvertebrate community change with the wildfire occurrence; iii) the area and depth of stones play an important role in the survival and composition of the ground-dwelling macroinvertebrate community.

2.2 Materials & methods

2.2.1 Study area

The study took place in the municipalities of Pedrogão Grande, Figueiró dos Vinhos and Castanheira da Pêra in the Leiria District, located in Central Portugal, in an area affected by one of the most dramatic and devastating wildfires in the country that occurred in June 2017. This wildfire affected a total area of 45000 ha, being classified as a medium-to-high severity fire (ICNF, 2017) (Figure 2.1). The climate of area is identified as Csa and Csb according to the Köeppen-Geiger classification system, with an average annual temperature of 14.9°C and an average annual rainfall of 1010 mm. The soils are mainly derived from schist but locally from granite and quartz. The characteristic climax vegetation is a forest dominated by cork oak (*Quercus suber*), black oak (*Quercus pyrenaica*), and strawberry tree (*Arbutus unedo*) (Costa *et al*., 1998), but it is nowadays reduced to small patches. At present, mono-specific plantations of eucalypt (*Eucalyptus globulus*) and maritime pine (*Pinus pinaster*) are now the main forest types in the region (Oliveira *et al*., 2017).

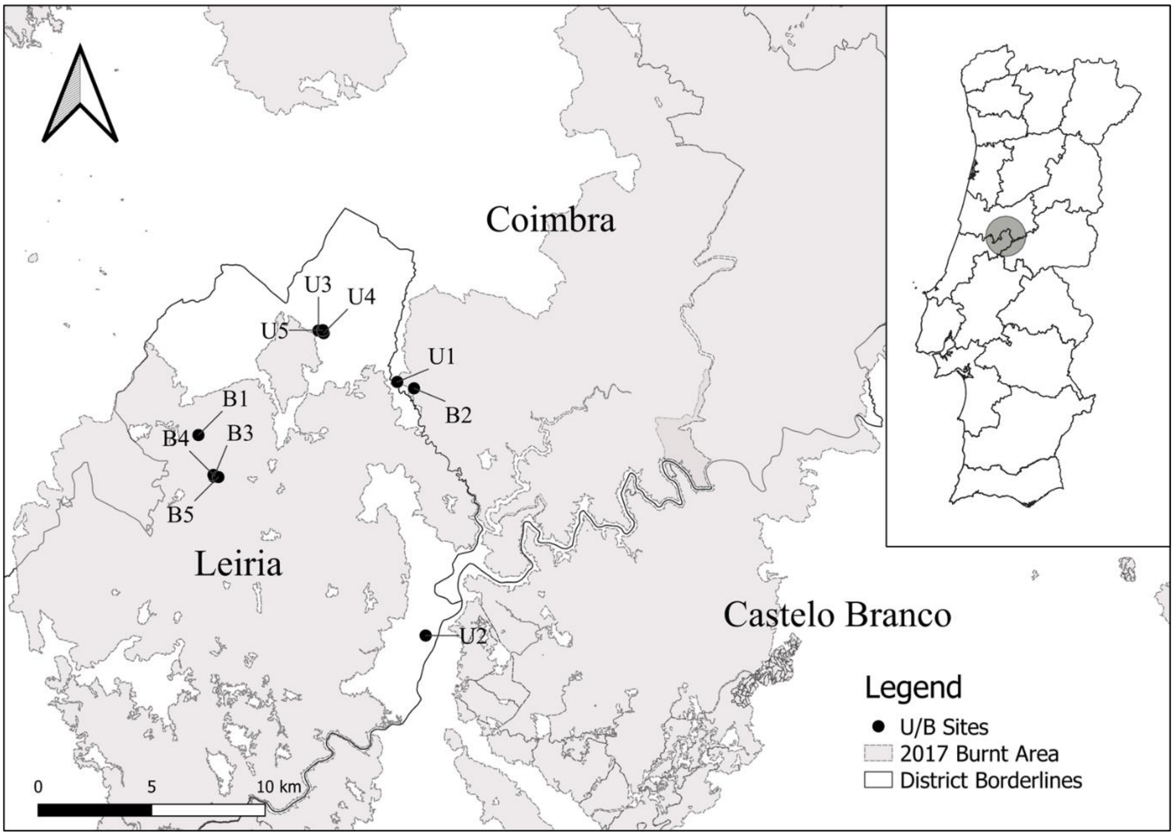

Figure 2.1 Location of the study area and sampling sites. Unburnt sites (U): U1, U2, U3, U4, U5; Burnt sites (B): B1, B2, B3, B4, B5.

2.2.2 Sampling design

The available CORINE land cover and Google Satellite maps (EEA, 2017; Google Earth Pro, 2017) were combined with the burnt area map (EFFIS, 2017), to pre-select stands of burnt and unburnt oak forest in the region (Figure 2.1). Field inspection of these sites then resulted in the selection of a total of 5 burnt and 5 unburnt study sites that were at least 500 m apart and were similar in terms elevation (500-800 m a.s.l.), orientation (S-SW), parent material (schist), tree age (> 50 years), land use, and, in the case of the burnt sites, fire severity using soil and vegetation assessments (Parson *et al.*, 2010). The selected sampling sites corresponded to mixed cork and black oak forest patches on former agricultural lands abandoned several decades ago.

The sampling of the ground-dwelling macroinvertebrate community took place less than one week after the wildfire. Each site was distanced at least 1 km apart and integrated 3 linear transects with 50 m, each divided into 11 points separated by 5 m. At each sampling point, the nearest stone that was larger than roughly 50 cm^2 but could still be lifted by hand was selected as sampling unit, so that a total of 330 stones was sampled across all 10 sites.

2.2.3 Stone characterization and ground cover

Each sampled stone was measured in terms of maximum length and perpendicular to it, width (to the nearest 1 cm) as well as its depth into the soil. Furthermore, next to each stone, a plot of 1m by 1m was laid out to visually estimate the percentage ground cover of stones (U = 23%; B = 31%), bare soil (U = 0%; B = 55%), litter (U = 58%; B = 0%), and vegetation (U = 19%; B = 14%).

2.2.4 Ground-dwelling macroinvertebrate sampling and identification

Immediately after removal a selected stone, all living and dead ground-dwelling macroinvertebrates were collected using aspiration (pooter) and direct collection by hand. All collected organisms were then immediately preserved in ethanol.

In the case of ant and termite nests with living specimen, a specific sampling approach was used: a) for smaller *taxa* the sampling consisted of 1-minute aspiration; b) for bigger *taxa* only a few specimens were captured by hand. All collected organisms were also preserved in ethanol. This data was analyzed separately to quantify the total number of nests and included only in the richness analyses.

For this study, only the ground-dwelling macroinvertebrate fauna (> 2 mm) was included in the analysis and identified as morphospecies. All collected specimens were identified to Family level, except for the snails and ants that were identified to Order and Genus level, respectively. The latter was done because there are only a reduced number of ant Families in Portugal (Harde & Severa, 1984; Goulet & Huber, 1993; Roberts, 1995; Barrientos, 1998; Czechowski *et al*., 2002).

2.2.5 Data analysis

The diversity indices of abundance (*n*), *taxon* richness (*s*), Shannon-Weiner's diversity index (H'), and Pielou's equitability index (J') were calculated for the individual sampled stones. Boxplots were used to contrast the data distribution between burnt or unburnt areas. Living and dead animals collected in the burnt area were treated and plotted individually, being designated by Burnt_L and Burnt_D, respectively. No dead animals were found in the unburnt area. Differences in the abundance, richness, diversity and equitability between the 1) Unburnt, 2) Burnt L and 3) Burnt D were tested for statistical significance using the non-parametric Kruskal-Wallis test. In the case of significant differences, the contrasts between the individual treatments were tested for statistical significance using the post hoc Least Significant Difference (LSD) test. Non-parametric tests were preferred as the various data sets did not meet the ANOVA assumptions of normality and homoscedasticity, following the Shapiro-Wilk and Levene tests.

Sample-based species rarefaction (Mao's tau) for the *taxa* with living specimens was assessed for the burnt and unburnt sites separately to estimate overall richness as a function of the number of samples (Colwell *et al*., 2004)

Non-metric multidimensional scaling (NMDS) was used to analyze the variation in community composition among the 10 study sites, combining the live animal data of all stones at each site. The measure of dissimilarity used in NMDS was Euclidean distance. In addition to NDMS, the differences in community composition between the burnt and unburn sites were tested for statistical significance using analysis of similarities (ANOSIM) with Euclidean distance as similarity index.

To evaluate the fire effects on community function, each *taxon* was classified according to their diet/feeding behavior and habitat preferences. Based on the existing bibliography, the feeding habits were divided into five categories: predator, omnivore, herbivore, fungivore, and detritivore. Also based on bibliography, habitat associations were divided into three categories: ground-dwellers, underground-dwellers, and plant-dwellers.

Then the distribution frequency among each class was calculated using family richness and total abundance of live specimens in the unburnt and burnt areas. Chi-square tests were then used to verify if the distribution of the feeding habits and habitat association of the ground-dwelling invertebrate community differed between the burnt and unburnt sites. In case they did, a post-hoc analysis was performed with adjusted residuals and a corrected Bonferroni p-value to identify which groups differed significantly between the classes of feeding habits and dominant habitat association. The Spearman rank correlation coefficient was used to quantify and test the relationship of the indices of abundance and richness with stone depth and area. The distribution of the ground cover classes in each unburnt and burnt site was obtained using the average class cover in each. All statistical tests used an alpha of 0.05.

2.3 Results

2.3.1 Immediate post-fire effects on community structure and diversity

Significant differences were found between the burnt and unburnt area for the abundance ($H = 88.05$, $p < 0.001$), richness ($H = 100.9$, $p < 0.001$), diversity ($H = 54.28$, $p < 0.001$) and evenness ($H = 14.69$, $p < 0.001$) of the ground-dwelling macroinvertebrate community found under stones (Figure 2.2).

Comparing the abundance and richness values obtained for live invertebrates in the unburnt area and in the burnt area (Burnt L) significantly higher values in the unburnt area were found ($H = 20.85$; $p < 0.001$ and $H = 21.83$; $p < 0.001$, respectively) (Figure 2.2). Higher diversity values were also observed in the unburnt area ($H = 19.15$; $p < 0.001$), while for evenness significantly higher values were observed in the burnt area ($H = 11.97$; $p < 0.001$) (Figure 2.2). A similar pattern was observed for dead animals (Burnt D), with significantly higher values of abundance ($H = 9.04$; $p < 0.01$), richness ($H = 17.03$; $p < 0.001$) and diversity ($H = 21.77$; $p < 0.001$) in the unburnt area, and higher values of evenness in the Burnt D ($H =4.31$; $p < 0.05$) (Figure 2.2).

Concerning the abundance and richness of live (Burnt L) and dead (Burnt D) invertebrates found in the burnt area on the same sample, slight but significantly higher values were found in the Burnt D for abundance ($H = 5.89$; $p < 0.05$) and richness ($H = 5.69$; $p < 0.05$) (Figure 2.2). The diversity values were similar, and no significant differences were found between the Burnt L and Burnt D sites ($H = 3.93$; $p > 0.05$). Evenness values were significantly higher in the Burnt L ($H =3.91$; $p < 0.05$) (Figure 2.2).

The NMDS also illustrates segregation between unburnt and burnt sites, (Figure 2.3). Likewise, the ANOSIM analysis showed significant differences between the burnt and unburnt areas (R = 0.2366; $p < 0.0001$).

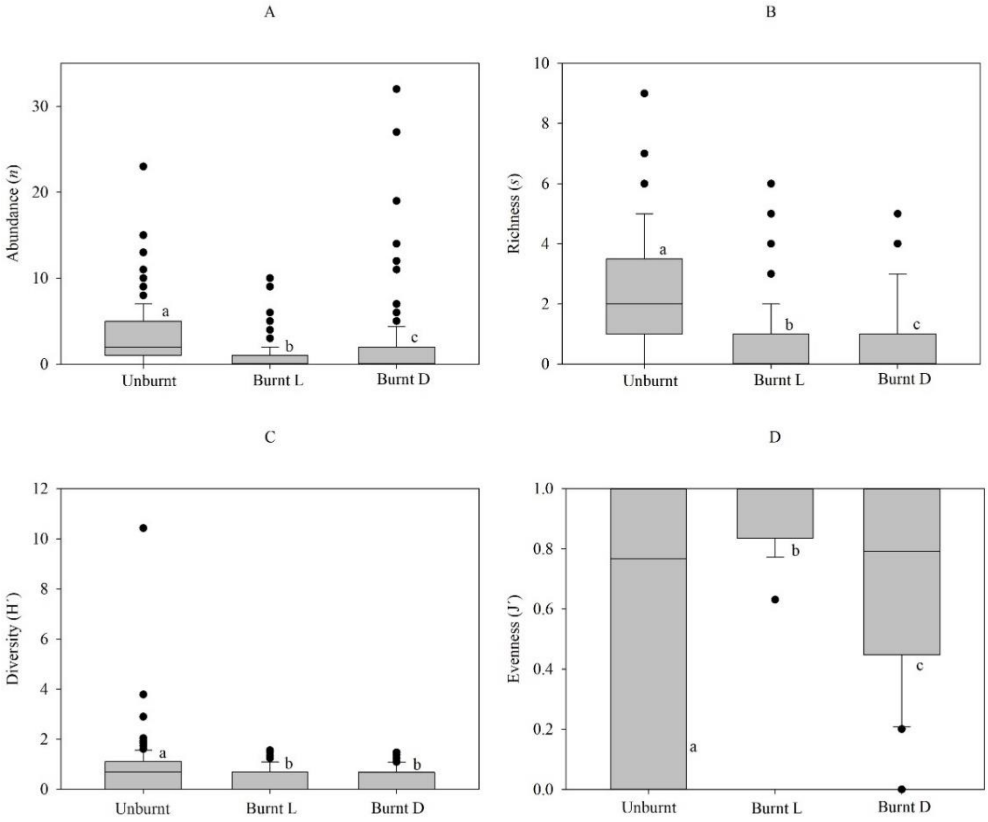

Figure 2.2 Abundance (*n*) (A), richness (*s*) (B), Diversity (H') index (C) and Evenness (J') index (D) of ground-dwelling invertebrates per stone (n = 165). Burnt L – live organisms collected in the Burnt area; Burnt D – dead organisms collected in the burnt area. Horizontal lines are the standard deviation. Distinct letters indicate significant differences between the three groups ($p < 0.05$).

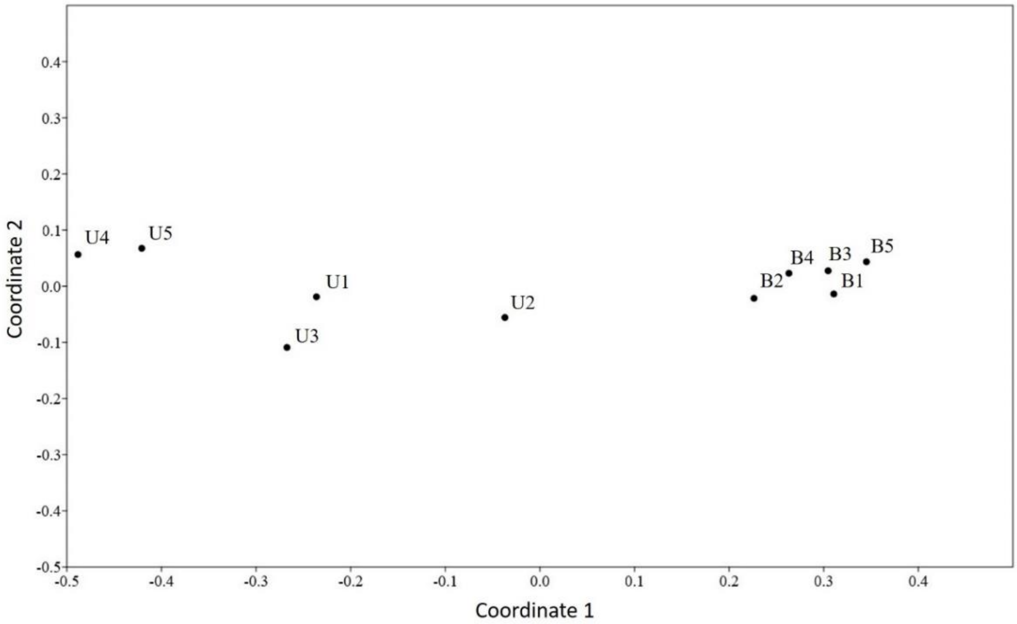

Figure 2.3 Non-metric multidimensional scaling (NMDS) plot for unburnt (U) and burnt (B) sites. Stress = 0.01.

A total of 72 distinct invertebrate *taxa* were identified in this study from a total of 4782 individuals. Of the 72 *taxa* found, 40 were exclusively found in the unburnt area, while 7 were only found in the burnt area.

In the unburnt area, the total number of individuals found was 4038, while in the burnt area was 744. From the total of organisms found under stones in the unburnt area, 3515 were ants living in nests. In the burnt area from 744 individuals, 359 were found alive, from which 251 were ants living in nests. The remaining individuals (385) were found dead under the stones, from which 93 were ants in nests.

Among live specimens in both areas, the more abundant groups were Araneae, Hymenoptera, and Isopoda (Figure 2.4). Other groups such as Coleoptera, Microcoryphia, and Orthoptera were less numerous, especially in the unburnt area, where several other less represented *taxa* also occur (Annex 1; Figure 2.4). In the burnt area, the dominant groups were the same as in the unburnt area, but Blattodea and Chilopoda have increased representativity. In other groups such as Lepidoptera, Zygentoma, Neuroptera, Pseudoscorpionida, Thysanoptera, and Pulmonata no live specimens were found (Annex 1).

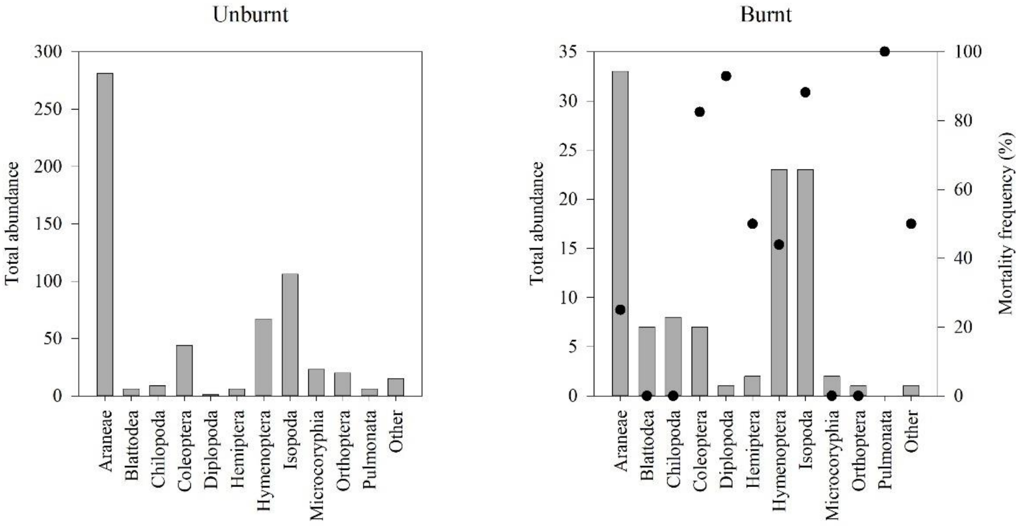

Figure 2.4 Total abundance per *taxa* (Order level), found in Unburnt and Burnt areas; Other *taxa* includes: Dermaptera; Lepidoptera, Neuroptera, Pseudoscorpionida, Thysanoptera, and Zygentoma); Black dots show the mortality frequency in each *taxa*.

The comparison between the list of live *taxa* (at Order level) in the unburnt and burnt areas showed that the most represented groups that compose the community in the unburnt area still occur in the burnt area, though their abundance was reduced (Figure 2.4). Fire induced high mortality in all burnt sites, exterminating more than half of the existent specimens. At the order level the most affected *taxa* were Pulmonata, Isopoda, Diplopoda, and Coleoptera, with a mortality rate between 80% and 100%; and less than 50% for both Araneae, Hemiptera, and Hymenoptera. Microcoryphia was the less affected *taxa* within the community. Concerning the Family level (Annex 1), a pronounced difference was observed between both areas, with 65 *taxa* found in the unburnt area and 29 in the burnt area. The number of families found in the burnt area was reduced, being in most cases half or less of the number of families found in the unburnt area. This reduction was also clearly evidenced by the rarefaction analysis (Figure 2.5). Despite the general decrease in biodiversity observed in most Orders in the burnt area compared to the unburnt, 7 *taxa* were identified only in the burnt area, corresponding to 4 Araneae, 1 Coleoptera, 1 Hemiptera, and 1 Isopoda families (Annex 1).

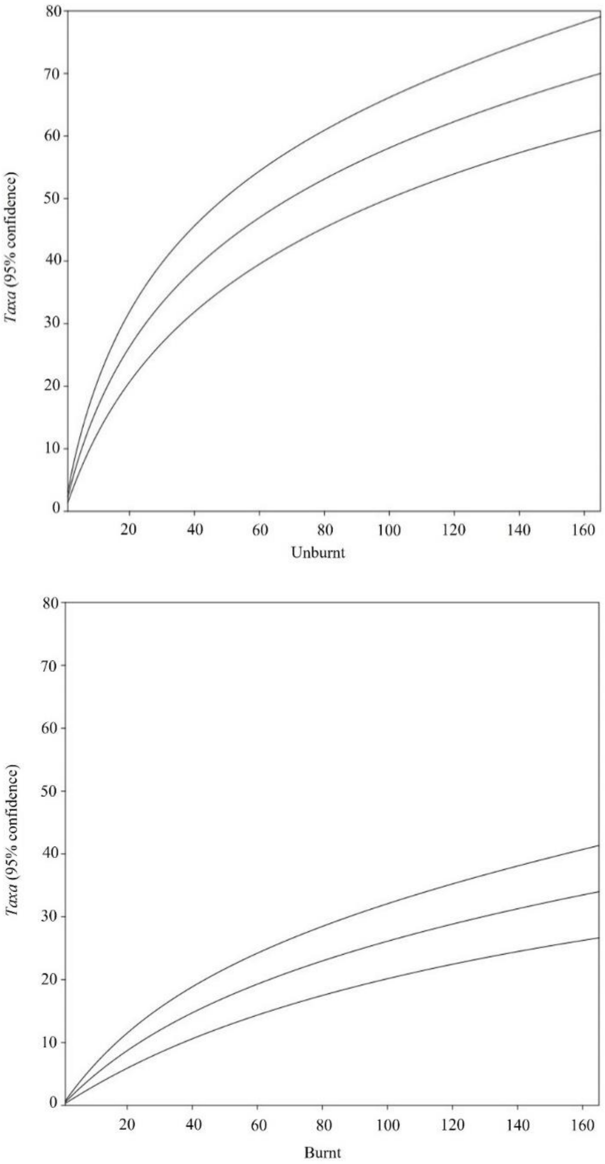

Figure 2.5 Sample-based rarefaction comparison between the unburnt and burnt areas (live *taxa* only). n = 165; upper and lower lines represent the 95% confidence intervals.

The number of alive ant nests found in the burnt area was lower than in the unburnt area, for most of the identified *taxa* (Figure 2.6), except for the genus *Camponotus*. Genus *Plagiolepis* was not found in the burnt area despite being the most abundant in the unburnt area. ANOVA results showed significant differences in the number of ant nests found between areas (H = 3.692, $p < 0.05$).

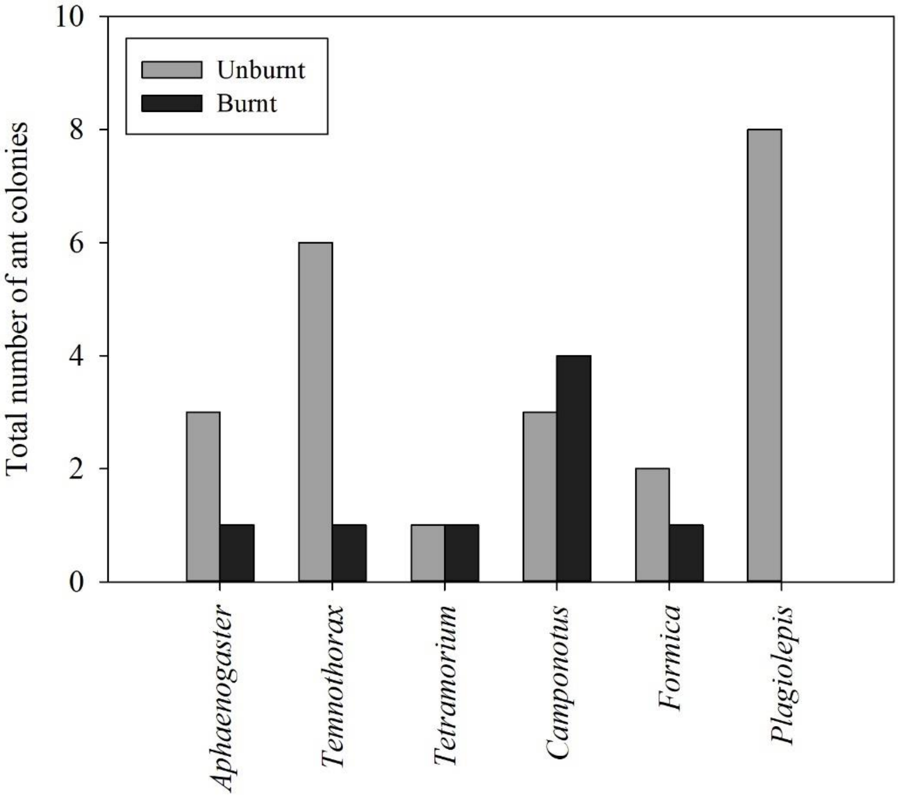

Figure 2.6 Total number of alive ant nests found (genus level) in unburnt and burnt areas.

2.3.2 Immediate post-fire effects on community function

Changes in the functional feeding behavior and habitat associations were observed in the burnt area, immediately after the wildfire, at family level richness (Figure 2.7A and 2.7B), and total abundance (Figure 2.7C and 2.7D). However, significant differences were only found between areas for diet (Chi-square = 18.963; $p < 0.001$) and habitat associations (Chi-square = 6.398; $p < 0.05$) regarding the overall abundance. A reduction in the number of predators of about 20% and fungivores to 0 was observed in the burnt area. In contrast, the relative number of omnivores, herbivores, and detritivores increased 10%, 5%, and 3%, respectively. Concerning the analysis at the family level, although no significant differences were detected, there was an increase in the number of predator and detritivore *taxa* of 3% and 11%, respectively, and a decrease of 11% in herbivore *taxa* and fungivores to 0, while omnivore *taxa* frequency remained unchanged.

Concerning the habitat association of the overall community an increase of 9% of plant-associated individuals was observed in the burnt area compared with the unburnt area, while the ground dwellers decrease 7% and the underground specimens became less than 1%. Family relative richness showed a similar trend in the burnt area, with ground dwellers being the dominant group totalizing 76% of the *taxa*, plant-associated *taxa* 21%, and underground dwellers 3%.

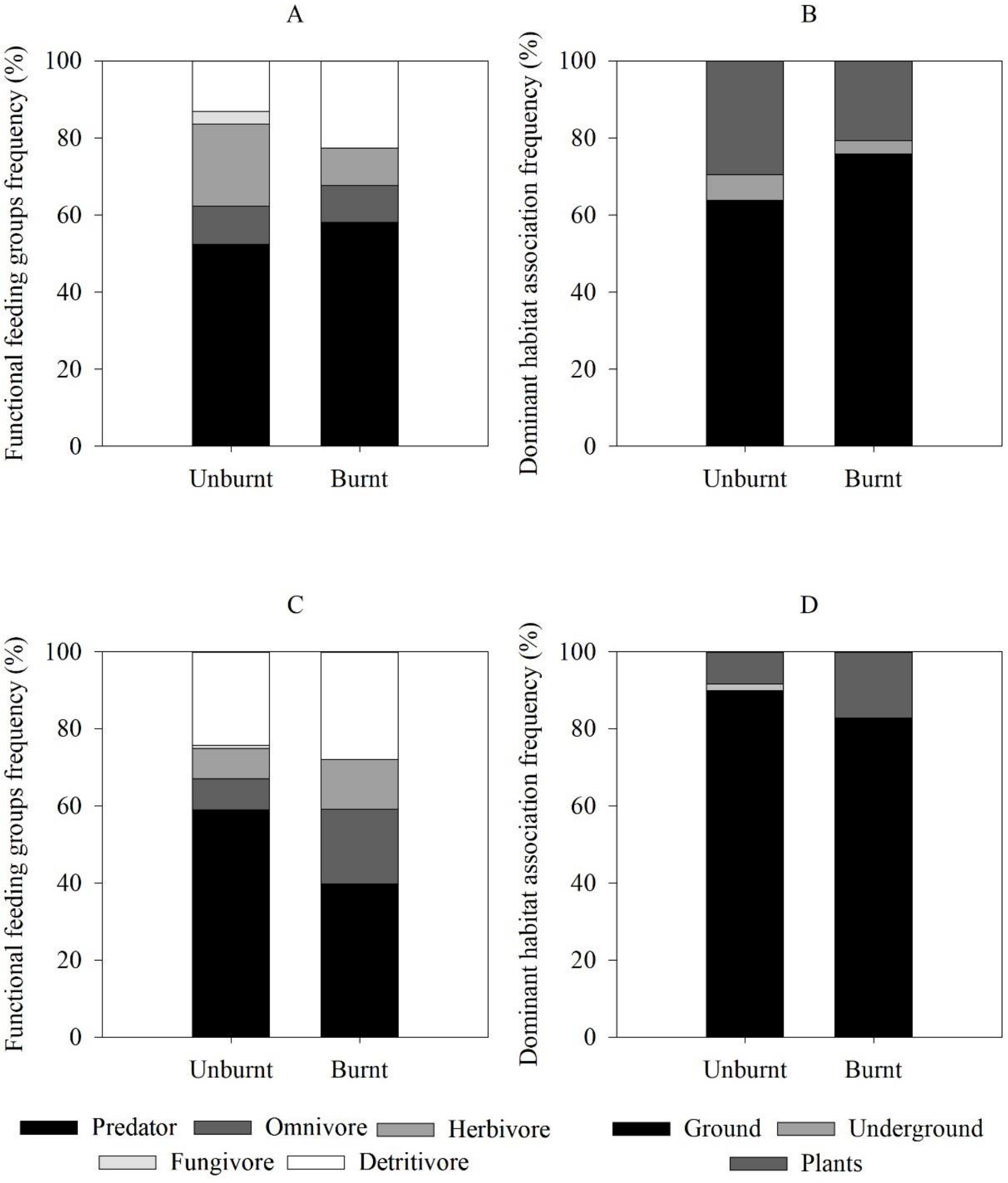

Figure 2.7 Ground-dwelling macroinvertebrate community functional feeding behavior and dominant habitat distribution frequency at the family level richness (AB) and for total abundance (CD) in unburnt and burnt areas.

2.3.3 Stone depth and stone area

Regarding the stones' features they varied between 50 and 2160 cm^2 of area and between 0.5 and 25 cm depth. The sampled stones showed an average area of was 594 cm^2 and an average depth of 6.9 cm, while in the burnt area, the average area was 499 cm^2 and the average depth 5.1 cm (Figure 2.8 and Figure 2.9).

The rate of occupancy of individuals living under stones differed between unburnt and burnt areas. In the unburnt area, 85% of the sampled stones showed specimens, while in the burnt area only 69% of the stones revealed individuals, but only 33% were alive. In the unburnt area, 92% of the stones without individuals had less than 500 cm^2, while in the burnt sites was 73%. In the unburnt area, of 15% of the samples without arthropods, 11% were buried above 5 cm in the ground, while in the burnt area that value was about 49%. Significant differences were found in the unburnt area between sampled stones with 0 individuals and stones with at least 1 individual (Chi-square = 18.857; $p < 0.001$) for depths under 5 cm regardless of stone area. In the burnt area, significant differences were only found between sampled stones with 0 individuals and stones with at least 1 individual (Chi-square = 15.436; $p < 0.001$) for stones with less than 500 cm^2 and less than 5 cm depth, and for stones with more than 500 cm^2 and more than 5 cm depth, regarding live individuals.

Concerning the relationships between the stone's characteristics and the ground-dwelling macroinvertebrate, was found that deeper buried stones were positively correlated with a high number of individuals ($rs = 0.230$; $p < 0.05$) and *taxa* ($rs = 0.253$; $p < 0.05$) in the burnt area. The same was observed for larger stones in the burnt area, which housed high number of individuals ($rs = 0.268$; $p < 0.05$) and *taxa* ($rs = 0.242$; $p < 0.05$). In the unburnt area the same relation between stone depth and stone area, with the number of individuals and *taxa*, was also observed. Stones buried deeper in the soil have a high number of individuals ($rs = 0.231$; $p < 0.05$) and *taxa* ($rs = 0.183$; $p < 0.05$). Likewise, larger stones also have a high number of individuals ($rs = 0.169$; $p < 0.05$) and *taxa* ($rs = 0.168$; $p < 0.05$).

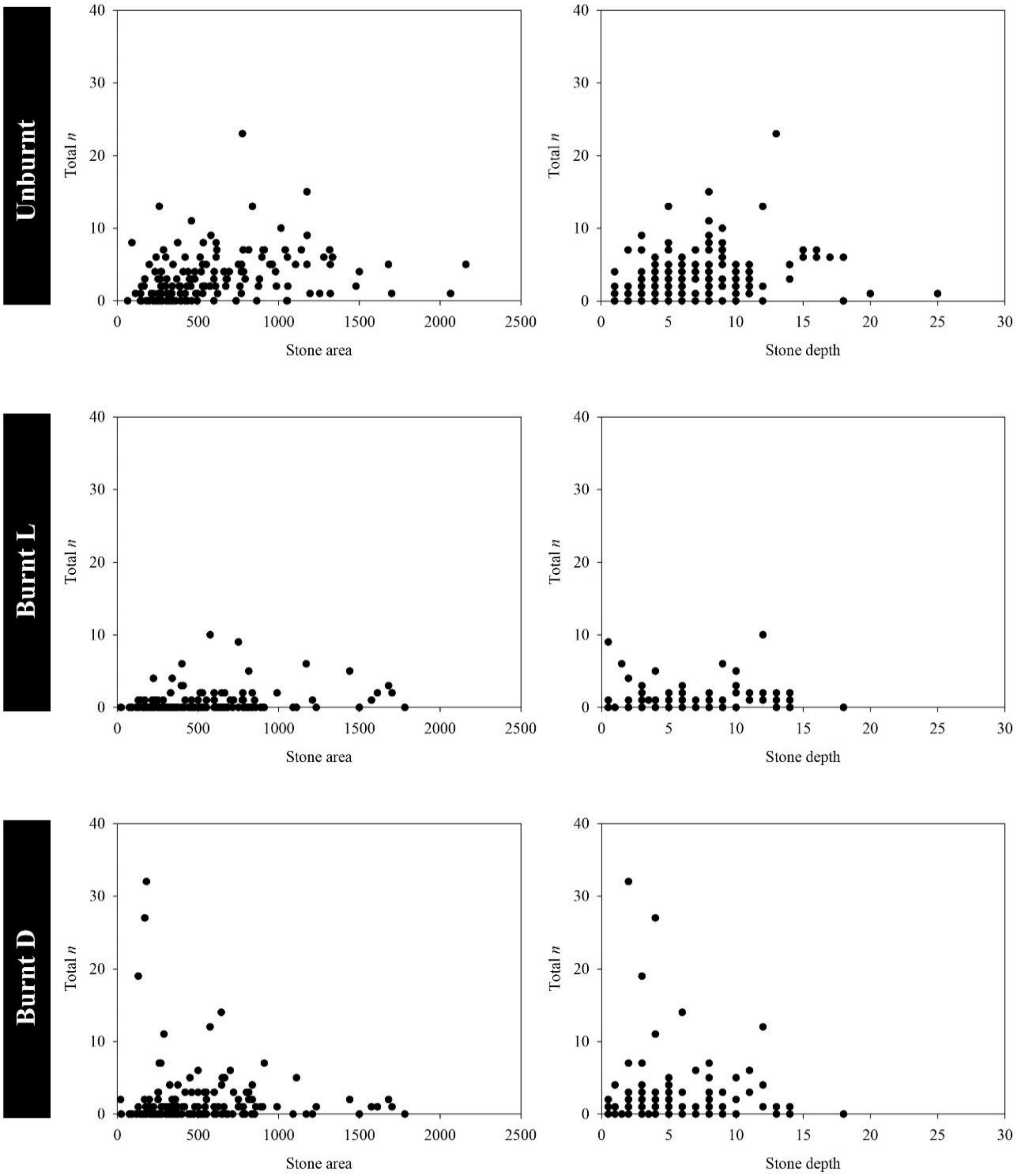

Figure 2.8 Total number of individuals (*n*) collected under each stone sampled according to stone area (left) and stone depth (right) in the unburnt and burnt areas. Burnt L – live organisms collected in the Burnt area; Burnt D – dead organisms collected in the burnt area.

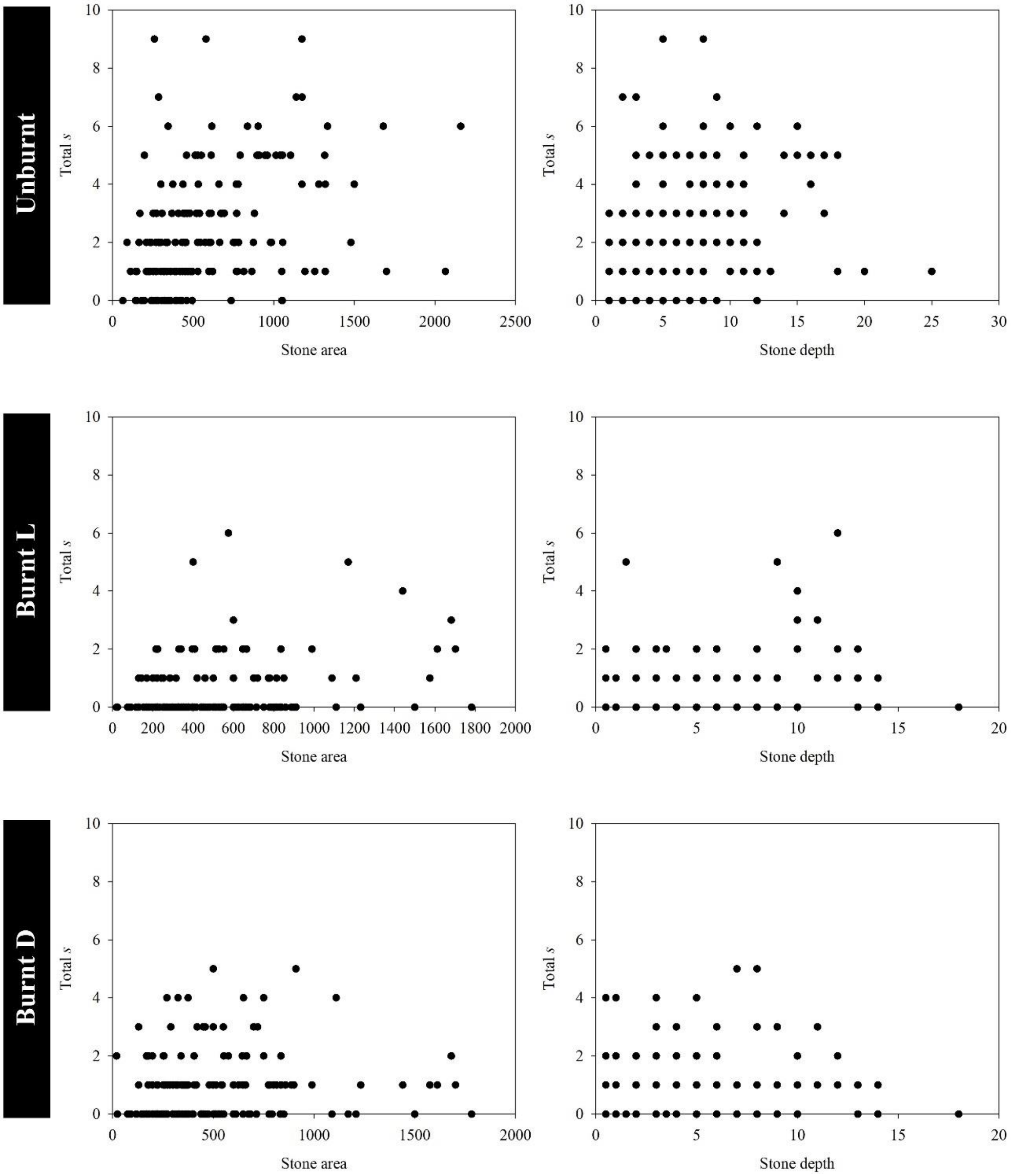

Figure 2.9 Total number of *taxa* (*s*) collected under each stone sampled according to stone area (left) and stone depth (right) in the unburnt and burnt areas. Burnt L – live organisms collected in the Burnt area; Burnt D – dead organisms collected in the burnt area.

2.4 Discussion

Several factors can influence the direct effects of fire on the soil faunal community, like the characteristics of the fire itself, soil humidity, available vegetation in the area, percentage of organic matter, and geography (Moreira *et al*., 2010; Pressler *et al*., 2018).

However, the role of microhabitats, such as ground stones, has been largely ignored. In this regard, the present study provided a new insight by revealing that stones are a safeguard microhabitat and can be a refuge for the ground-dwelling macroinvertebrate community in catastrophic events such as wildfires. Despite a generalized loss in richness, abundance, and diversity derived from mortality due to fire, stones effectively allowed the survival of a wide range of *taxa*.

The community found in burnt sites was mainly composed of ground-dwelling groups such as the Araneae, Hymenoptera (primarily ants), Coleoptera, and Isopoda. These results are like other studies on post-fire effects in ground-dwelling invertebrates in the Mediterranean basin (Sgardelis *et al*., 1995; García-Domínguez *et al*., 2010; Puga *et al*., 2016), as well as in other geographical regions (York, 1999; Wikars & Schimmel, 2001; Collet, 2003; Coleman & Rieske, 2006; Verble-Pearson & Yanoviak, 2014; Kwok & Eldridge, 2015).

The wildfire impacted the ground-dwelling macroinvertebrate diversity. Likewise, the multivariate analysis also segregated unburnt from burnt sites. Abundance and richness decreased in the burnt area shortly after the wildfire, as also observed by Wikars & Schimmel (2001), Coleman & Rieske (2006), or Verble-Pearson & Yanoviak (2014). Hence, the direct effects of fire in the ground-dwelling macroinvertebrate community lead to a loss in diversity in the affected areas, reducing it under each stone up to half of the initial value encountered in non-affected areas. Sample-based rarefaction analysis also confirmed this, between the diversity found in the burnt area in comparison with the unburnt area. Despite the reduction of the abundance of predators and the decline of fungivores, followed by an unequal increase in the number of omnivores, herbivores, and detritivores, the community functions were not compromised by the wildfire, as most Orders found in the unburnt area remained represented in the burnt area, despite an overall reduction in their number of families and reduced equitability. Kaynas (2016) found identical abundance and richness results on surface-dwelling arthropod communities of pine forests of Anatolia a month after a fire. Mason *et al*. (2021) also supports these results by showing several examples of the after-fire invertebrate resilience, even if, at some given moment, the community shaped to the changes that occurred in the ecosystem.

Regarding mortality, our results indicate that wildfire events can directly affect most of the *taxa* that form the community living under stones in native oak forests. The mortality rate obtained in the burnt sites in this specific community was above 50%. In Europe, Wikars & Schimmel (2001) also observed a high mortality due to the wildfire in soil invertebrates of boreal pine forests; the same was noticed by Trucchi *et al*. (2009) in different Italian native forest types of *Quercus*, *Pinus*, and *Populus*, and by Sgardelis *et al*. (1995) in Greek native phryganic vegetation ecosystems.

Mortality differently affected the community, and if for some groups it was high, for several other *taxa* the direct fire effects were less marked. As observed in other studies, a high rate of mortality was observed in animals whose specific traits makes them highly vulnerable to fire, including slow locomotion, moisture dependency, and body size, as previously observed by Moretti & Legg (2009) and Trucchi *et al*. (2009). As such, the ground-dwelling community can be divided in three groups according to their resilience to fire. The first group, with low mortality rates when comparing with the total abundance values found in the unburnt area, includes cockroaches (Blattodea), centipedes (Chilopoda), rock bristletails (Microcoryphia), and crickets (Orthoptera). Abbot *et al*. (2003) found that cockroach and cricket populations can rapidly recover from fire events, and results from Arnold *et al*. (2016) also suggest that cockroaches can survive to wildfires despite a reduction in diversity, in comparison with areas outside the burnt area. Trucchi *et al*. (2009) identified that despite changes in centipede composition, some species could survive to fires in deeper soil layers, which was also observed in the present study. As for rock bristletails, information is scarce, as they seem to be the less affected of all groups when comparing the total abundance values between the unburnt and the burnt areas. As suggested by Lewis (1981) for centipedes, our field information at the time of collection also showed that rock bristletails benefit from aestivation behavior by sheltering under deeper buried stones. The second group includes spiders (Araneae) and ants (Hymenoptera), on which the mortality rate was high, but the abundance of live animals found in the burnt area remained high when compared with the rest of the *taxa*. Notwithstanding, the richness and abundance of spiders decreased in the burnt area as observed by previous studies (Wikars & Schimmel (2001; Verble-Pearson & Yanoviak 2014). In our study, spider families associated with vegetation were the most affected, with several *taxa* absent from the burnt area. Most of the remaining spiders in the burnt area were ground-dwelling spiders using stones as hunting and nesting grounds. As mentioned earlier, behavior may have been a decisive factor for the differences in mortality among Araneae. Yekwayo *et al*. (2019) found similar results for spiders in rocky fynbos habitats 3 months after the fire, where a decrease in abundance and richness was also observed, and the survivability was attributed to potential existing refuges such as rocks and plants, as also suggested before by Pryke & Samways (2012). As for ants, both wandering individuals and ant nests suffered a high reduction in their number for all inventoried genera. *Plagiolepis* sp. nests were absent from the burnt area, despite being the most abundant in the unburnt area. This result raises questions about the wildfire's ability to shift the ground-dwelling macroinvertebrate community, whether temporarily or more permanently, particularly in areas subject to repeated wildfire, which deserves a special attention. In fact, previous studies that focused on fire effects on ants showed that these community shifts occur, favoring open land ant species at the expense of forest specialist ants (Andersen *et al*. (2009), Matsuda *et al*. (2011), and Anjos *et al*. (2017)). The absence of wandering individuals of *Crematogaster* sp. in the burnt area corroborates this assumption, as this *taxon* is associated with vegetation and commonly builds its nests in trees.

A third group, with the highest rates of mortality, includes the Orders Pulmonata, Isopoda, and Diplopoda, being all of them highly moisture dependent, and the first have slow locomotion, making them more susceptible to the direct effects of fire. Similar results were disclosed by Moretti *et al*. (2004) that identified a reduction of isopod's richness and abundance in burnt areas and by Sgardelis *et al*. (1995) that also observed a decrease of Isopoda and Diplopoda abundance after-fire, from which the latter remained for at least 2 years since the fire. Concerning the high mortality rate of snails, immediate after-fire effects on their populations can drastically reduce their abundance and richness, as also observed by Kiss & Magnin (2006). However, it seems that land snail populations are resilient to fire events, which in part is explained by the presence of refuges that allow their initial survival (Nekola, 2002; Kiss *et al*., 2004; Kiss & Magnin, 2006). Despite the ecological value of stones as microhabitat, the high number of isopods recorded (dead and live) in the burnt area compared to the unburn suggests that these animals besides using stones for nesting and foraging, could also have sought refuge under stones during the wildfire, thus explaining the anomaly high number of dead isopods under some of the stones sampled. This search for protection under stones during fire events could also have influenced the Coleoptera's mortality, which seems associated with body size, since mortality occurred mainly in larger specimens found nearer the outer perimeter of the stones sampled. Previous studies on immediate post-fire effects on ground-dwelling coleopterans have showed negative effects on both abundance and richness (Wikars & Schimmel, 2001; Moretti *et al*., 2004), followed by a shift in composition favoring pyrophilous species (Sasal *et al*., 2010; Fredriksson *et al*., 2020). Additionally, regarding the relationship between mortality and body size, it is important to consider that smaller individuals may have been totally consumed by fire. Therefore, the impacts of the wildfire on the lethality of the ground-dwelling invertebrates associated with stones may have been underestimated.

Notwithstanding, all groups have *taxa* with specific traits and behaviors, that when combined with the use of stones as a permanent or temporary refuge, shields part of their populations from the direct effects of fire.

Regarding the stones' characteristics, the abundance and richness of the ground-dwelling organisms tend to increase with larger stones and greater depths in both areas. In terms of habitat, larger stones support more individuals and species, contributing to higher biodiversity. Greater depths open the possibility of the co-existence of additional ecological niches and strategies that promote an increase in the number of species. Also, the bigger and more buried in the soil each stone is, the more protection it offers against fire. Between 5 to 15 cm seems to be a greater chance of encountering live specimens than above that depth. These results are in line with other studies showing that direct fire effects can be negligible few centimetres below the soil surface, as the high temperatures above soil surface tend to dissipate and rapidly decrease (New, 2014; DeBano, 2000; Moreira *et al*., 2010; Caut *et al*., 2013). In terms of stone area, it seems reasonable to assume that the larger the stone, the more isolated the center of each stone will be from the fire, providing a safer area for the animals living or seeking refuge under it.

As observed by other studies, refuges can assume crucial roles for many species during catastrophic events (Brennan *et al*., 2011; Gongalsky *et al*., 2012; Pryke & Samways, 2012). When considering the ground-dwelling invertebrate community associated with stones in Mediterranean oak forests, this study revealed a vast and diverse community, considering the average size of stones available in the forest ground. These microhabitats support many invertebrate species, mostly ground dwellers, which use stones as part of their ecological requirements. Evidence of the use of stones as nesting sites were found for several families of spiders (*e.g.*, Oecobiidae, Dysderidae, Zoridae), woodlice (Stenoniscidae, Cylisticidae, and Porcellionidae), rock bristletails (Meinertellidae), silverfish (Nicoletiidae), and for most of the identified ant and termite *taxa*. Evidence of stones used as hunting grounds for most of the predators, mainly spiders and centipedes (Lithobiidae and Geophilidae), and as foraging grounds for cockroaches (Ectobiidae), woodlice, rock bristletails, silverfish, crickets (Gryllidae), and ground beetles (Staphylinidae, Carabidae, Scarabidae, and Tenebridae), was also found. According to our data, stones also seem to be used as aestivation sites for Geophilidae and Meinertellidae species. All these *taxa* form the core community associated with stones in oak forests and are most of the community that prevails after a wildfire. At a trophic level, the invertebrate community directly associated with stones is dominated by predators, particularly ground spiders, either in terms of abundance or diversity, making them especially relevant in this microhabitat. In addition, several other *taxa* dwell under stones, where they can find temporary refuge, but its association is commonly attributed to plants and vegetation, being these mostly true bugs, moths, and beetles with low abundances, compared to the *taxa* described earlier.

The destruction of the vegetation and litter by the wildfire also reduces the number of plant and litter associated *taxa*, also consumed by the fire. Only few individuals belonging to families typically associated with plants such as Oxyopidae, and Philodromidae spiders, Cucujidae beetles, or Lygaeidae bugs, were found under stones, meaning that these animals actively sought refuge under the stones as a strategy to survive the fire.

Despite the mortality and the reduced abundance and diversity, many *taxa* survived and remained in the burnt area, suggesting that post-fire recolonization, in addition to the entrance of organisms from the unburnt peripheric areas, as suggested by previous studies (Zaitsev *et al*., 2014; Yekwayo *et al*., 2016; Swart *et al*., 2017), may also occur from refuge sites within the burnt area, such as stones.

The present study discloses the importance of stones in promoting the survival of ground dwelling organisms during the fire occurrence, emphasizing their particularly role as refuge. This information gains relevance as insufficient evidence about invertebrate diversity and ecology, particularly their responses to fire, limits the capacity to make evidence-informed decisions about post-fire land restoration and funding (Saunders *et al*., 2021).

2.5 Conclusions

The main conclusions of the present study were the following:

a) Stones act as refuge or habitat, safeguarding part of the ground-dwelling invertebrate community from the direct effects of fire.
b) Stones can influence the immediate after-fire recolonization process within the affected area as starting points for the ground-dwelling invertebrate community.
c) Feeding behavior and habitat association in the community changes after a wildfire, decreasing the overall abundance of specialist *taxa* while benefitting other more tolerant.
d) Stone area and depth are directly related to the survival and post-fire structure of the community, with higher rates of survivability for larger and deeper buried stones.

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Chapter 3

To be submitted to *Forest Ecology and Management* until 25th December of 2021 as "Immediate impacts of wildfires on ground-dwelling macroinvertebrates under stones in Mediterranean eucalypt and pine planted-forests" by Puga, J. R. L., Keizer, J., Moreira, F., Abrantes, N.

3 Immediate impacts of wildfires on ground-dwelling macroinvertebrates under stones in Mediterranean eucalypt and pine planted-forests

3.1 Introduction

Forest plantations are commonly associated with intensive land management that enhances production at biodiversity's expense, especially when involves tree species that need periodic silvicultural maintenance or have short-rotation regimes (Silva, 2007; Chaudhary *et al*., 2016). Plantation forests have lower levels of biodiversity than mixed natural forests and are less capable of providing ecosystem services linked to biodiversity (Brockerhoff *et al*., 2013). In non-native plantations, the biodiversity levels and ecosystem services tend to be even lower (Silva, 2007; Zhan *et al*., 2009; Calviño-Cancela *et al*., 2012; Wang *et al*., 2019). The Mediterranean basin is one of the most affected regions worldwide by wildfires each year (Doerr & Santín, 2013; Rego & Silva, 2014), further increasing human-related pressure on its forests. Climatic changes, inappropriate land management policies, and extensive monoculture plantations of highly fire-prone species, are the most cited reasons for this increase (Flannigan *et al*., 2013; San-Miguel-Ayanz *et al*., 2017).

Wildfires are crucial natural phenomena in many regions of the world, as they contribute to vegetation renewal, return nutrients to the soil, and promote the life cycle of many species that depend on it (Pausas *et al*., 2008). However, when the same areas are recurrently affected by them, and in conjugation with other human-induced factors, wildfires can promote an extensive list of direct and indirect harmful effects in the environment and to biodiversity. Soil erosion and degradation, hydrological regime alterations, water contamination, plant and animal mortality, and habitat alterations are some examples of the extent of potential effects of wildfires (Certini, 2005; Moreira *et al*., 2010; Belcher, 2013).

Soil properties can suffer a wide range of effects that vary over time, sometimes becoming permanent. The destruction of organic matter, the loss of nutrients and alterations in pH, bulk density, water repellence, structure stability, particle-size distribution, and decreased albedo are some of the most common effects on the physical and chemical properties of the soil induced by wildfires (Certini, 2005; Shakesby, 2011; Belcher, 2013). Soils are also affected by wildfires at a biological level, with changes in composition and abundance covering the entire soil fauna.

Soil fauna studies mainly focus on medium-to-long-term fire effects and the overall community or specific groups but rarely focus on microhabitats, especially those that existed before and that remain after a fire event. Burnt trees can prevent the death of saproxylic beetles in less severe fires (Ulyshen *et al*., 2010), but information related to other microhabitat-promoting structures is scarce (Abbot & Maitre, 2010; Ross *et al*., 2017). Several studies show that the depth of burn determines patterns of survival, colonization, and regrowth of plants and invertebrates (Schimmel & Granström, 1996; Wikars & Schimmel, 2001). Other studies refer invertebrates may survive fire events by burrowing in the soil, escaping to less burnt patches, and occupying refugia (Wikars & Schimmel, 2001; Trucchi *et al*., 2009; Verble-Pearson & Yanoviak, 2014; Zaitsev *et al*., 2014), but very few experiments were able to add more information about the processes behind this post-fire survivability. Research reports that *taxa* related to vegetation and litter, specialist *taxa*, and niche-specific *taxa* are the most affected (Vogel *et al*., 2010; Kim & Holt, 2012). Regional location may also be a differentiating factor in the post-fire response of some groups of invertebrates. But ecosystem's characteristics, in particular the type of forest associated with it and its management, seem to be the most relevant factors in the short-to-long term fire effects at a physical and chemical level and to the response of most invertebrate groups to fire worldwide (Barbéro *et al*., 2004; Kiss *et al*., 2004). Each passing year more studies add knowledge about the effects of fires on animals, however available information is still insufficient to counter the trend of increasing fires worldwide, as well as to minimize its impacts on ecosystems (Swengel, 2000; Doblas-Miranda *et al*., 2009; Saunders *et al*., 2021).

In Portugal, there has been a substantial increase in the area occupied by forest plantations during the last decades, first with pine (*Pinus pinaster*) and recently with blue gum eucalypt (*Eucalyptus globulus*). These plantations progressively replaced formerly agricultural and grazing areas in which the associated dominant vegetation was native. In the last decades, eucalypt plantations started to replace pine plantations because of their higher short-term profitability associated with the pulp paper industry (Mendes *et al*., 2004; Collins *et al*., 2013), and are now one of the most extensive forested habitats in the country (Oliveira *et al*. 2017). Since 2017 wildfires, have been an increase in public concern about these matters due to much of the national forest territory being fire-prone plantations, especially regarding the impact of wildfires in biodiversity, public health, and forestry-related economy (Comissão Técnica Independente, 2017).

In this study, the immediate effects of fire were investigated in the two higher occupancy types of plantation forestry in Portugal (maritime pine and blue gum eucalypt) by focusing on the role of stones as a microhabitat and its associated ground-dwelling macroinvertebrate community. Mortality and community structure of ground-dwelling macroinvertebrate assemblages living under rocks were compared in correspondent burnt and unburnt forest patches. It was also hypothesized that distinct types of forest management can lead to different post-fire responses of the ground-dwelling macroinvertebrate community at a regional level.

3.2 Materials & methods

3.2.1 Study area

The study area is between Pedrogão Grande, Figueiró dos Vinhos and Castanheira de Pêra (Leiria District, Central Portugal) within and in the vicinity of a recently burnt area affected by a large high severity wildfire that burned 45.000 ha at the end of June 2017 around these locations (Figure 3.1).

According to the Köeppen-Geiger climate classification system, the area is described as Csa and Csb and has 14.9 ºC of average annual temperature and 1010 mm of average rainfall. The soils in this region are schist or granite and quartz. Overall altitude is between 500 and 800 m. The native climax vegetation that characterizes this region is cork oak (*Quercus rubur*), strawberry tree (*Arbutus unedo*), and black oak (*Quercus pyrenaica*) (Costa *et al*., 1998). The native understory is *Pterospartum*, *Cistus*, *Erica*, and *Ulex* shrubs (Costa *et al*., 1998).

This region of Portugal is now occupied by extensive maritime pine (*Pinus pinaster*) and blue gum eucalypt (*Eucalyptus globulus*) plantations that reflect the expansion of paper pulp production and logging industries and the regression of the once-dominant native forest. In the last decades, eucalypt plantations began to replace pine plantations and are now the most common type of forest in the area (Oliveira *et al*., 2017).

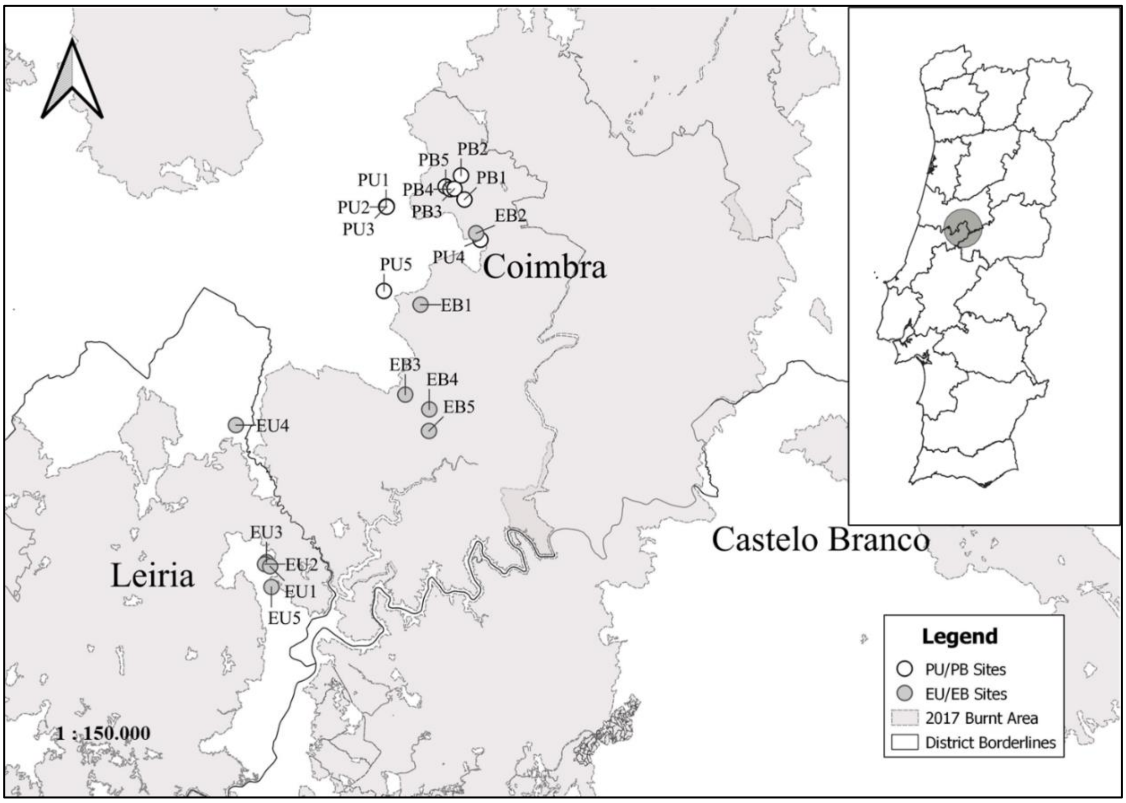

Figure 3.1 Location of the study area and sampling sites. U1, U2, U3, U4, U5 show pine (P) and eucalypt (E) unburnt sites. B1, B2, B3, B4, B5 show pine (P) and eucalypt (E) burnt sites.

3.2.2 Sampling design

The study was conducted between 26 of June and 13 of July (burnt areas between 26 of June and 3 of July; unburnt areas between 4 and 13 of July), immediately after the 2017 fires that devastated the region between 17 and 24 of June (Figure 3.1). For each type of forest plantations, pine and eucalypt, 5 sites were selected in the burnt area and 5 sites in the unburnt area. All sites were similar within each group regarding tree age (between 20 to 40 years for pines; between 8 to 12 years for eucalyptus), orientation (S-SW for all sites), land and forest management (pine sites were all plantations; eucalypt sites were all linear terrace plantations), altitude (between 600 to 800 meters for all sites), and soil characteristics (schist for all sites).

Each site was distanced at least 1 km apart and integrated 3 linear transects with 50 m, each divided into 11 points separated by 5 m. The largest stone (> 50 cm^2) nearest to each point was searched. A total of 330 stones were sampled for each stand type, 165 in the unburnt area and 165 in the burnt area. Rolling and not removable stones were excluded from the *in situ* selection. Each stone was also measured for width, length, and depth into the soil.

Soil cover surrounding the sampled stones was mostly litter in unburnt pine sites and stones in unburnt eucalypt sites. In both plantations burnt sites, soil cover was mostly stones and bare soil.

3.2.3 Invertebrate sampling and identification

In each site, stones were sampled for all alive macroinvertebrates under it through aspiration (using a pooter) and hand collection. In the burnt area, all dead specimens were also collected. All collected specimens were preserved in ethanol.

Only macrofauna (> 2 mm) was included in the analysis, registering separately live and dead animals. Specimens were identified as morphospecies to the Family level except for ants, which were identified to genera level because this group is represented by a reduced number of families in the Portuguese fauna (Harde & Severa, 1984; Goulet & Huber, 1993; Roberts, 1995; Barrientos, 1998; Czechowski *et al*., 2002), and land snails, which were identified at the Order level.

For ant and termite nests was used a different methodology, 1-minute aspiration per stone for smaller *taxa*, and hand-collected larger *taxa*. Nest-related data was analyzed separately to quantify the total number of nests found in each plantation type.

3.2.4 Data analysis

Indices of abundance (*n*), species richness (*s*), Shannon-Weiner diversity index (H’), and Pielou equitability index (J’) were calculated for each sampled stone and used to compare the data distribution between burnt and unburnt areas for each plantation type. Living and dead animals were analyzed separately only for burnt sites, as the latter were absent in the unburnt sites. Differences in abundance, richness, diversity and equitability between burnt and unburnt (for live animals) were tested for statistical significance using the non-parametric Kruskal-Wallis test. In the presence of significant differences, the contrasts between the individual treatments were tested for statistical significance using the post hoc Mann-Whitney test. On the rare occasions when the Kruskal-Wallis test was rejected, Dunn's post hoc test was used. As most data sets did not meet the ANOVA assumptions of normality and homoscedasticity, non-parametric tests were used, following the Shapiro-Wilk and Levene tests.

For each forest type, sample-based species rarefaction (Mao’s tau) was used to assess burnt and unburnt sites and estimate overall richness as a function of the number of samples (Colwell *et al*., 2004).

Non-metric multidimensional scaling (NMDS) was used to analyze the variation in community composition under stones among the 10 study sites (unburnt and burnt) of each forest type combining the live animal data of all stones at each site, using Euclidean distance as the measure of dissimilarity. In addition to NDMS, the differences in community composition between the burnt and unburn sites for each forest type were tested for statistical significance using analysis of similarities (ANOSIM) with Euclidean distance as similarity index.

For the evaluation of the fire effects on the community function, each *taxon* was classified according to diet/feeding behavior and habitat preferences. Based on the existing bibliography (Harde & Severa, 1984; Barrientos, 1988; Goulet et Huber, 1993; Buddle *et al*., 2000; Czechowski *et al*., 2002; Collet, 2003: Moretti *et al*., 2006; Andersen *et al*., 2009; Gongalsky *et al*., 2012; Kim & Holt, 2012; New, 2014; Lissner, 2014; Zaitsev *et al*., 2014; Barton *et al*., 2017; Nentwig *et al*., 2021; Oger, 2021), feeding habits were divided into four categories (predator, omnivore, herbivore, and detritivore) and habitat associations into three categories (ground-dwellers, underground-dwellers, and plant-dwellers). Distribution frequencies of richness and total abundance of live specimens in the unburnt and burnt areas were then calculated. Chi-square tests were used to verify if the distribution of the feeding habits and habitat association of the ground-dwelling invertebrate community differed between the burnt and unburnt sites of each forest type. In case they did, a post hoc analysis was performed with adjusted residuals and a corrected Bonferroni *p*-value to identify which groups differed significantly between the classes of feeding habits and dominant habitat association. Spearman's rank correlation coefficient was used to quantify and test the relationship of abundance and *taxa* richness with stone depth and stone area.

3.3 Results

3.3.1 Fire effects on community structure and diversity

Rarefaction analysis shows a reduction to nearly half the *taxa* in burnt areas compared to unburnt for both forest types (Figure 3.2). In pine stands, it decreased from 39 to 17, and in eucalypt stands from 38 to 14.

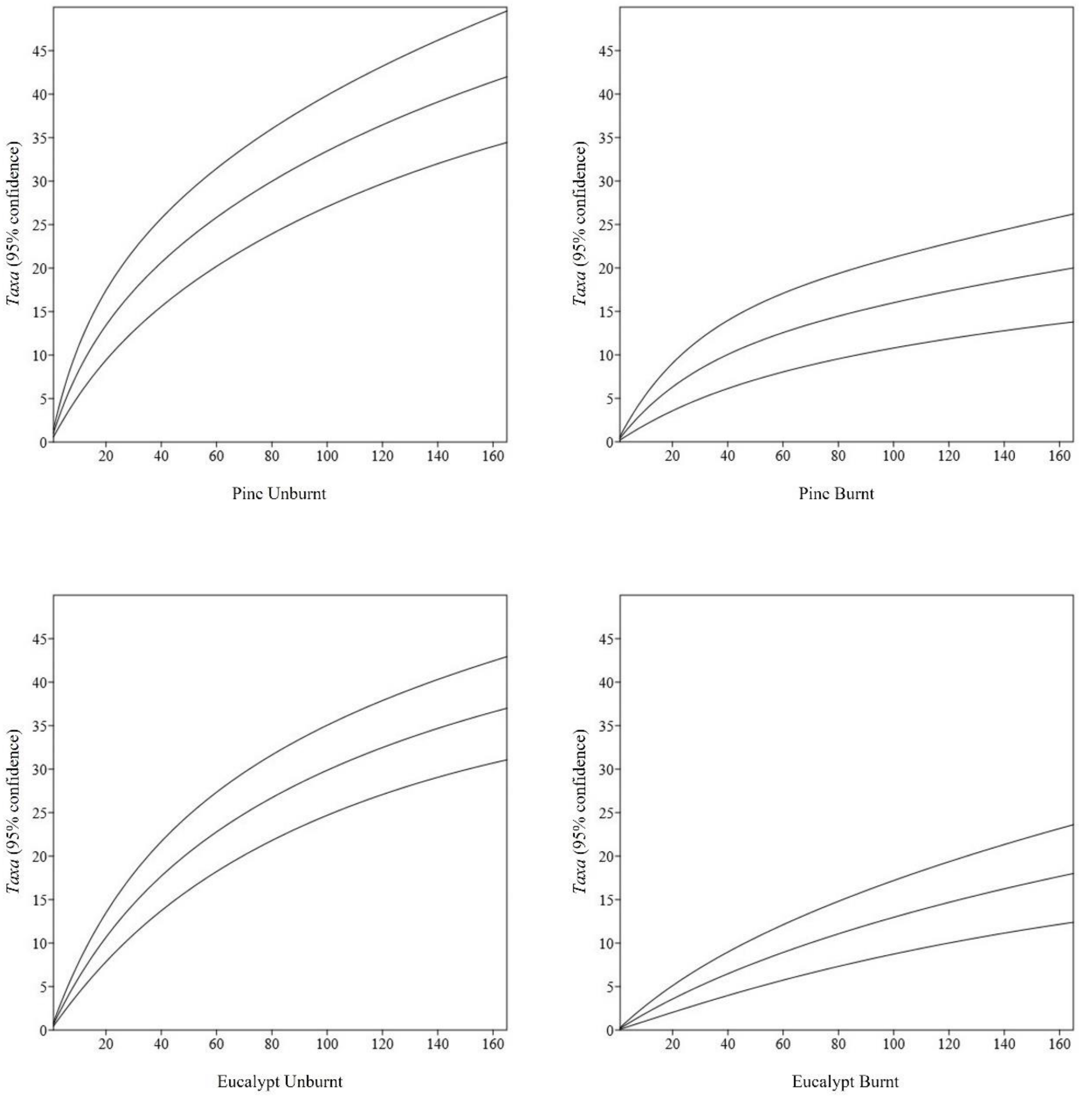

Figure 3.2 Sample-based rarefaction for each type of forest (pine and eucalypt) in the unburnt and burnt areas. *n* = 165; upper and lower lines represent the 95% confidence intervals.

Significant differences were found between unburnt and burnt areas of pine plantations with a decrease in the abundance (H = 28.550, $p < 0.001$), richness (H = 54.845, $p < 0.001$), and diversity (H = 10.554, $p < 0.005$), but not in the evenness of the ground-dwelling macroinvertebrate community found under each stone (Figure 3.3). Significant differences were also found between unburnt and burnt areas of eucalypt plantations with a decrease in the abundance (H = 29.328, $p < 0.001$), richness (H = 35.732, $p < 0.001$), and diversity (H = 15.105, $p < 0.001$), but also not for evenness (Figure 3.3). Between pine and eucalypt plantations unburnt areas significant differences were found regarding abundance (H = 9.747; $p < 0.001$) and richness (H = 15.577; $p < 0.001$) under each stone, while between burnt areas significant differences were also only found for abundance (H = 16.757; $p < 0.001$) and richness (H = 12.364; $p < 0.001$) under each stone (Figure 3.3).

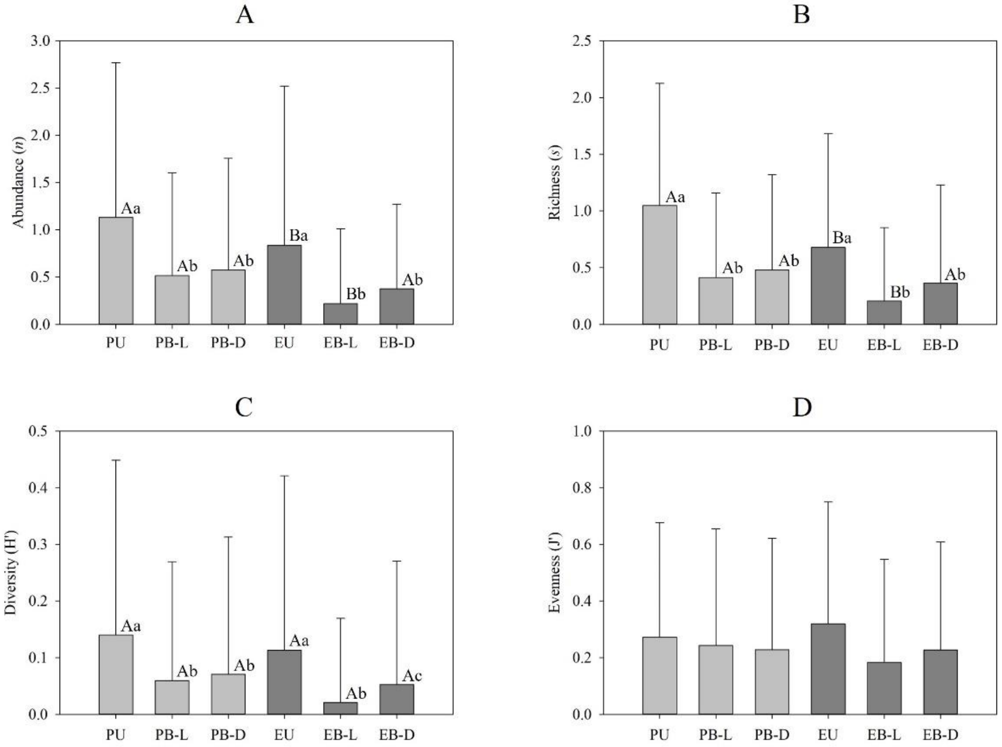

Figure 3.3 Average abundance (A), richness (B), diversity (C) and evenness (D) found under each stone in unburnt (U) and burnt (B) pine (P) and eucalypt (E) plantation areas. In the burnt areas live (B-L) and dead (B-D) specimens were analyzed separately. Horizontal lines are the standard deviation. Distinct letters indicate significant differences ($p < 0.05$): capital letters – between plantations; lower case letters – within each plantation. Stone is the sample unit (n =165).

NMDS results separate unburnt from burnt sites for both forest types and at the same time show overall homogeneity among sites within the same area, except for some eucalypt burnt sites where homogeneity is lower (Figure 3.4). The ANOSIM analysis supports NMDS results, showing high homogeneity among sites and minor but significant differences between the burnt and unburnt sites belonging to pine (R = 0.0658; $p < 0.0001$) and to eucalypt (R = 0.0459; $p < 0.0001$) stands, and between pine and eucalypt plantations unburnt (R = 0.027; $p < 0.001$) and burnt (R = 0.02072; $p < 0.001$) areas

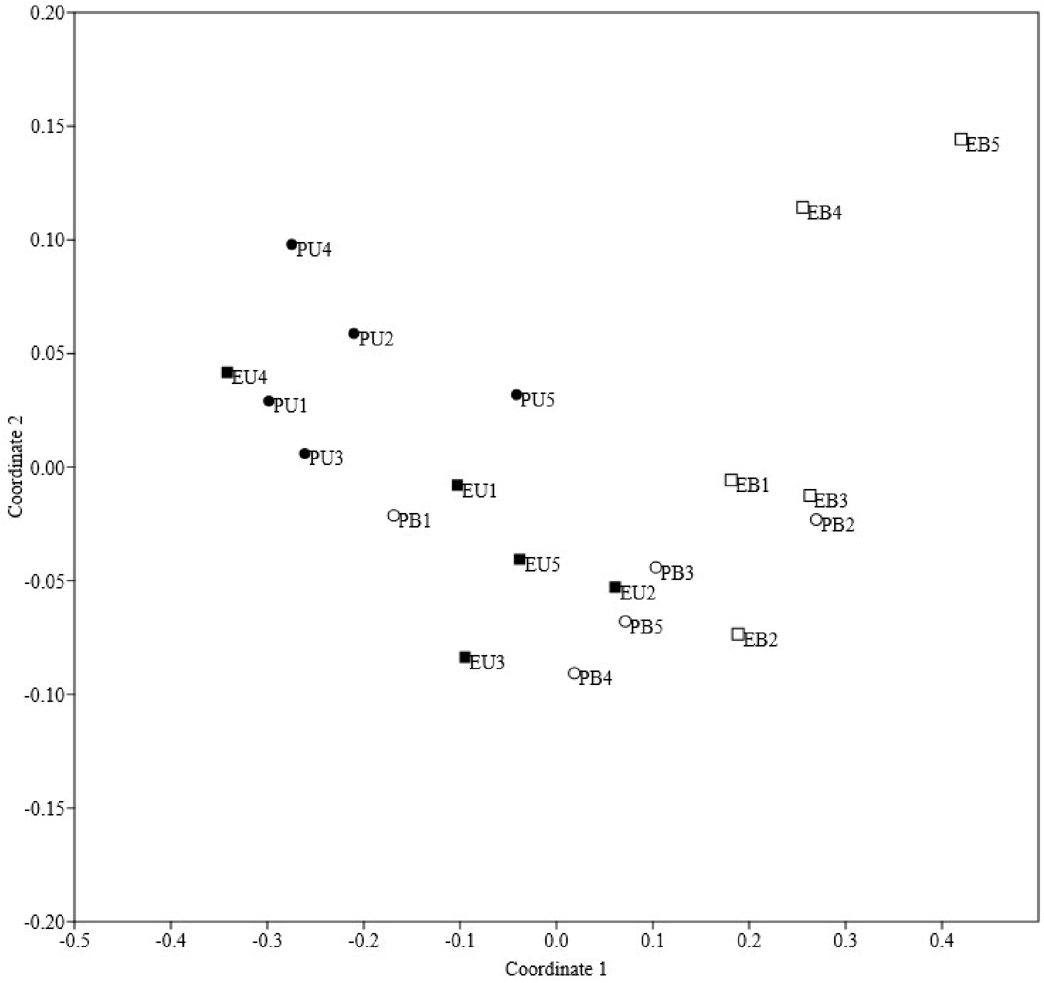

Figure 3.4 Non-metric multidimensional scaling (NMDS) plot for unburnt (U) and burnt (B) pine (PU and PB) and eucalypt (EU and EB) stand sites regarding abundance, richness, diversity and evenness. U1, U2, U3, U4, U5 show unburnt sites. B1, B2, B3, B4, B5 show burnt sites; Stress = 0.01.

A total of 2752 specimens associated with stones was found in pine stands. From the 38 *taxa* found, 20 were exclusively found in the unburnt area and 2 only in the burnt area. In the unburnt area, the total number of individuals found was 2503, while in the burnt area was 249. In the burnt area from 249 individuals, 153 were alive, of which 68 were ants in nests. The remaining 95 individuals were found dead under the stones.

The invertebrate community found in eucalypt stands accounted for 1599 specimens. Among the 37 *taxa* detected, 23 were found exclusively in the unburnt area and 3 only in the burnt area. In the unburnt area, the total number of individuals found was 1181, while in the burnt area was 418. In the burnt area from 418 individuals, 330 were alive. The remaining 88 individuals were found dead under the stones.

Spiders (Araneae) and ants (Hymenoptera) dominate the community found in unburnt pine sites, which includes other abundant *taxa*, mostly ground dwellers related to litter, such as rock bristletails (Meinertellidae) and woodlice (Isopoda) (Annex 1; Figure 3.5). Beetles (Coleoptera) have some relevance due to their overall diversity, and cockroaches (Blattodea) and harvestmen (Opiliones) due to their abundance. In burnt pine sites, the dominant and more representative groups of the community found there are the same as in the unburnt area, except for ants (Hymenoptera), in which the number of genera found compared to unburnt sites decreased from 7 to 1. No specimens from Order Hemiptera, Orthoptera, Pseudoscorpionida, and Pulmonata were found alive in burnt pine sites.

Spiders and ants are the dominant *taxa* found under stones in unburnt eucalypt sites, and together with cockroaches, beetles, and rock bristletails, form most of the macroinvertebrate community (Annex 1; Figure 3.5). In burnt eucalypt sites, these same *taxa* maintained their dominance, while several other *taxa* encountered in unburnt eucalypt sites such as true bugs (Hemiptera), woodlice, crickets (Orthoptera), and land snails (Pulmonata) were not found in the burnt area.

In both forest plantations, the comparison between the list of alive *taxa* (at Order level) shows that the most represented groups of the invertebrate community in unburnt still occur in burnt areas (Figure 5), despite a decrease in biodiversity at the Family level (Annex 1). Fire-induced mortality was high and decreased the overall abundance of most groups to half or less of the values encountered in both pine and eucalypt unburnt areas (Figure 3.5).

High mortality was found among spiders, beetles, and woodlice in burnt pine sites, varying between 60% and 80%. As for land snails, mortality reached 100%. Crickets and true bugs were also not found in burnt pine sites after the wildfire. Evidence of high mortality on ants was not found, but the number of live specimens collected in burnt pine sites suffered a 95% reduction compared to unburnt pine sites. Cockroaches, centipedes (Chilopoda), rock bristletails, harvestmen, and ground spiders were the less affected.

In burnt eucalypt sites, the mortality for spiders and cockroaches was below 60%, and for beetles, woodlice, and land snails ranged from 80% to 100%. True bugs, crickets, and earwigs were not found in the burnt eucalypt area after the wildfire. Ant mortality suffered a reduction of about 65% compared to unburnt eucalypt sites. The less affected groups in eucalypt burnt sites were centipedes, rock bristletails, and harvestmen.

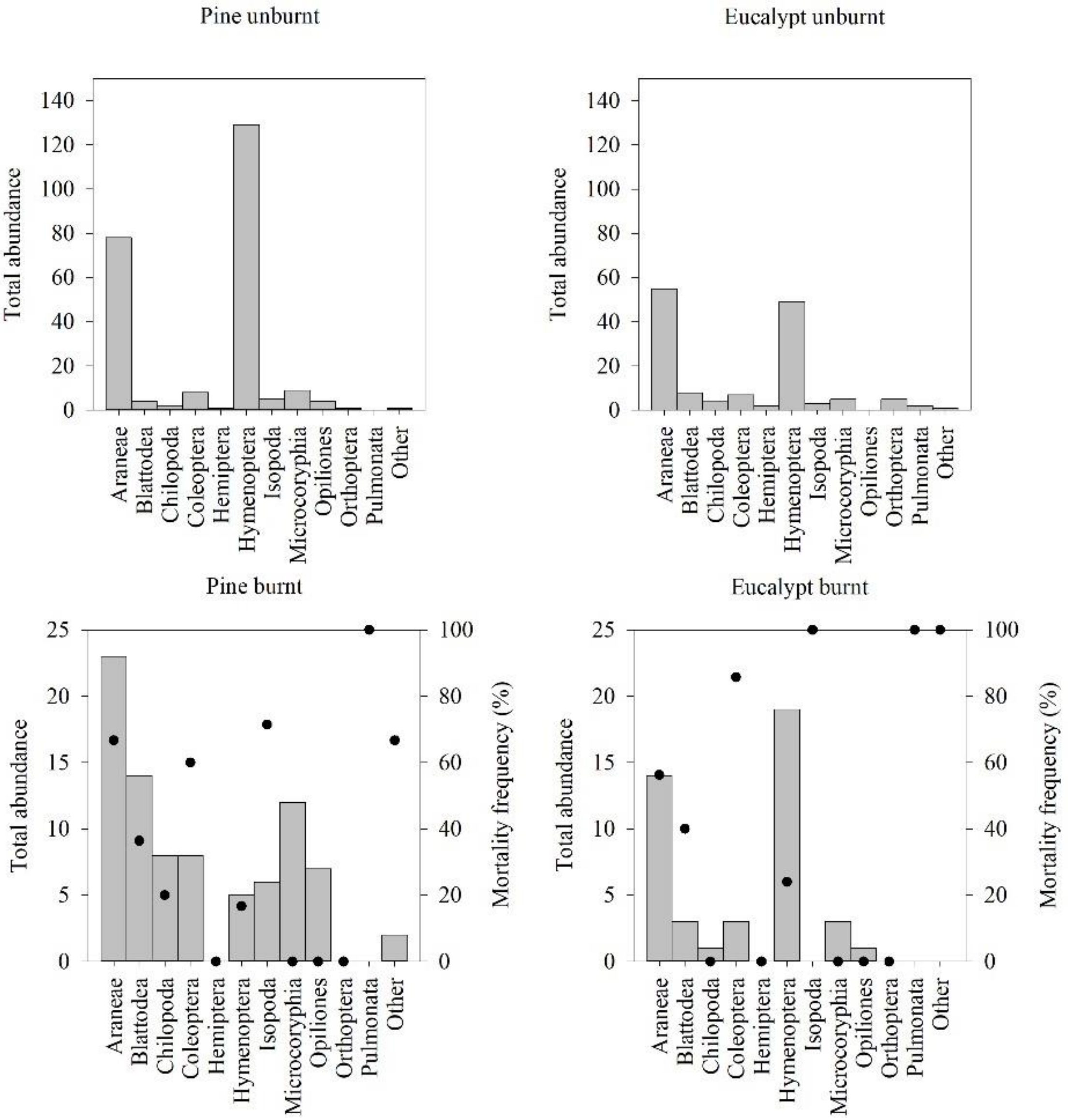

Figure 3.5 Total number of alive specimens per *taxa*, found in pine and eucalypt unburnt and burnt areas (Order level; Other *taxa* includes: Dermaptera; Lepidoptera and Pseudoscorpionida); Black dots show mortality frequency in each *taxa* observed in pine and eucalypt burnt areas.

Colonial insects found under stones in the study area comprise two major groups: ants and termites. Ant nests were more abundant and diverse than termite nests in both forest type stands. A total of 29 ant nests from 7 genera was found in the unburnt pine sites and only one in the burnt pine sites, corresponding to a decrease of 97% of ant nests in the pine burnt area (Figure 3.6).

In eucalypt unburnt sites, the total number of ant nests was 14 from 4 genera, and in the burnt area, while maintaining most ant genera, the number of nests suffered a reduction of 79% (Figure 3.6). The dominant genus in both unburnt forest stands was

Plagiolepis sp., but at the same time, it was also the only absent in both plantation's burnt areas. Regarding termites, 3 Rhinotermitidae nests were found, one in each unburnt plantation area and one in the eucalypt burnt area (Figure 3.6).

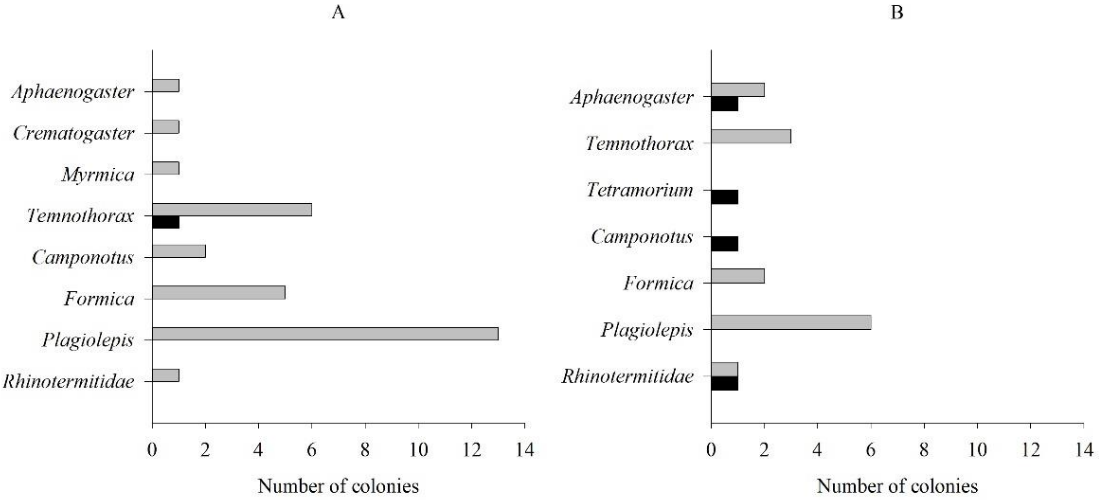

Figure 3.6 Total number of alive ant and termite nests found in pine (A) and eucalypt (B) unburnt (gray bars) and burnt (black bars) areas.

3.3.2 Immediate post-fire effects on community traits

After the wildfire, minor changes were observed in feeding behavior and habitat associations in burnt pine and eucalypt areas regarding richness and total abundance (Figure 3.7 and Figure 3.8). However, significant differences were only found between unburnt and burnt pine areas for diet (Chi-square = 21.713; $p < 0.001$) regarding the overall abundance of detritivores.

The frequency of feeding habits regarding richness, among the ground-dwelling macroinvertebrate community in the burnt pine area, remained identical to the unburnt area (Figure 3.7). Habitat association also suffered some transformations in the burnt area, with the absence of underground *taxa* and an increase of 10% of ground dwellers frequency (Figure 3.7). Omnivores and herbivores abundance's decreased from 32% to 18% and 12% to 7% in the burnt pine area. The frequency of predators remained identical, but detritivores increased from 5% to 23% in the burnt pine area (Figure 3.7). Habitat association in the burnt pine area showed a 7% decrease of plant-associated specimens and an increase of

6% of ground dwellers, while underground-associated individuals became more than 1% (Figure 3.7).

At the richness level, predators increased in the burnt eucalypt area from 58% to 71% and omnivores from 12% to 14% (Figure 3.8). Herbivores decreased from 18% to 7% and detritivores from 12% to 7% (Figure 3.8). Regarding habitat association, an increase of ground dwellers was observed from 70% to 79% in the burnt eucalypt area, while underground dwellers and plant-associated *taxa* decreased from 9% to 7% and from 21% to 14%, respectively (Figure 3.8). In terms of abundance, the diet association comparison between the unburnt and burnt eucalypt areas showed an increase in predators from 50% to 69% and in herbivores from 17% to 19%, while omnivores decreased from 26% to 12% and detritivores from 6% to 0% (Figure 3.8). The same comparison, but regarding habitat association, showed a 16% decrease in the number of ground dwellers in EB while underground and plant dwellers increased from 11% to 17% and 11% to 21%, respectively (Figure 3.8).

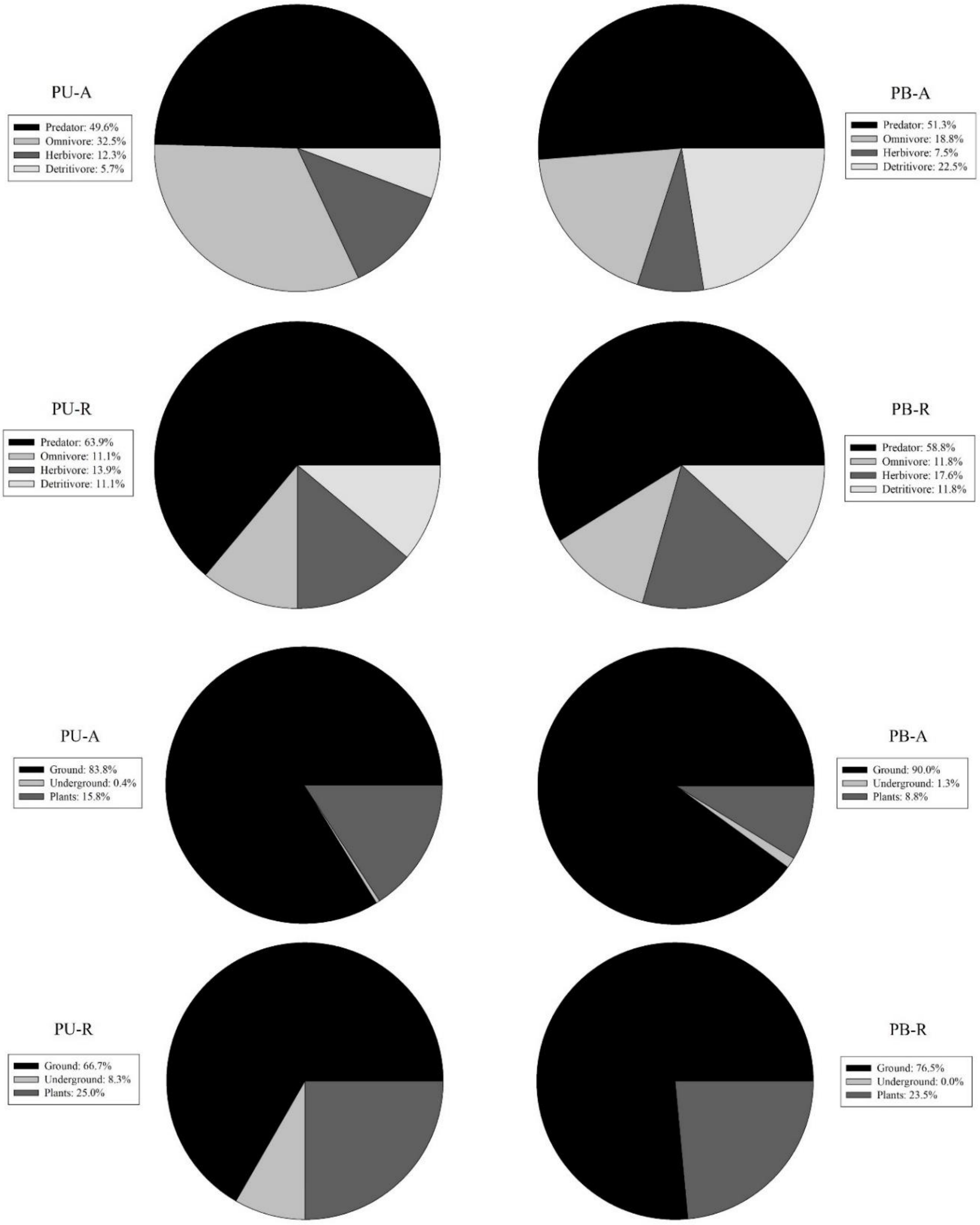

Figure 3.7 Influence of ground-dwelling macroinvertebrate community functional feeding behavior and dominant habitat distribution frequency per abundance (A) and per richness (R) in unburnt (U) and burnt (B) pine stands.

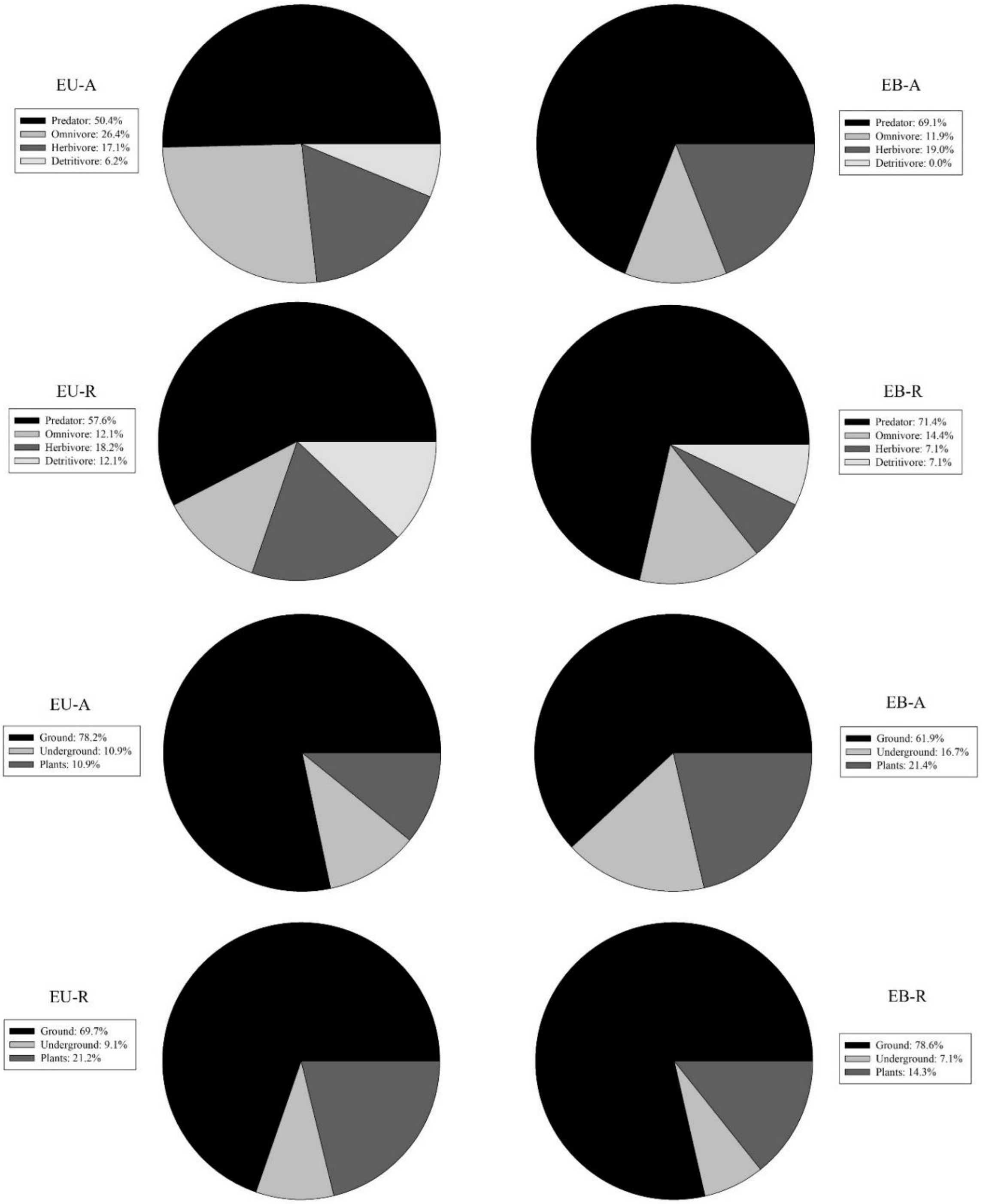

Figure 3.8 Influence of ground-dwelling macroinvertebrate community functional feeding behavior and dominant habitat distribution frequency per abundance (A) and per richness (R) in unburnt (U) and burnt (B) eucalypt stands.

3.3.3 The role of stone depth and stone surface area

Stone surface area and stone depth in pine stands varied between 50 and 1960 cm^2 and between 0.5 and 24 cm, respectively. Sampled stones in unburnt pine sites had an average area of 248 cm^2 and an average depth of 4.6 cm, while in the burnt, the average was 343 cm^2 and the average depth 4.9 cm.

In eucalypt stands surface area and depth of stones varied between 50 and 1943 cm^2 and between 0.5 and 29 cm, respectively. Stones sampled in unburnt eucalypt sites had an average surface area of 489 cm^2 and an average depth of 5.7 cm, while in the burnt was 267 cm^2 and the average depth 4.4 cm, respectively.

For each forest type, the occupancy rate of macroinvertebrates under stones varied between unburnt and burnt areas. In unburnt pine sites, 55% of the sampled stones had at least one individual. In burnt pine sites, 50% of the stones had specimens, but only in 17% of those stones, the animals were alive. In the unburnt pine area, of 45% stones without individuals, 96% had less than 500 cm^2, while in the burnt area of a 50% total of stones without individuals, the equivalent value was 90%. Of the 55% stones without arthropods found in the unburnt pine area, 66% were buried above 5 cm in the ground, while in the burnt pine area, those values were 50% and 75%, respectively. In the unburnt pine area, significant differences between sampled stones with 0 individuals and stones with at least 1 individual (Chi-square = 18.857; $p < 0.001$) were found for depths under 5 cm and areas under 500 cm^2 and stones with depths above 5 cm and areas above 500 cm^2. In the burnt pine area, significant differences between sampled stones with 0 individuals and stones with at least 1 individual (Chi-square = 16.185; $p < 0.001$) were found regarding stones with less than 500 cm^2 and less than 5 cm depth.

In the unburnt eucalypt sites, 36% of the sampled stones had at least one individual. In burnt eucalypt sites, 32% of the stones had individuals, but only in 11% of those stones, the animals were alive. In the unburnt eucalypt area, of 74% stones without individuals, 69% had less than 500 cm^2, while in the burnt area of a 68% total of stones without individuals, the equivalent value was 92%. Of the 64% stones without arthropods found in the unburnt eucalypt area, 54% were buried above 5 cm in the ground, while in the burnt eucalypt area, those values were 69% and 66%, respectively. Nor in the unburnt nor burnt eucalypt areas were found significant differences regarding stone depth, stone area, and presence or absence of individuals living under stones.

Regarding the relationships between stone characteristics and the ground-dwelling macroinvertebrate community, deeper buried stones were positively correlated with abundance (*rs* pine = 0.777; *rs* eucalypt = 0.860; *p* < 0.05) and richness (*rs* pine = 0.987; *rs* eucalypt = 0.830; *p* < 0.05), but only in the unburnt sites of both plantation types.

3.4 Discussion

Some studies (e.g., Brennan *et al*. (2011); Gongalsky *et al*. (2012); Pryke & Samways, 2012) suggested that refuges could have vital roles for many *taxa* during natural disasters, such as wildfires. In this study, to test that hypothesis regarding the role of stones in wildfires, the community of animals associated with stones was studied for the first time, and, despite the human-made alterations and management that both plantations are periodically submitted, stones as microhabitats, still support a relevant community of invertebrates. The present study revealed that stones are microhabitats that shield, to a certain extent, the ground-dwelling macroinvertebrate community from wildfire effects. Stones successfully allowed the survival of a broad range of *taxa* on maritime pine and blue gum eucalypt plantation forests, but with a decrease in richness, abundance, and diversity derived from mortality due to fire. The abundance and richness found under each stone were significantly higher for pine plantations, for both burnt and unburnt areas, compared to eucalypt plantations. Diversity and equitability, however, remained identical after the wildfire, regardless of forest type.

Abundance and richness decreased in the burnt pine sites soon after the wildfire, as also observed by Wikars & Schimmel (2001), Coleman & Rieske (2006), Verble-Pearson & Yanoviak (2014) and Kaynas (2016) in pine woodlands, leading to a loss in diversity of more than half of the initial value found in unburnt sites. Identical results were obtained in the burnt eucalypt sites, in line with other studies performed in eucalyptus forests where invertebrate diversity decreased after the wildfire (York, 1999a; York, 1999b; Bradshaw *et al*., 2018).

Macroinvertebrate biodiversity associated with stones in pine and eucalypt plantations is similar and formed by ground-dwellers that use stones as nesting sites and as hunting and foraging grounds, from which spiders (Araneae) and ants (Hymenoptera) are the dominant *taxa*. The most abundant groups of ground-dwelling macroinvertebrates are ground spiders (e.g., Agelenidae, Dysderidae, Gnaphosidae, Lycosidae), rock bristletails (Meinertellidae), cockroaches (Ectobiidae), harvestmen (Phalangiidae), and ants (*Aphaenogaster* sp., *Camponotus* sp., *Formica* sp. and *Plagiolepis* sp.). Several other *taxa*, more commonly associated with vegetation, are also found under stones such as snout and bark beetles (e.g., Curculionidae, Scolytidae), true bugs (e.g., Anthocaridae, Reduviidae), and some spider families (e.g., Araneidae, Mimetidae, Thomiisidae). Also associated with stones in both plantations are *taxa* with underground habits such as centipedes (Geophilidae) and termites (Rhinotermitidae). Despite the diversity found on unburnt pine and eucalypt plantations, ground-dwellers are what remains of the macroinvertebrate community immediately after a wildfire, forming the core community associated with stones in both plantation types. In a non-fire scenario, eucalypt plantations present lower values of abundance, richness, and diversity per stone than pine plantations, which tend to promote less balanced communities. These differences can be attributed essentially to the type of forest itself, as maritime pine is native and blue gum eucalypt is a non-native species, and to differences in the associated land management used for each type of plantation.

On a function level, predators dominate the stone-associated community of unburnt pine and eucalypt plantations, particularly ground-spiders and ants. Centipedes (Chilopoda) and harvestmen (Opiliones) are predators associated with stones but less abundant. Omnivores are the second more abundant feeding behavior, from which cockroaches (Blattodea) and ants are the more common *taxa*. Herbivores and detritivores are less abundant and have lower diversity, probably because most of the *taxa* within these two groups is associated with plants and litter, seeking stones for temporary refuge rather than for feeding. While litter availability can be high, plant diversity and availability are low on both unburnt plantation areas, making herbivore and detritivore abundance and diversity lower. After the wildfire, diet and habitat associations suffered minor changes in abundance and Family richness for both plantation's burnt sites. Overall, there is an increase in the relative abundance of predators, favored by lower mortality rates among ground-spiders, centipedes, and harvestmen, but their richness decreases. The frequency of all other feeding strategies tends to decrease abundance and richness, except for detritivores in burnt pine sites, which increased because of low mortality among rock bristletails (Microcoryphia). Changes in habitat association are related with plant-associated *taxa*, whose abundance and richness decrease due to their high mortality rate.

The wildfire caused high mortality among the ground-dwelling macroinvertebrate community, but its extent varied with each *taxon*. The less affected groups were centipedes, rock bristletails, and harvestmen. The few studies related to fire effects on centipedes show that these animals can survive fires in deeper soil layers, but not without changes in composition (Trucchi *et al*., 2009), as also observed in this study. In both plantations, the abundance and richness of centipedes varied little from unburnt to burnt sites. For rock bristletails the information regarding fire effects is scarce, but our results show that they were the least affected by the wildfire in both plantations, and that may be due to aestivation or avoidance behaviors as suggested for different *taxa* in other studies (Lewis, 1981; Moretti

& Legg, 2009). Recently, Brantley (2020) added information related to wildfire effects on harvestmen concluding that, as for several ground-spiders, the fire effects were less severe, and differences in their populations were related more to distinct habitat characteristics than to the wildfire. High mortality among spiders and ants was identified, and for the latter, an abrupt reduction in the number of nests in both burnt pine and eucalypt areas. For these two groups, the mortality rate was high, but the abundance of live individuals found in both burnt plantation areas continued high in comparison with the rest of the ground-dwelling macroinvertebrate groups. The few studies focusing on short-term fire effects also observed a decrease in spider and ant abundance and richness (Wikars & Schimmel, 2001; Verble-Pearson & Yanoviak 2014). In our study, however, the mortality rate and loss of spider diversity were higher among plant-associated spiders, but less in ground-dwelling spiders, being the latter most of the spider fauna that survived the wildfire. Authors such as Pryke & Samways (2012) and Yekwayo *et al*. (2019) have suggested that spider survivability could be related to the potential existing refuges such as rocks and plants, which has proven to be true in this study. However, among spiders, behavior also seems to be a relevant mortality factor because if, on the one hand, it favored *taxa* that use stones as hunting grounds and refuge areas, for spiders that use stones as their nesting grounds, it was a cause of higher mortality. In the burnt pine sites, cell spider (Dysderideae) mortality was very high, and it seems related to behavior as most mature individuals died in the nest with their brood. The mortality among wandering ants and the abrupt reduction in all ant genera nests were also high in both plantations. Vasconcelos *et al*. (2017), in a meta-analysis about the fire effects on ants at a global scale, identified strong negative effects of wildfires in ant diversity, as ant assemblages depend on the complexity of vegetation and magnitude of fire-associated changes in vegetation structure. In this study, the severity of the wildfire was high, destroying all vegetation and litter on both plantation's burnt sites where biomass loads were high, and at the same time exposing stones to high temperatures for longer periods, resulting in higher mortality. For *Plagiolepis* sp., the high mortality observed also seems to be related to its behavior, as it was the most abundant in both plantations' unburnt areas, becoming absent in both plantations' burnt areas. Results such as the observed in this study regarding cell spiders and ants such as *Plagiolepis* sp., identify wildfire's ability to change the ground-dwelling macroinvertebrate community, even if only temporarily, and are particularly relevant in areas subject to repeated wildfire, where it can lead to a permanent loss of biodiversity. Apart from behavior, other traits such as slow locomotion, humidity dependency, and larger body size also induced mortality among the ground-dwelling macroinvertebrate community, as also mentioned in other studies (Moretti & Legg, 2009;

Trucchi *et al*., 2009). Orders Pulmonata and Isopoda were the most affected in both burnt areas, as they depend on specific air humidity intervals to survive (Sgardelis *et al*. 1995; Moreno-Rueda *et al*., 2009), which can rapidly become intolerable during a wildfire. Order Pulmonata also has slow locomotion, such as Diplopoda, reducing the success of escape movements to safer areas. Kiss & Magnin (2006) described a drastic reduction of abundance and richness in land snails as a direct fire effect shortly after the fire, while Sgardelis *et al*. (1995) observed a decrease of Diplopoda abundance after-fire, that remained for at least 2 years. Body size seems to affect the ease of each specimen being able to protect itself under stones, in the most interior and potentially safer zone, from the direct effects of fire, as observed in Coleoptera's mortality that affected mostly larger specimens from Carabidae, Scarabidae, and Tenebrionidae families. Wikars & Schimmel (2001) and Moretti *et al*. (2006) also identified negative effects on both abundance and richness of beetles, particularly when affected by high severity fires (Saint-Germain *et al*., 2005). Contrary to several studies that observed immediate post-fire colonization by pyrophilous species of beetles (Sasal *et al*., 2010; Fredriksson *et al*., 2020), particularly in pine stands (Moreira *et al*., 2010), at the time of sampling, any evidence of it was found in the macroinvertebrate community associated with stones. This information shows that pyrophilous species are not related to the stone-associated community and probably come from adjacent unburnt areas. Associated with body size mortality, smaller-sized individuals, regardless of the *taxon*, may have been entirely consumed by fire, leading to underestimated fire-related lethality of some of the ground-dwelling macroinvertebrates associated with stones. Most of the larger dead specimens collected had visible burn marks, but many physical traits were lost, particularly the most fragile. This study limitation can partially explain the reduced number of smaller specimens with visible burnt marks, regardless of *taxa*. Also, in addition to the influx of other animals from unburnt areas in the vicinity of the affected area, the survivability and permanence of several *taxa* identified in this study in burnt areas immediately after the wildfire suggest that post-fire recolonization is influenced by communities such as the one associated with stones that live and shelter in natural structures, as implied in previous studies (Zaitsev *et al*., 2014; Yekwayo *et al*., 2016; Swart *et al*., 2017).

It is likely that stone cover is one of the main factors regarding the differences between both plantations in the region and is directly related to old and new management practices. For millennia, transforming natural into agricultural land was a priority for humans in Europe (Williams, 2008), and removing stones from the soil was and still is part of the process to accomplish it (Saini & Grant, 1980). Stones were then used for constructing houses, rural walls or were accumulated in marginal zones, leaving most of those areas with a low stone cover. In late-19th century, after many changes concerning forestation, government policies changed again, favoring the plantation of maritime pine across the country, and most of the rural areas and mountain slopes in the study area were transformed into pine plantations as a strategy to develop wood and resin industries (Silva, 2007). Since then, the concern about stones in the forest floor, due to faster and easier mechanical methods of transforming the land to plantation purposes and the implementation of newer management practices, diminished, until recently with the coming of studies that focus on the effects of land managing practices on soil and biodiversity (Chaudhary *et al*., 2016). These transformations, however, led to an increase of stone availability in the newer pine plantation's areas, that persisted until today. With the pulp production expansion initiated in the 1960s, the area occupied by eucalypt plantations increased (Silva, 2007), leading to more erosion-inducing management methods (Chaudhary *et al*., 2016). The recurrent use of linear terraces in eucalypt plantations to ease seasonal management, planting, cutting, and collecting of the wood (Alves *et al*., 2007), combined with increasingly shorter periods of plantation rotation (Chaudhary *et al*., 2016), has led to a high frequency of smaller stones, now the main ground cover. It is likely that lower stone cover percentage is now an indicator of less soil disturbance. Despite stone cover not being directly associated with wildfire effects, it is the outcome of land management practices that physically alter the ecosystem (Johnston & Crossley Jr., 2002). These alterations combined with wildfires promote more severe effects in the community by reducing biological legacies and changing ecosystem dynamics (Lindenmayer & Noss, 2006; Foster & Orwig, 2006; Perry & Herms, 2019). As mentioned before, stone surface area and depth are crucial factors for biodiversity, with strong positive correlations with abundance and richness, particularly in the case of stone depth. Several studies show that fire effects can be negligible from 5 cm depth (New, 2014) to 40 cm (DeBano, 2000; Moreira *et al*., 2010; Caut *et al*., 2013), which proved to also be true in this study. Large and deep buried stones had a higher abundance and richness, adding more protection from fire and lowering mortality rates. Considering the management history of each forest type, these results are particularly relevant. Pine plantations have lesser availability of stones, but larger and more deeply buried stones, which reflects a less frequently disturbed ecosystem than eucalypt plantations where the opposite occurs. This difference in stone availability is due to today’s heavily mechanized management methods, that apart from several other negative effects, decrease the size and alter the depth of stones, and promote soil displacement, increasing the availability of smaller stones (NCASI, 2004; Cambi *et al*., 2015). However, this higher availability of smaller and less buried stones does not promote biodiversity. The comparison between the number of unoccupied stones found in each plantation type reinforces this idea. It reveals that stones need to meet certain conditions to be colonized by the ground-dwelling macroinvertebrate community, being surface area and depth part of those conditions.

The present study uncovers the value of stones in the survival of several *taxa* during fire events, emphasizing their specific role as a refuge. This information gains significance as limited data about invertebrate diversity and ecology, particularly their responses to fire, restrains the ability to create evidence-informed decisions about post-fire land restoration (Saunders *et al*., 2021), particularly regarding highly modified and periodically managed ecosystems such as forest plantations. The information presented in this study is also relevant for stakeholders concerned with conservation purposes as it shows distinct biodiversity values between native and non-native plantations in both current and post-fire scenarios.

Several important matters related to this study still need further research and clarification in follow-up studies, especially questions related to soil and stone properties, fire behavior, and *taxa*-specific responses.

3.5 Conclusion

This study presents new information about the immediate fire effects in the ground-dwelling macroinvertebrate communities of native and non-native plantation forests in the Mediterranean region. It also explores the potential implications of historical land management alterations in these ecosystems and their influence on the ground-dwelling macroinvertebrate community.

The main conclusions of the present study were the following:

a) Stones shelter and safeguard part of the ground-dwelling invertebrate community from the direct effects of fire.
b) By allowing the survival of ground-dwelling macroinvertebrates that remain in the affected areas after the wildfire, stones can have a crucial role in the immediate recolonization process of burnt areas.
c) Feeding behavior in the community changed after the wildfire, but only in the pine burnt area.
d) Ground-dwelling macroinvertebrate abundance and richness per stone is higher in pine unburnt and burnt areas than in their eucalypt counterparts.

e) Diversity and equitability per stone remained identical after the wildfire in both plantations types.
f) Stone area and depth are directly related to abundance and richness of the community, but only in pine and eucalypt unburnt areas.
g) Stone availability, surface area and depth are likely to be the main factor of differences between the abundance and richness found under stones in pine and eucalypt plantations unburnt and burnt areas.

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Chapter 4

Submitted to *Land Degradation & Development* in 20th December of 2021 as “Short-term impacts of two post-fire mulching application rates on ground-dwelling arthropod communities in eucalypt planted forests” by Puga, J. R. L., Silva, T., Abrantes, N., Keizer, J.

4 Short-term impacts of two post-fire mulching application rates on ground-dwelling arthropod communities in eucalypt planted forests

4.1 Introduction

Wildfires are a natural disturbance common in many terrestrial ecosystems, especially in Mediterranean climate regions, but the increasing number of fires and its severity over the past decades has been raising important concerns on fire-induced land and soil degradation (Shakesby, 2011). Portugal is one of the countries in the world that is most affected by fire, with an average burnt area of 140 000 ha/year (ICNF, 2014), due to climate conditions propitious to fire ignition and spreading, and to elevated fuel loads associated with land abandonment as well as widely planting of fire-prone tree species (Shakesby, 2011).

The direct and indirect effects of wildfires on soil fauna have been poorly studied compared to their effects on soil physical and chemical properties (Certini, 2005; Faria *et al*., 2015; Campos *et al*., 2016), in spite the importance of these organisms for soil quality and functioning (Buckingham *et al*., 2015; Orgiazzi *et al*., 2016). The most observed effects of fire on soil fauna are mortality, changes in diversity, loss of habitats, and changes in community composition (Brown & Smith, 2000; Gongalsky *et al*., 2012; Jhariya & Raj, 2014; Buckingham *et al*., 2015). Also, the direct and indirect effects of post-fire land management on soil fauna have been poorly studied especially compared to its effects on runoff generation and soil erosion. This is also true for mulching, which is now widely accepted as a highly effective post-fire emergency stabilization measure for reducing runoff and especially soil losses in recently burnt areas (Robichaud *et al*., 2013; Fernández & Vega, 2014; Prats *et al*., 2014a, 2016). To the best of the authors' knowledge, Puga *et al*. (2017) is one of the few published SCI-paper on the effects of post-fire mulching on soil fauna, focusing on ground-dwelling arthropods and dealing with the mid-term effects of eucalypt bark mulch.

Puga *et al*. (2017) found these mid-term mulching effects on the ground-dwelling arthropods in a eucalypt plantation to be minor but did argue that the same might not be true for the immediate and short-term effects. Wildfires tend to reduce the diversity of soil arthropods, although responses can vary markedly between different *taxa* as well as between different ecosystems (Collet, 2003; Baker *et al*., 2004; Apigian *et al*., 2006). Post-fire mulching may influence soil arthropod communities directly through the introduction of organisms. It can further be expected to affect soil arthropod communities indirectly but in contrasting manners: (i) in a positive manner, through improving soil nutrient and water availability, moderating topsoil temperature regime and stimulating soil microbial activity (Chalker-Scott, 2007; Gill *et al*., 2011); (ii) in a negative manner, through reducing light availability and acting as a physical barrier against plant recovery (Maia *et al*., 2012; Fernández *et al*., 2016) and, possibly, reducing habitat diversity, especially in the case of standard mulch application rates as they typically aim at achieving a protective mulch cover of at least 60 % (Prats *et al*., 2014b; 2016). These possible mulch impacts on soil arthropods can interfere with post-fire ecosystem recovery in general, as soil invertebrates play a key role in forest ecosystems. They not only are of major importance in the food chain but also promote organic matter decomposition and nutrient cycling, contributing to the bulk of natural processes in the topsoil (García-Domínguez *et al*., 2010; Menta, 2012; Briones, 2014).

The present study was carried out as a follow-up of Puga *et al*. (2017), addressing the immediate and short-term impacts of mulching with eucalypt logging residues on the abundance, richness, diversity, and evenness of ground-dwelling arthropod communities in planted eucalypt forest. More specifically, these impacts were studied for the same two distinct mulch application rates that were applied in the previous study, and for two contrasting eucalypt plantations and seven sampling occasions during the first year after a wildfire.

4.2 Materials and methods

4.2.1 Study area and sites

The present study was carried out in the Semide - Rio de Vide parish union in the Miranda do Corvo municipality (40º 9.977’ N, 8º 19.506’ W), north-central Portugal. The study area was burnt by a wildfire that started on 9 of August 2015 and consumed a total of 715 ha of mainly forest stands (96 %) and, particularly, planted eucalypt forest (ICNF, 2015). Within the burnt area, two eucalypt plantations located at approximately 2 km distance were selected on similarly steep slopes (A: 27 ± 2 º; B: 30 ± 3 º) with similar expositions (A: ENE; B: NNE) but with contrasting pre-fire conditions, in the sense that site B had been logged just before the fire so that large amounts of logging residues had been lying on the forest floor by the time of the fire (pers. comm. land owner), which could have caused in that area a higher fire severity. Despite this difference, fire severity appeared to have been similar at both sites. Complete combustion of the litter layer and the predominantly black colour of the ash layer suggested a moderate soil burn severity, following Shakesby and Doerr (2006).

The Twig Diameter Index likewise indicated a moderate burn severity fire at both sites (Maia *et al*., 2012).

The soils at the two study sites were described in the field through various soil profiles that were cleared at the side of the trail at the bottom of the plantations and dug up 5 meters upslope. They were classified as Epileptic umbrisol in the case of site A, and as a complex of Epileptic and Cambic umbrisols in the case of site B (IUSS Working Group WRB, 2015). The soils at both sites were developed from pre-Ordovician schists of the Hesperic Massif (Pereira & FitzPatrick, 1995) and had an A horizon with a loamy texture, a pH of 4.6-4.8 and a high organic matter content (15-20 %).

4.2.2 Experimental design

At both study sites, an experiment was set up in the framework of the EU-FP7 project RECARE to assess the effectiveness of applying eucalypt logging residues at two distinct rates to reduce post-fire runoff and erosion. This set-up involved three treatments: NT (untreated or control), LT (low mulch application rate (2.6 Kg ha^{-1})) and HT (high mulch application rate (8.0 Kg ha^{-1})). A total of three plots, each with a subplot for each different treatment were installed at both sites. One extra subplot for each different treatment was also installed and named destructive plot. These destructive plots were used to install the pitfall traps and to collect soil samples within the RECARE project framework. Each subplot had 2 m wide by 8 m long and was surrounded by a fence cloth, except the destructive plots. Plot installation was accomplished by 7 September 2015 on site A and exactly one week later on site B, while mulching was applied on 15 September 2015 on both sites.

4.2.3 Ground cover

Ground cover in the immediate vicinity of the pitfall traps was estimated by taking vertical pictures on nine occasions between October 2015 and August 2016, and by determining the relative frequency of six cover categories, assigning a category to each of 100 intersection nodes of a virtual grid that was laid out over the digital picture. The six cover categories were: stones, bare soil, ashes (including charcoal), litter (including mulch), moss, and other vegetation.

4.2.4 Sampling and identification of ground-dwelling arthropods

Sampling of the ground-dwelling arthropod communities of the destructive plots was done using pitfall traps, an efficient and commonly used method for that purpose (Baars, 1979; Wang *et al*., 2001). As in the preceding study (Puga *et al*., 2017), the traps consisted of the bottom part of a 1.5 L plastic bottle with a diameter of 8 cm, used to commercialize drinking water, cut off at a height of 12 cm. In the field, the traps were first filled with a small amount of ethanol 70%, to which was then added a few drops of glycerine (as preserving agent) and a drop of detergent (to break the ionic tension at the top of the solution and to ensure that trapped invertebrates cannot escape), and then placed in a 12 cm deep hole. After seven days in the field, the contents of the traps were collected, put in vials filled with ethanol 70% and glycerine, and then stored until further processing. In each destructive plot, three pitfall traps were (re-)installed at a fixed 2.5 m distance, a totalling 18 traps.

Sampling of the ground-dwelling arthropod communities took place at seven occasions during the first year after the wildfire. The first sampling events were done in September and October 2015 to assess the ground-dwelling arthropod community immediately after the wildfire and immediately after the mulching, respectively. Subsequent sampling occasions were conducted in November 2015 (1-2 months after application (a.a)) and January (3-4 months a.a.), April (6-7 months a.a.), June (8-9 months a.a.) and September 2016 (11-12 months a.a.).

All the invertebrates that were collected could be identified to the *taxon*omic rank of order, using standard *taxon*omic keys (Harde & Severa, 1984; Goulet & Huber, 1993; Roberts, 1995; Barrientos, 1998; Czechowski *et al*., 2002) and a stereoscopic magnifier. Identification at order level is normally used to assess the ecological complexity of the soil community in a simpler way (Stork & Eggleton, 1992), providing valuable information related to each group function in the soil. Due to the lack of relevant *taxon*omic differentiation content of the samples (high homogeneous data) the identification at order level was used to facilitate the processing of the large number of specimens collected (4611), while maintaining a level of identification that allows inference of the data regarding potential richness differences within treatments Satisfactory results had been already obtained with this level of identification in other studies (York, 1999; Santos *et al*., 2007; García-Domínguez *et al*., 2010; Gill *et al*., 2011; Jacobs *et al*., 2015) and also in the precursor study of (Puga *et al*. 2017). Also, as in Puga *et al*. (2017), Acari, Diptera, Lepidoptera and larvae were not considered here, as pitfall trap are less suited to sample these *taxa*/life stage.

4.2.5 Data analysis

The ground-dwelling arthropod communities were characterised using the following four structural parameters: total abundance, richness, Shannon-Weiner diversity index and Pielou evenness index, the latter three of which at the *taxon*omic rank of Order.

Two-way repeated measures ANOVA were used to test community differences between treatments, sites, and cover at significance level α of 0.05. In the case of the ground cover categories, specific differences between treatments at the individual study sites as well as specific differences between the two sites for the individual treatments were analysed using the Tukey multiple comparison test. In all tests, normality and homogeneity of variances was analysed using the Shapiro-Wilk test and the Levene median test, respectively, and, if rejected, data was transformed to neperian logarithm (Ln). A one-way ANOSIM test was employed to determine similarities between treatments and sites using total abundance at rank order and Euclidean distance.

4.3 Results and discussion

4.3.1 Ground cover

The application of eucalypt logging residues proved to be effective in that it produced significant differences in litter cover between the three treatments over the entire study period at both study sites (Table 4.1). These significant differences are also readily perceived in Figure 4.1, with litter cover increasing from control (NT) to low (LT) and to high (HT) mulch application rate. Furthermore, the litter cover at the two sites did not differ significantly for either of the mulch application rates.

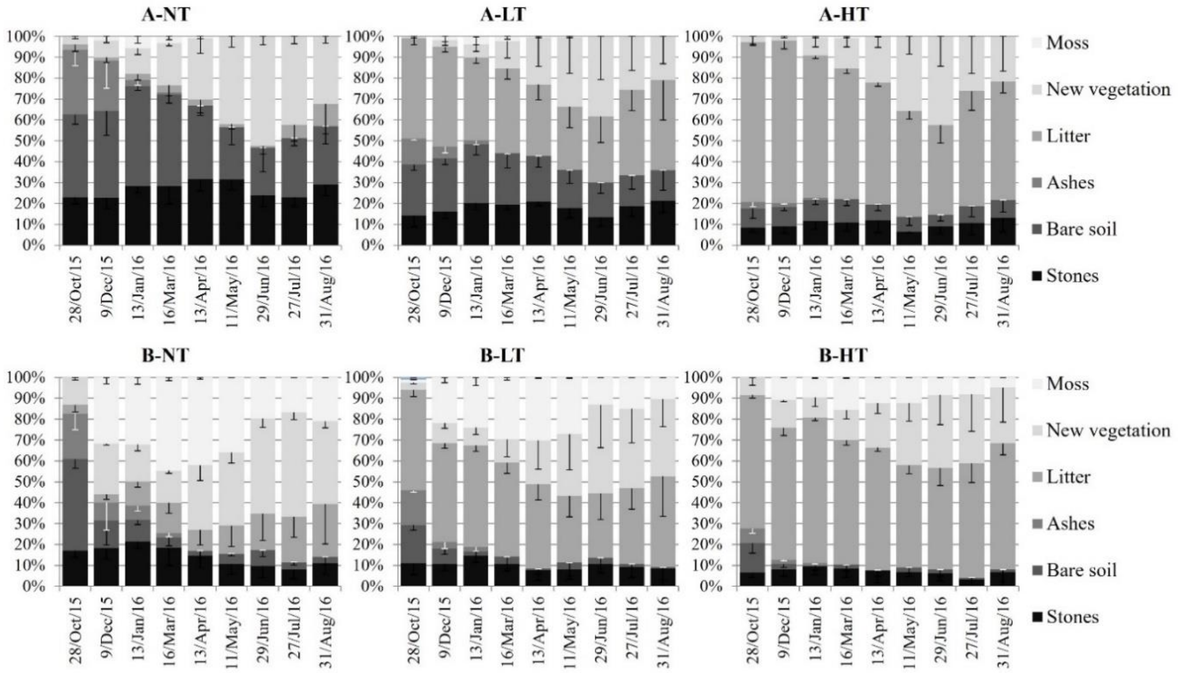

Figure 4.1 Average values and standard deviations of the six ground cover categories for the three treatments (NT = no mulching; LT = mulching at low application rate; HT = mulching at high application rate) at the two study sites (A and B) between October 2015 and August 2016

Table 4.1 Summary of the Tukey multiple comparison tests results for the five ground cover categories that revealed significant differences between the three treatments (NT = control; LT = low mulch application rate; HT = high mulch application rate) and/or between the two study sites (A and B) for the individual treatments. Significant differences at α = 0.05 are marked in bold.

Variable	**Source of variation**		**Cover**		
			Diff. means	***t* value**	***p value***
Stones	Between treatments at site A	NT vs HT	16.678	16.713	**< 0.01**
		NT vs LT	8.815	8.833	**< 0.01**
		LT vs HT	7.864	7.880	**< 0.01**
	Between treatments at site B	NT vs HT	7.375	7.390	**< 0.01**
		NT vs LT	4.205	4.213	**< 0.01**
		LT vs HT	3.170	3,177	**< 0.01**
	Between sites for treatments	NT	12.465	8.663	**< 0.01**
		LT	7.855	5.459	**< 0.01**
		HT	3.162	2.197	**0.04**
Bare soil	Between treatments at site A	NT vs HT	26.197	12.375	**< 0.01**
		NT vs LT	13.770	6.505	**< 0.01**
		LT vs HT	12.427	5.870	**< 0.01**
	Between treatments at site B	NT vs HT	7.562	3.572	**< 0.01**
		NT vs LT	5.952	2.812	**< 0.01**
		LT vs HT	1.610	0.760	0.45
	Between sites for treatments	NT	24.301	8.354	**< 0.01**
		LT	16.483	5.667	**< 0.01**
		HT	5.666	1.948	0.07
Litter	Between treatments at site A	NT vs HT	57.242	18.284	**< 0.01**
		NT vs LT	35.914	11.472	**< 0.01**
		LT vs HT	21.328	6.813	**< 0.01**
	Between treatments at site B	NT vs HT	45.052	14.390	**< 0.01**
		NT vs LT	27.772	8.871	**< 0.01**
		LT vs HT	17.279	5.519	**< 0.01**
	Between sites for treatments	NT	9.918	5.327	**< 0.01**
		LT	1.776	0.954	0.352
		HT	2.272	1.221	0.237
Other vegetation	Between treatments at sites A and B	NT vs HT	8.304	7.602	**< 0.01**
		NT vs LT	8.122	7.435	**< 0.01**
		LT vs HT	0.182	0.167	0.87
Moss	Between treatments at site A	NT vs HT	1.154	0.673	0.51
		NT vs LT	0.802	0.468	0.64
		LT vs HT	0.352	0.205	0.84
	Between treatments at site B	NT vs HT	18.000	10.492	**< 0.01**
		NT vs LT	10.095	5.884	**< 0.01**
		LT vs HT	7.905	4.608	**< 0.01**
	Between sites for treatments	NT	25.777	8.024	**< 0.01**
		LT	18.224	5.673	**< 0.01**
		HT	8.932	2.780	**0.02**

Stone cover revealed the same statistical differences between the three treatments at the two sites as litter cover. As easily observed in Figure 4.1, however, these differences were in the opposite sense, with stone cover decreasing with mulch application and more so at higher than lower application rate. Also, unlike litter cover, stone cover did differ significantly between the two study sites for both mulching rates, with values being somewhat lower at the B than A site.

These findings for stone cover were closely matched by the results for bare soil cover, except that bare soil cover at site B did not differ significantly between the two mulch application rates. The same was true for the recovery of the other vegetation, including the lack of a significant difference between the two mulching rates, even though the differences between the untreated vs. mulched plots were only significant when the data of the two sites were pooled.

Moss cover stood out for revealing a clear contrast between the two study sites, being typically absent at site A as opposed to markedly present at site B, except immediately after the mulching in October 2015. At site B, however, moss cover revealed the same treatment-wise significant differences as, for example, stone cover, being highest without mulch application and lowest at the highest application rate.

4.3.2 Ground-dwelling arthropods

A total of 4611 ground-dwelling invertebrates pertaining to 14 orders were captured and identified over the entire study period. Site A had a total of 2661 specimens and site B a total of 1950 specimens. Puga *et al*. (2017) found almost the same number of ground-dwelling arthropod orders (13) in a eucalypt plantation in north-central Portugal that had been burnt by a wildfire then partially mulched with eucalypt logging residues some five years earlier.

The results of two-way repeated-measure ANOVAs revealed that none of the four structural arthropod community parameters (total abundance, richness, Shannon-Weiner diversity index and Pielou evenness index) differed significantly between the three treatments and/or the two study sites (Table 4.2). The most conspicuous feature was that at specific sampling occasions diversity was clearly lower without mulching than with mulching. These specific occasions, however, differed for the two sites, suggesting an immediate impact of mulching at site B as opposed to a delayed impact at site A (winter

2015-spring 2016). A common aspect at the two sites was that the diversity of the treatments tended to be at its maximum during summer 2016.

Table 4.2 Summary of the two-way repeated measure ANOVA results regarding total abundance, richness, diversity and evenness of the ground-dwelling arthropod communities of the three treatments (NT = no mulching; LT = low mulch application rate; HT = high mulch application rate) at the two study sites (A and B). *Df* = degrees of freedom; MS = mean squares; *f* = *f* test; *p* = *p* value

Variable	**Source of variation**	***Df***	**MS**	***f***	***p***
Abundance	Month	5	183.499		
	Site	1	13.011	0.786	0.416
	Site x Month	5	16.557		
	Treatment	2	31.166	1670	0.237
	Treatment x Month	10	18.662		
	Site x Treatment	2	1.953	0.219	0.807
Richness	Month	5	8.450		
	Site	1	0.694	0.312	0.601
	Site x Month	5	2.228		
	Treatment	2	5.083	1.968	0.19
	Treatment x Month	10	2.583		
	Site x Treatment	2	0.361	0.082	0.922
Diversity	Month	5	0.554		
	Site	1	0.08	1.553	0.268
	Site x Month	5	0.051		
	Treatment	2	0.269	4.064	0.051
	Treatment x Month	10	0.066		
	Site x Treatment	2	0.146	1.033	0.391
Diversity	Month	5	0.008		
	Site	1	0.001	0.870	0.394
	Site x Month	5	0.001		
	Treatment	2	0.002	1.265	0.324
	Treatment x Month	10	0.002		
	Site x Treatment	2	0.003	1.770	0.220

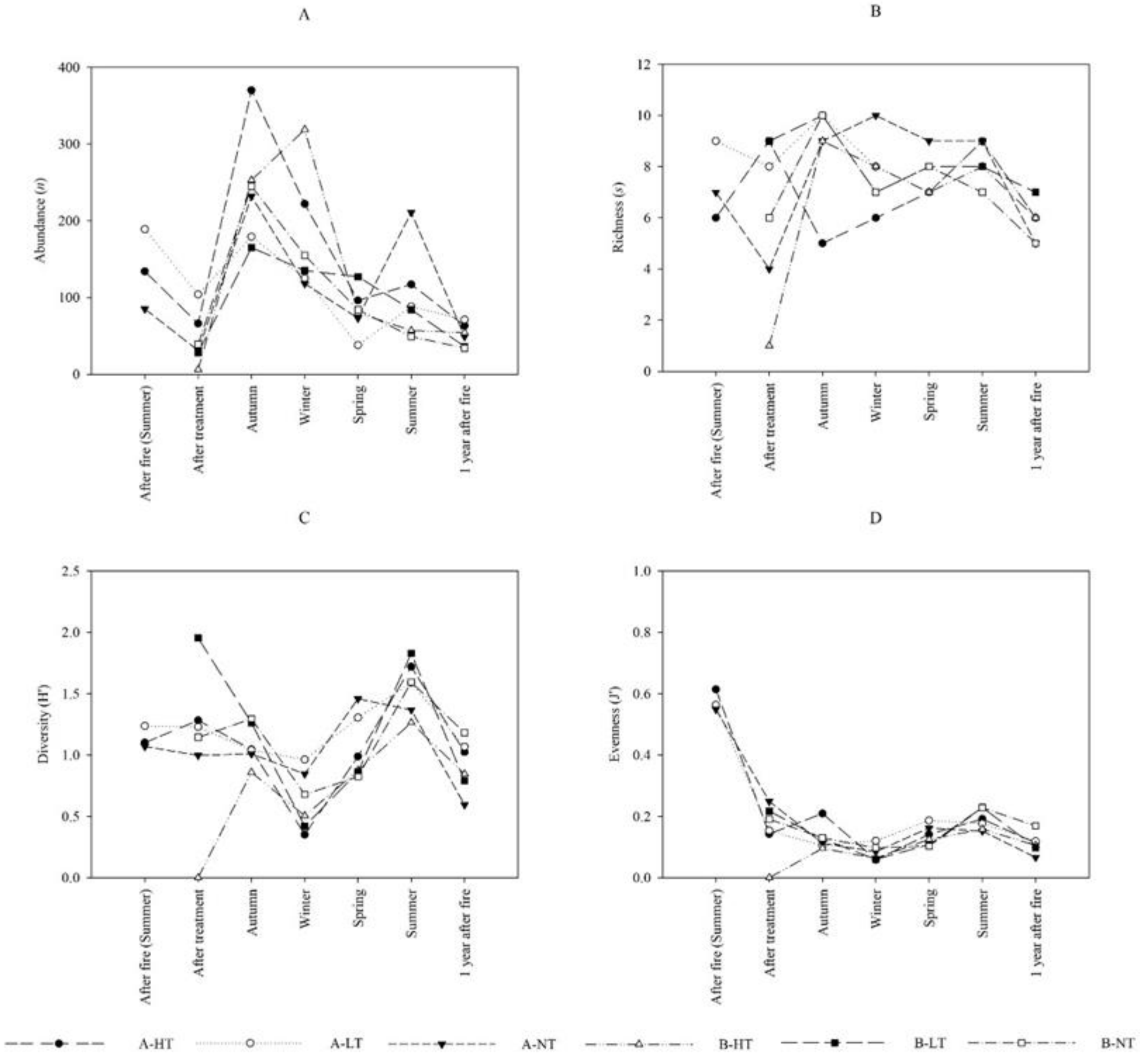

Figure 4.2 Total abundance, total number of orders, Shannon-Weiner diversity index and Pielou's evenness index values of the ground-dwelling arthropod communities for the three treatments (NT = no mulching; LT = mulching at low application rate; HT = mulching at high application rate) at the two study sites (A and B) on the seven sampling occasions during the first year after the wildfire.

There was some suggestion that treatment played a relevant role in total abundance when it was highest (Figure 4.2). Average total abundance was clearly lower for the mulched than untreated plots at site A during autumn and winter 2015 and at site B during winter 2015. In overall ANOSIM results are in concordance with these findings suggesting a lack of evident differentiation between sites and treatments related to abundance (Table 4.3) despite the values obtained for NT showing a greater distance of the remaining treatments in both sites.

Table 4.3 Summary of the one-way ANOSIM results regarding total abundance at order rank of the ground-dwelling arthropod communities of the three treatments (NT = no mulching; LT = low mulch application rate; HT = high mulch application rate) at the two study sites (A and B). Permutation n = 9999; mean rank within = 333.6; mean rank between = 312.5; *p* = *p* value; *r* = *r* value.

Treatment	**Total abundance per Order**	
	***p* value**	***r* value**
A NT vs A LT	0.8582	-0.07037
A NT vs A HT	0.9073	-0.07222
A NT vs B NT	0.7861	-0.05926
A NT vs B LT	0.3601	**-0.001852**
A NT vs B HT	0.6815	-0.03889
A LT vs A HT	0.9399	-0.1093
A LT vs B NT	0.5496	-0.02593
A LT vs B LT	0.7063	-0.08704
A LT vs B HT	0.834	-0.1019
A HT vs B NT	0.5901	-0.04444
A HT vs B LT	0.7769	-0.07778
A HT vs B HT	0.811	-0.07963
B NT vs B LT	0.421	**-0.005556**
B NT vs B HT	0.6931	-0.05556
B LT vs B HT	0.9842	-0.1278

Richness showed an absence of pattern regarding the community response to the treatments at each site immediately after the mulch application. Only a few months after its application it was possible to see more similar results between treatments at each site (Figure 4.2). These results may have been due to differences between sites especially during the winter and spring periods when many physical alterations occur. However, by the end of spring the richness values for each treatment and site started to be much similar. The evenness results revealed as most conspicuous feature a marked drop from immediately after the fire to immediately after the mulching (Figure 4.2).

The present results on the four structural community parameters were in line with those of Puga *et al*. (2017), finding that none of the four parameters differed significantly between plots that had and had not been mulched with eucalypt logging residues some five years earlier, immediately after a moderate-severity wildfire. At the same time, Puga *et al*. (2017) did find some suggestion that mulching had reduced total abundance, as was also the case here when abundances reached maximum values. The present results further agreed reasonably well with those of Puga *et al*. (2017) in terms of richness (6-10 orders) and diversity (1.1-1.4), but not of evenness (0.5-0.7), except immediately after fire (only data from site A). Evenness stood out among the other structural parameters by the pronounced drop between the first and second sampling occasions, immediately after fire and immediately after mulching. This could suggest that evenness was least affected by the direct fire impacts but most affected by the early indirect fire impacts and requiring most time (> 1 year) to recover. In contrast, Pitzalis *et al*. (2013) found that the evenness of the soil fauna hardly changed during the first year after fire, like, alias, its diversity.

The community composition at the order rank did not suggest an obvious role of mulching but did hint at differences between the two sites (Figure 4.2). While the Collembola tended to be the clearly dominant order at both study sites across the various sampling occasions and the three treatments, the Hymenoptera become increasingly more abundant at both sites with the coming of summer. Order Hemiptera seemed more abundant at site B than at site A, especially immediately after the mulching. The Araneae and Coleoptera were typically present but with comparatively low abundances, except in one instance when the Coleoptera were the dominant order (site A, autumn 2016, low mulching rate). Predominance of a limited number of *taxa* and the resulting homogeneous community composition could be related to the presence of a reduced number of habitat types (Sgardelis *et al*., 1995; Ober & DeGroote, 2011; Jacobs *et al*., 2015) as is the case of the mulch layer.

A lack of clear differences in community composition at the order level between mulched and control plots was also observed by Puga *et al*. (2017), some five years after wildfire and mulching with eucalypt logging residues. The present study and that of Puga *et al*. (2017) further agreed in that Collembola and Hymenoptera were clearly the most abundant orders, typical accompanied by Araneae and Coleoptera. This indicated that both resistance to fire and colonization ability were key traits in determining community composition during the initial post-fire period (Sgardelis *et al*., 1995; Moretti *et al*., 2006). Matsuda *et al*., (2011) found Hymenoptera to be resilient to fire, as only 2% of the population of a mature ant nest was active on the surface, so that most of the nest survived the fire itself. Underwood & Quinn (2010) reported that Araneae could survive the direct wildfire effects by hiding under rocks, from which they could then quickly colonize the burnt area.

4.4 Conclusions

The main conclusions of this study into the immediate and short-term impacts of post-fire mulching with eucalypt logging residues at two distinct application rates, on the ground-dwelling arthropod communities of two planted eucalypt forests were the following:

(i) neither the total abundance of the communities nor their richness, diversity or evenness at the *taxon*omic rank of order were affected in an obvious manner by mulching during the first year following the wildfire;

(ii) the same was true for the relative contributions of the individual orders to the communities' total abundances;

(iii) total abundance and diversity varied more markedly with time-since-mulching than richness and evenness but temporal patterns in all four parameters were typically unrelated across the two sites and poorly associated with changes in ground cover;

(iv) the Collembola were the most abundant order throughout the entire study period at one of the study sites and most of the period at the other site, becoming outnumbered by the Hymenoptera by the first summer after the wildfire.

(v) Based on an erosion mitigation perspective this study recommends the use of eucalypt bark mulching after fire on previously burnt eucalypt stands, at the rate that best fits the soil loss prevention, given that there is no evidence of important changes on the soil community one year after mulch application. However, the potential application of different types of mulch material for this particular ecosystem should always be properly assessed concerning the soil invertebrate community. Similar procedures should be taken for each different ecosystem prone to fire before recommending mulch application

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Chapter 5

Submitted to *Ecology and Evolution* in 20th December of 2021 as “Short-term impacts of wildfires on the diversity and presence of medium-sized mammals in Mediterranean coastal pine forests” by Puga, J. R. L., Abrantes, N., Moreira, F., Keizer, J.

5 Short-term impacts of wildfires on the diversity and presence of medium-sized mammals in Mediterranean coastal pine forests

5.1 Introduction

Wildfires are natural phenomena of great importance in various regions of the world that promote the regeneration of ecosystems, integrate the life cycle of many species that depend on it, and are therefore an evolutionary force that has shaped the communities and ecosystems affected by it (Belcher, 2013). Wildfires are particularly important in the Mediterranean region, which is why natural ecosystems of these areas have adapted to it over time (Keeley *et al*., 2012). Human action has changed wildfire regimes through the ages, but this human impact has become particularly pronounced since the early 20th century. The progressive transformation and degradation of natural ecosystems has changed how fire interacts with nature, making it an increasingly destructive force whose negative effects have become gradually more difficult to counter in recent years (Pausas *et al*., 2008; Moreira *et al*., 2011). Furthermore, future predictions point to a further intensification of wildfire regimes in many parts of the world (Moritz *et al*., 2012; Flannigan *et al*., 2013). In Europe, Portugal is amongst the most fire-prone countries, with burnt areas exceeding 300,000 ha in three out of the last 20 years (Doerr & Santín, 2013; Rego & Silva, 2014).

Wildfires affect ecosystems directly through the destruction of vegetation and killing and injuring of fauna as well as indirectly through, for example, changes in habitat conditions and food availability (Moreira *et al*., 2010; Hradsky *et al*., 2017; Certini *et al*., 2021). These wildfire effects can be short-lived but also be permanent, at least within the human live time (Certini, 2005; Belcher, 2013), depending on a panoply of factors such as the timing, severity and size of the fire, the time since the previous fire or other disturbance, and post-fire weather conditions (Pastro *et al*., 2014). These effects are not necessarily negative for all species, as some can benefit from fire-induced ecosystem changes (DellaSalla & Hanson, 2015).

Wildfire effects haven been studied far more extensively for vegetation than for fauna (Clarke, 2008; Chia *et al*., 2016; Pausas, 2019). There continues to exist a large knowledge gap on how fires affect many of the animal groups, because of the difficulties to study fire–wildlife interactions (Fontaine & Kennedy, 2012; Pausas, 2019; Soyumert *et al*., 2019). This is especially true for mammals, since their communities and interactions with abiotic and biotic factors are typically highly complex and since their census and tracking are often very challenging (Fisher & Wilkinson, 2005; Monasmith *et al*., 2010; Pastro *et al*., 2014; Geary *et al*., 2019). Species diet, feeding behaviour, habitat selection and predator-prey relations are key factors in wildfire effects on mammals (Vieira & Briani, 2012; Griffiths & Brook, 2014; Leonard & Koprowski, 2010). For example, squirrels tended to abandon burnt forest areas and avoid them during the initial succession stages (Fisher & Wilkinson, 2005; Leonard & Koprowski, 2010), while large African herbivores were attracted to burnt areas because of new plant growth and increased safety from predators (Eby *et al*., 2014).

This study seized the opportunity of the - dramatic and huge - October-2017 wildfires to address this knowledge gap for one of the country's most emblematic and protected ecosystems, i.e., the coastal woodlands of maritime pine. The specific objectives were to assess the immediate as well as short-term fire effects by: (i) comparing the occurrence of the full assemblage of medium-sized mammal species in burnt and unburnt pine stands immediately after fire; (ii) repeating this comparison one year later.

5.2 Materials and methods

5.2.1 Study area

The study area is in the coastal region Central Portugal between the hamlets of Quiaios and Tocha of the Figueira da Foz and Cantanhede municipalities, Coimbra District (Figure 5.1). The study area is part of an approximately 100 km-long coastal strip of woodlands designated as "Matas Litorais" and, more specifically, of the Natura-2000 area of Mira, Gândara and Gafanhas Dunes (PTCON0055). The study area basically corresponds to the Coastal Woodlands of the Quiaios Dunes (MNDQ). The MNDQ has a temperate climate with Mediterranean characteristics of warm and dry summers, and soft and rainy winters but with some influences from the Atlantic Ocean (AFN, 2010). Mean annual temperature is between 12.5 and 15 °C, and mean precipitation between 700 and 1000 mm (DGA, 2021). Wind conditions constitute an important ecological factor, especially in the parts closest to the Ocean, with North being the predominant direction. The soils of MNDQ are predominantly Regosols, i.e., unconsolidated poorly-developed sandy soils with very low organic matter contents, reduced water holding capacity and poor nutrient status. Towards the eastern boundary of the MNDQ, however, the soils are mainly Podzols but offer similarly poor conditions for plant growth in terms of water and nutrient availability.

The 6039 ha comprising the MNDQ are private domain of the Portuguese state and, as such, are managed by the Institute for Nature Conservation and Forests (ICNF). The current management plan of MNDQ aims to foster silviculture, recreation and landscape aesthetics, protection (especially against wind erosion), and conservation of habitats, fauna and flora species and geo-monuments (AFN, 2010). In turn, forest management of MNDQ (as of the rest of the Matas Litorais) has traditionally focused on plantations of maritime pine (*Pinus pinaster*) (Rego, 2001). For this purpose, the MNDQ is divided into a total of 190 management units of typically 400 m by 800 m (i.e., except along the coast) that are separated by forest tracks. In 2010, the MNDQ consisted for 94% of pure and mixed forest stands of maritime pine and invasive *Acacia* spp. (including *A*. *dealbata*, *A*. *longifolia*, *A*. *melanoxylon*), the remaining area consisting of shrubland (4%), surface water bodies (the Braças, Salgueira, Teixoeiros and Vela lagoons, located along the eastern border of the MNDQ; 3%) and built-up area (2%).

On 15 October 2017, 67% of the 36563 ha of Matas Litorais were affected by wildfires (Tomé, 2018). This included 3777 ha of the MNDQ, especially of its inland part (Figure 5.1). Based on the Relative difference Normalized Burn Ratio derived from Sentinel SENTINEL-2 Level-2A imagery, fire severity within the MNDQ was classified as predominantly extreme (38%), very high (31%) and high (20%) (Fernandes & Guiomar, 2018). Following the fire, hunting was prohibited during the study period, while post-fire salvage logging and wood extraction commenced during the period of the second monitoring occasion but had not taken place at any of the monitoring sites before the actual field data collection.

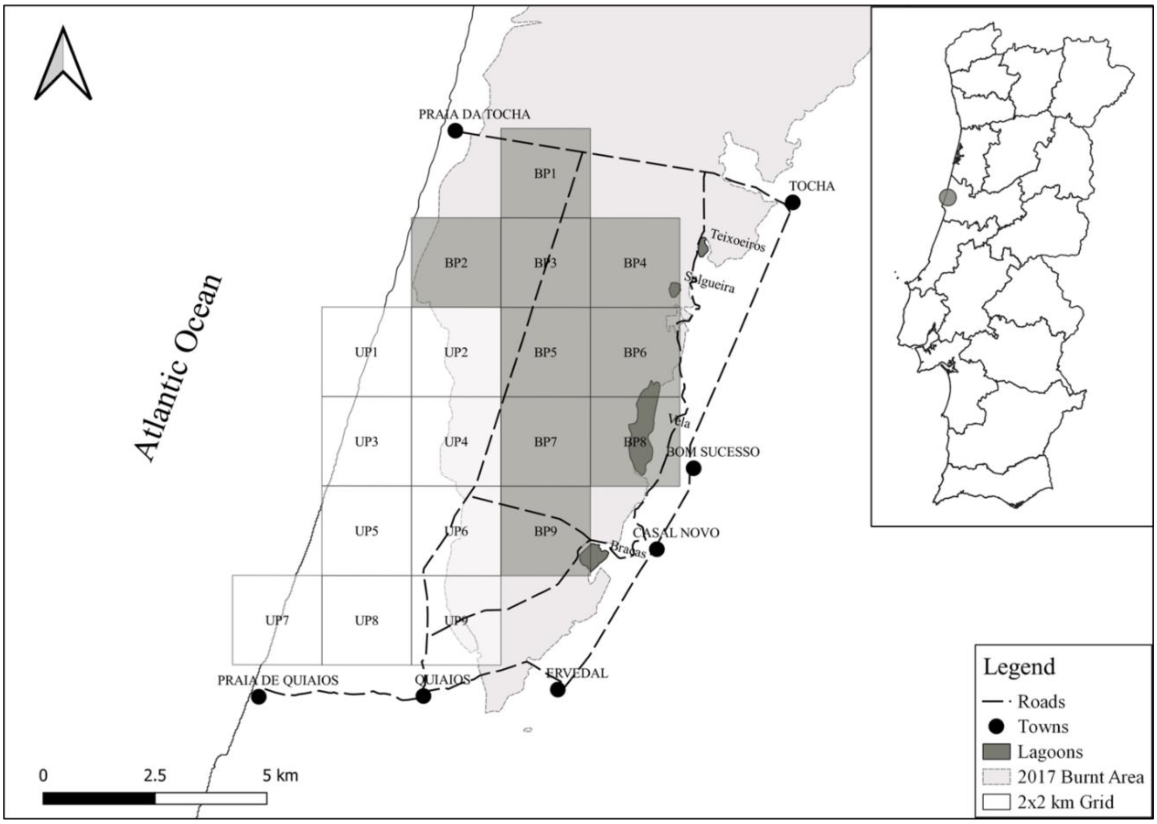

Figure 5.1 Study area and its division in 18 2 km by 2 km grid cells for the purpose of this study, in particular to achieve an equal number of grids cells that were burnt (BP1-BP9) and partly unburnt (UP1-UP9) during the October 2017 wildfire.

5.2.2 Experimental design

The study area was selected for allowing a balanced experimental design for assessing wildfire impacts on the diversity and area-use of medium-sized mammals. More specifically, the study area could be divided in an equal number of grid cells that represented burnt vs. unburnt conditions. The nine burnt grid cells (BP1-BP9) were (almost) completely within the burnt area of the October-2017 wildfire, whereas four of the unburnt grid cells were partially within the burnt area (UP2, UP4, UP6, UP9) and the other five completely outside it. The former four unburnt cells, however, did allow to sample unburnt conditions. Sampling was done along the existing, unpaved forest tracks within the grid cells (see Sutherland, 2006). The actual observations were carried out at 10 checkpoints in each grid cell, located at 100-m distances. At each checkpoint, the presence of evidence for the recent presence of the selected mammal species was recorded *in-situ* (Sanz *et al*., 2004; Artigas, 2012), using multiple indicators as detailed underneath (Table 5.1). This was done for an area of 6000 m^2, i.e., 50 m in either direction along the track and 30 m in perpendicular direction on either side of the track. Sampling was done at two occasions, i.e., starting roughly two and a half months after the fire and lasting approximately one month (from 15 December 2017 to 15 January 2018) and again one year later (from 15 December 2018 to 15 January 2019). The winter period was preferred as the moisture retained in sandy soils allows using good quality footprints as indicators of the presence of all but one of the species.

In the absence of published studies on terrestrial medium-sized mammal species in the study area, a recent national atlas (Bencatel *et al*., 2019) was used to draw up a list of 13 potentially occurring species (Table 5.1). The list of 13 species also includes the Iberian mole (*Talpa occidentalis*), despite its size, because of the conspicuousness and uniqueness of its signs of area-use and its importance as an endemic species. For each species, one or more indicators were then defined as evidence of its recent occurrence. The field identification of these three to nine indicators per species was done by the one and the same person (the first author) on both monitoring occasions, to guarantee the required consistency in the field data. In parallel, presence/absence of recent micromammal area-use along the same checkpoints was collected, through the observation of footprints, burrows, and gnawed pine nut accumulation piles (Sanz *et al*., 2004; Artigas, 2012) only to verify the availability of this group of animals in the study area as potential prey, as most medium-sized mammal species in the area can prey them.

Table 5.1 List of the 13 medium-sized mammal species targeted by this study and of the indicators used as evidence of their recent presence at the checkpoints.

Taxa	**Family**	**Diet**	**Burrows, dreys and/or beddings**	**Droppings**	**Footprints**	**Gnawed vegetation and/or fruit**	**Ground and/or tree markings**	**Pathways**	**Sightings**	**Skulls and carrion**	**Wallowing grounds**
Red fox *(Vulpes vulpes)*	Canidae	Carnivore	•	•	•		•	•	•	•	
Egyptian mongoose *(Herpestes ichneumon)*	Herpestidae	Carnivore	•	•	•				•	•	
Stone marten *(Martes foina)*	Mustelidae	Carnivore	•	•	•				•	•	
European badger *(Meles meles)*	Mustelidae	Carnivore	•	•	•		•	•	•	•	
Common weasel *(Mustela nivalis)*	Mustelidae	Carnivore		•	•				•	•	
Eurasian otter *(Lutra lutra)*	Mustelidae	Carnivore	•	•	•				•		•
Common genet *(Genetta genetta)*	Viverridae	Carnivore		•	•				•	•	
Wild boar *(Sus scrofa)*	Suidae	Omnivore	•	•	•	•	•	•	•	•	•
European rabbit *(Oryctolagus cuniculus)*	Leporidae	Herbivore	•	•	•	•	•	•	•	•	
Iberian hare *(Lepus granatensis)*	Leporidae	Herbivore	•	•	•	•	•	•	•	•	
Red squirrel *(Sciurus vulgaris)*	Sciuridae	Herbivore	•	•	•	•	•		•	•	
European hedgehog *(Erinaceus europaeus)*	Erinaceidae	Insectivore	•	•	•				•	•	
Iberian mole *(Talpa occidentalis)*	Talpidae	Insectivore					•		•	•	

5.2.3 Data analysis

The data on the various indicators for each species were merged for each checkpoint into presence or absence, and the sum of a species' presences in each grid cell was then coined as its frequency of presence, ranging from 0 to 10 (checkpoints). The total number of species present in a grid cell was coined as its species richness, while the sum of the frequencies of these species in a grid cell was coined its total species frequency. Species richness, total species frequency and frequency of presence of individual species were tested for significant differences related to fire and sampling occasion. This was done using two-way ANOVA or the Kruskal-Wallis test, depending on whether the ANOVA assumptions of normality of errors and homoscedasticity underlying were violated or not, following the Shapiro-Wilk tests. If the ANOVA or Kruskal-Wallis results indicated significant differences, the four relevant contrasts were tested for significance using the Dunn's post-hoc test and the Mann-Whitney U-test, respectively. The patterns in the species' co-occurrence in the grid cells were analyzed by means of non-metric multidimensional scaling (NMDS), using Euclidian distance as measure of dissimilarity. The scores of the grid cells on the principal NMDS-axes were related to potential confounding factors, in particular nearest distance to towns, roads, ocean, and to natural surface water sources. This was done using the Pearson's product-moment correlation coefficient. All statistical tests and NMDS analysis were carried out using PAST (Paleontological Statistics Software Package for Education and Data Analysis) (Hammer *et al*., 2001), using an α of 0.05.

5.3 Results

5.3.1 Species occurrence and frequency of indicators

Eleven of the list of 14 potentially occurring species were found to be present in the study area, the exceptions being the Iberian hare and Eurasian otter. The presence of these 11 species was observed a total of 1674 times across the various indicators, 967 times during the first monitoring occasion and 707 times during the second occasion. On both occasions, the species' presence was observed less frequently across the nine burnt grid cells than across the nine unburnt grid cells, with 455 *vs*. 512 times and 307 *vs*. 400 times, respectively.

The frequency of presence varied greatly among the 11 species. The two extremes were red fox and Iberian mole, having been detected 295 and 6 times, respectively, over both sampling occasions or, in other words, in 82% and 2% of the 360 cases (2 campaigns x 18 grid cells x 10 checkpoints). While wild boar was the only other species occurring in more than half of the cases (56%), the less frequent species included common weasel and European hedgehog, with overall frequencies of less than 5%.

From the nine indicators of the presence of the 11 species, droppings and footprints were clearly more frequently observed than the others. This was true for both monitoring occasions but there was a shift in prevalence between the first and second post-fire winter. During the first occasion, droppings amounted to 50% of the total number of presences as opposed to footprints 32%; during the second occasion, footprints amounted to 61% as opposed to droppings 37%. Droppings and footprints were also the principal indicators for all but two of the species, representing 80 to 100% of their total number of presences. The two exceptions are Iberian mole and red squirrel. In the case of the former, the only indicator observed were ground markings; in the case of the latter, 99% were gnawed pinecones. Ground markings also accounted 16% and 6% of the total presence signs found for wild boar and European badger, respectively.

5.3.2 Species richness and overall frequency of presence

Two-way ANOVA results showed that the wildfire had a significant overall impact on the number of species encountered in the grid cells, but that monitoring occasion had not (Table 5.2; Figure 5.2). The post-hoc tests, however, revealed that species richness was only significantly higher in the unburnt than burnt grid cells during the first monitoring occasion (7.0 *vs*. 4.4; $p < 0.01$) but not during the second occasion (6.1 *vs*. 5.2; $p = 0.31$). Such a year-specific role of wildfire was also suggested by the marginal significance of the fire-year interaction in the two-way ANOVA results ($p = 0.07$). It reflected contrasting patterns of decreasing and increasing average richness in the unburnt and burnt grid cells, respectively, between the first and second post-fire year.

Table 5.2 Two-way ANOVA results for species richness between areas and years after-fire.

Two-way ANOVA	**d. f.**	**Sum of squares**	**Mean square**	**F**	***P***
Fire	1	26.694	26.694	15.075	<0.001
Year	1	0.0278	0.0278	0.0157	0.901
Fire *vs* Year	1	6.25	6.25	3.529	0.069
Residual	32	56.667	1.771		
Total	35	89.639	2.561		

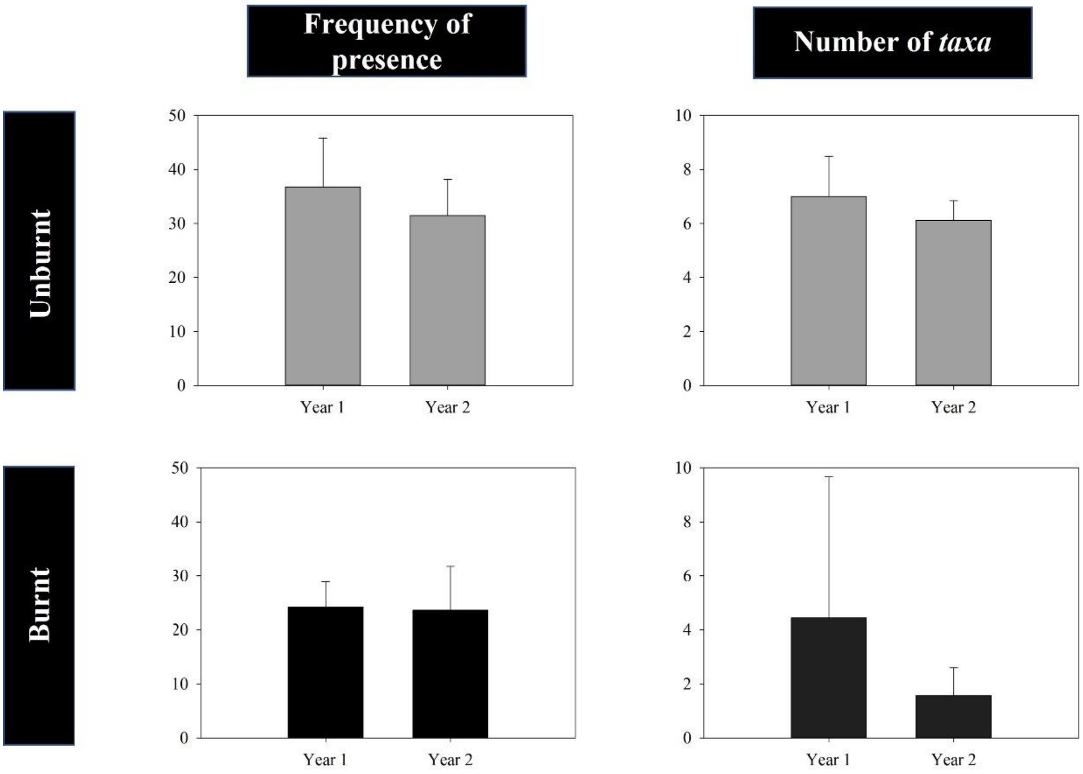

Figure 5.2 Average frequency of presence and number of *taxa* recorded in unburnt and burnt areas 3 months (Year 1) and 15 months (Year 2) after the fire.

The total area-use of all 11 species revealed similar statistical test results as their richness (Table 5.3; Figure 5.2). There was no significant interaction between wildfire and year, on the one hand, and, on the other, only fire played a significant overall role. Also, the year-specific differences between the unburnt and burnt grid cells were significant for the first monitoring occasion (37 *vs*. 24; *p* = 0.01) but not for the second occasion (31 *vs*. 24; *p* = 0.16), even if the fire-year interaction was clearly non-significant in the case of the total frequency of presence (*p* = 0.36). Like species richness, average total frequency of presence decreased between 2017 and 2018 in the unburnt grid cells but, in contrast with species richness, it remained unaltered in the burnt cells.

Table 5.3 Two-way ANOVA results for the total frequency of all species between areas and years after-fire.

Two-way ANOVA	**d. f.**	**Sum of squares**	**Mean square**	**F**	***P***
Fire	1	930.25	930.25	15.365	<0.001
Year	1	78.028	78.028	1.289	0.265
Fire *vs* Year	1	51.361	51.361	0.848	0.364
Residual	32	1937.333	60.542		
Total	35	2996.972	85.628		

5.3.3 Species-wise frequency of presence

The grid-wise frequency of presence data of none of the individual species passed the Shapiro-Wilk Normality Test ($p > 0.05$), so that the overall role of wildfire and monitoring occasion was tested by means of the Kruskal-Wallis test and, if significant, the four separate contrasts were tested by means of the Mann-Whitney U-test. The Kruskal-Wallis test indicated significant differences for 6 of the 11 species, with *p*'s ranging from < 0.01 to 0.02 (Table 5.4), and the Mann-Whitney U-tests for 5 of these 6 species, with *p*'s ranging from < 0.01 to 0.04 (Table 5.4; Figure 5.3). The sixth species, the Iberian mole occurred in too few grid cells to allow testing any of the four contrasts, while both the common genet and the red squirrel were too infrequent in the burnt grid cells to test the contrast between the two monitoring occasions. The red squirrel also did not occur in sufficient grid cells during the second monitoring occasion to test the contrast between burnt and unburnt grid cells.

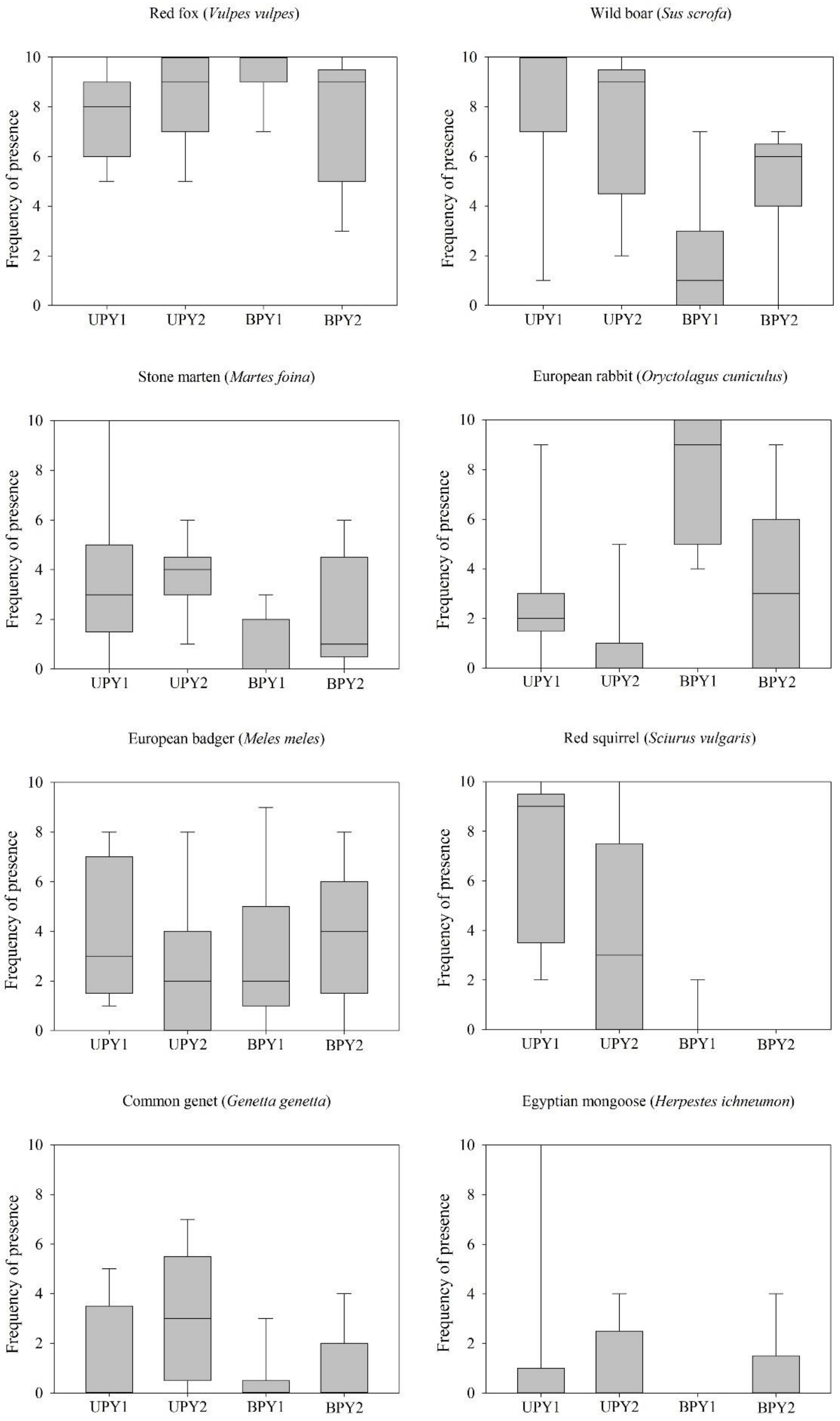

Figure 5.3 Recorded frequency of presence per species in UP and BP areas during year 1 (3 months after-fire) and year 2 (15 months after-fire) for the selected *taxa* in the study area.

Table 5.4 Kruskal-Wallis and Mann-Whitney test results for the frequency of presence of the individual species. UP and BP stand for unburnt and burnt grid cells, Y1 and Y2 for the two subsequent sampling occasions.

Taxa	**Fire *vs* Sampling occasion**		**UP Y1 *vs* Y2**			**BP Y1 *vs* Y2**			**Y1 UP *vs* BP**			**Y2 UP *vs* BP**		
	X^2	***p***	**U**	***z***	***p***	**U**	***z***	***p***	**U**	***z***	***p***	**U**	***z***	***p***
Red fox (*Vulpes vulpes*)	0.8131	0.3524												
Egyptian mongoose (*Herpestes ichneumon*)	0.2105	0.5461												
Stone marten (*Martes foina*)	7.403	0.0059	34.5	-0.492	0.6225	34	-0.546	0.5854	18.5	-1.957	0.0504	23.5	-1.486	0.1373
European badger (*Meles meles*)	0.1962	0.6549												
Common weasel (*Mustela nivalis*)	0.0811	0.5521												
Common genet (*Genetta genetta*)	4.295	0.0226	21	-1.723	0.0849				30	-1.053	0.2922	18	-2.036	0.0417
Wild boar (*Sus scrofa*)	13.94	0.0002	26	-1.297	0.1948	16	-2.153	0.0313	6	-3.073	0.0021	20.5	-1.743	0.0814
European rabbit (*Oryctolagus cuniculus*)	8.108	0.004	15	-2.286	0.0223	10.5	-2.625	0.0087	6	-3.053	0.0023	24.5	-1.442	0.1493
Red squirrel (*Sciurus vulgaris*)	15.27	0	20	-1.785	0.0743				0.5	-3.662	0.0003			
European hedgehog (*Erinaceus europaeus*)	0.3066	0.3102												
Iberian mole (*Talpa occidentalis*)	2.027	0.0177												

The significant Mann-Whitney U-test results for three of the five species were in line with the significant two-way ANOVA results for total frequency of presence. The frequency of presence of red squirrel, wild boar and stone marten was significantly higher in the unburnt than burnt grid cells during the first monitoring occasion, with mean ranks of 7.0 *vs*. 2.5 ($p < 0.01$), 6.7 *vs*. 2.8 ($p < 0.01$), and 6.0 *vs*. 3.5 (0.04), respectively. The common genet also had a significantly higher frequency of presence in the unburnt than burnt grid cells but during the second and not the first monitoring occasion (6.0 *vs*. 3.5, $p = 0.04$). By contrast, the mean rank of European rabbit was significantly lower in the unburnt than burnt grid cells, at least during the first monitoring occasion (2.8 *vs*. 6.7, $p < 0.01$).

The Mann-Whitney U-test results furthermore indicated that time-since-fire played a significant, albeit contrasting role in the frequency of presence of two of the species. The frequency of presence of European rabbit was significantly higher during the first than second monitoring occasion and both in the burnt and unburnt grid cells, with mean ranks of 6.2 *vs*. 3.3 ($p = 0.01$) and 6.4 *vs*. 3.1 ($p = 0.01$). By contrast, the frequency of presence of wild boar was significantly lower during the first than second monitoring occasion, at least in the burn grid cells with mean ranks of 3.4 *vs*. 6.1 ($p = 0.03$).

5.3.4 Species co-occurence

The first two axes of the grid cell-centred NMDS provided a satisfactory ordination of the species co-frequency of presence data of the two monitoring occasions, with a stress of 0.018. The first axis revealed a clear tendency of separating the burnt grid cells from the unburnt grid cells, particularly in the case of the first monitoring occasion (Figure 5.4). At the same time, the first-axis scores of the individual burnt grid cells were consistently smaller for the second than first monitoring occasion but these differences did not produce a clear-cut separation between the two data sets. By contrast, the scores of the unburnt cells did not reveal any obvious temporal pattern, either on the first or second axis. The second NMDS axis is clearly better correlated to the factor distance-to-nearest-town than to the other distance factors, with an overall Pearson's product-moment correlation coefficient of -0.67 as opposed to $<|0.40|$ (to roads: -0.38; to natural surface water sources: -0.33; to ocean: 0.18). In addition, the scores on the second axis are more strongly correlated with distance-to-nearest-town for the first than second monitoring occasion (Spearman's *r*: -0.79 *vs*. -0.54) as well as for the unburnt than burnt grid cells, not only on both occasions together (Spearman's *r*: -0.79 *vs*. -0.54) but also separately (Spearman's *r* year 1: -0.89 *vs*. 0.64; Spearman's *r* year 2: -0.66 *vs*. -0.51).

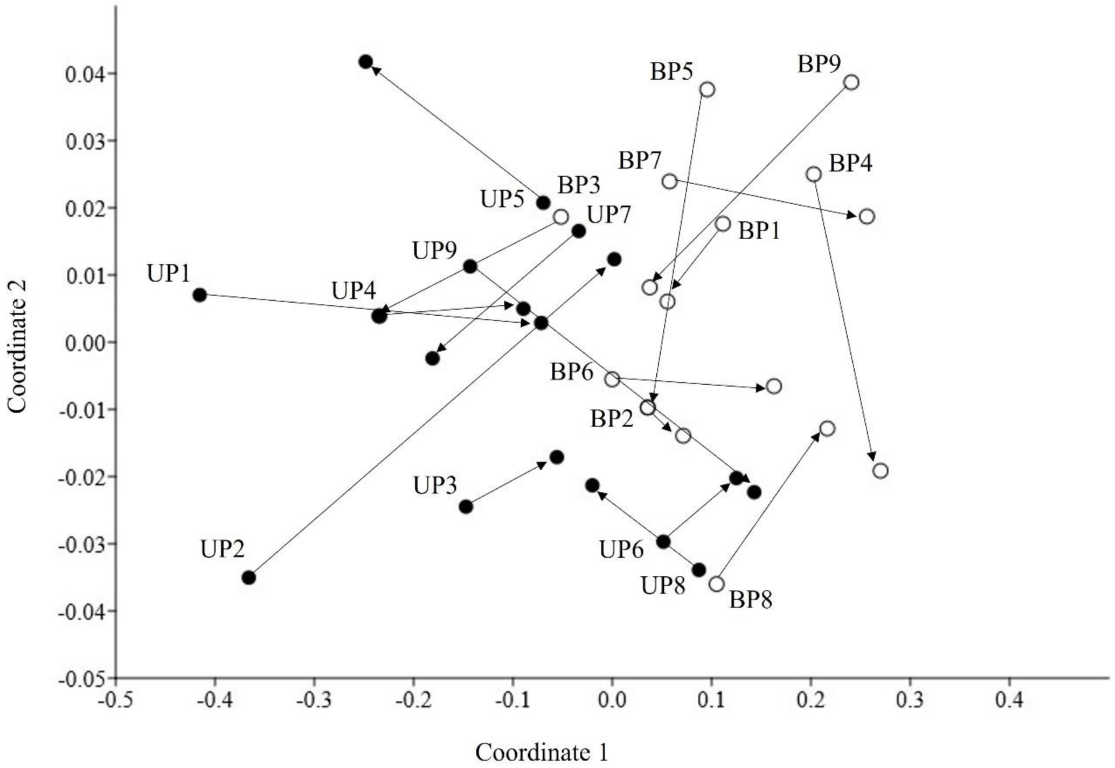

Figure 5.4 Grid cell-centered non-metric multidimensional scaling (NMDS) plot of species co-occurrence during the two sampling occasions. The unburnt (UP) and burnt (BP) grid cells are indicated by black and white dots, respectively, while the arrows connect the individual grid cells in the direction of the first to the second sampling occasion.

The first two axes of species-centred NMDS also provided a satisfactory ordination of the species co-frequency of presence data of the two monitoring occasions, with a stress of 0.07. The first axis arranged the species according to their frequency of occurrence (Figure 5.5). For example, the species occurring in 14 or more grid cells had positive scores on the first NMDS-axis, while the species occurring in fewer than 14 cells had negative scores. The second axis was broadly related to the above-mentioned wildfire impact on species frequency of presence. The scores of red squirrel and wild boar for the first monitoring occasion clearly contrasted with the score of the European rabbit, in line with their - significantly - higher respectively lower frequency of presence in unburnt than burnt grid cells. The separation of the species along the second axis was less pronounced for the second monitoring occasion, in line with the generalized absence of significant differences in species frequency of presence after the first post-fire year.

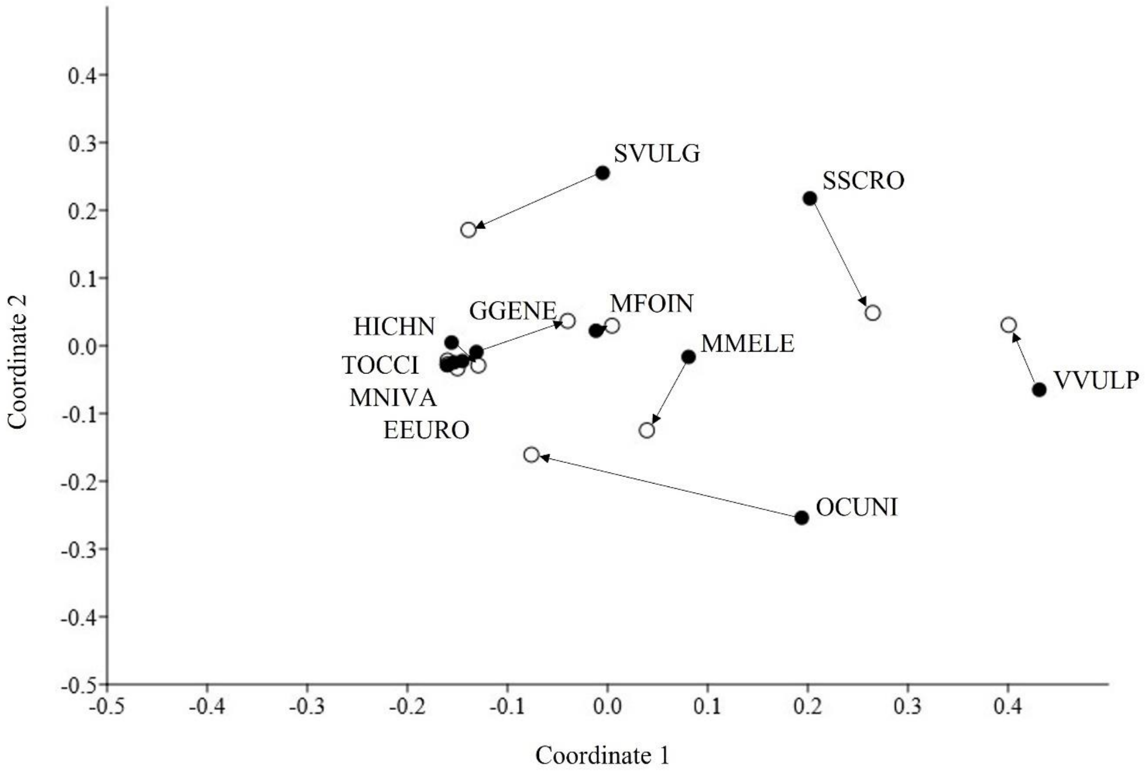

Figure 5.5 Species-centered non-metric multidimensional scaling (NMDS) plot of species co-occurrence during the two sampling occasions. The black and white dots correspond to the first and second sampling occasion, respectively, and are connected for each species by an arrow. The species names are abbreviated as follows: EEURO – *Erinaceus europaeus*, GGENE – *Genetta genetta*, HICH – *Herpestes ichneumon*, MFOIN – *Martes foina*, MMELE – *Meles meles*, MNIVA – *Mustela nivalis*, OCUNI – *Oryctolagus cuniculus*, SSCRO – *Sus scrofa*, SVULG – *Sciurus vulgaris*, TOCCI – *Talpa occidentalis*, VVULP – *Vulpes vulpes*.

5.4 Discussion

The wildfire promoted changes in the post-fire frequency of presence patterns of the mammal community, leading to a reduction in the overall frequency of presence in the burnt area during the first months after the wildfire comparing to the unburnt. Higher frequency of presence patterns in the unburnt area in the first months can be related to competition between *taxa* and between individuals of the same species due to the temporary scarcity of resources caused by the reduction of available habitat promoted by the wildfire (Geary *et al*., 2019). Rural areas with low human disturbance located in the vicinity of the unburnt area may also influenced the frequency of presence recorded at this time by favouring species with greater mobility and tolerance to human disturbance such as red fox (*Vulpes vulpes*) and others foraging for food such as the wild boar (*Sus scrofa*), that temporarily seem to have expanded their frequency of presence to these areas. Fifteen months after the wildfire, the overall frequency of presence of all *taxa* throughout the study area became more even, decreasing in the unburnt areas, but at the same time, some *taxa*-specific frequency of presence also declined or became absent in the burnt area, as observed for European rabbit (*Oryctolagus cuniculus*) and red squirrel (*Sciurus vulgaris*), respectively. The regeneration of the vegetation, particularly around streams and lagoons near the burnt area, seems to have had a relevant role in a more even frequency of presence between burnt and unburnt areas. These humid habitats are among the first to recover their vegetation (Johnson, 2005; Pettit & Naiman, 2007) and guarantee access to water, being particularly important in an ecosystem where water retention is naturally low. While these changes in vegetation may have benefited most *taxa*, they were not enough to maintain some of the identified *taxa* in the burnt area.

Red squirrel frequency of presence in the burnt area was almost non-existent after the wildfire, as its ecological requirements have altered. The absence of living pine trees and canopy deprives the red squirrel of refuge and access to pinecones, its primary food source (Ferreira *et al*., 2001; Fisher & Wilkinson, 2005; Leonard & Koprowski, 2010), which makes this species avoid burnt areas immediately after the wildfire. Wild boar also avoided burnt areas in the first months after the fire, regardless of the low frequency of presence observed in the boundary of the burnt area with rural areas, suggesting that in the initial post-fire period, the burnt area was a passage zone to the adjacent rural areas where the wild boar would feed (Thurfjell *et al*., 2009). Even so, several wild boar droppings were found in the burnt area during the first sampling period, mainly consisting of pine nuts, evidence that during the first winter, perhaps due to food scarcity, this species also sought the burnt area to feed. After 15 months, the frequency of presence of wild boar increased in the burnt area, but an extensive after-fire period may be necessary to achieve identical values to the obtained in the unburnt area, as observed by Soyumert *et al*. (2020) in a burnt pine forest of Turkey. The European rabbit was the only herbivory-oriented species whose frequency of presence benefited from the fire. The transformation of a mature pine forest into an open area promoted the expansion of the European rabbit in the burnt area early since the wildfire, as reported in other studies about lagomorphs (Fisher & Wilkinson, 2005). The sequence of natural events in the short period after the wildfire that includes the change of season and the arrival of precipitation benefited the European rabbit, by rapidly promoting extensive open areas with emergent vegetation, which are basic ecological requirements for the establishment of the species. The increase of European rabbit frequency of presence in the burnt area also acted as an attracting factor for predator species frequency of presence in that same area. The high number of new European rabbit burrows found along the burnt forest paths was evidence of the species expansion. European rabbit is the main diet of some of the predator species detected, acting as a driver for the spatial distribution of several predator species in the study area throughout the monitoring period, particularly in the first sampling period for the red fox, and later, during the second sampling period, for the Egyptian mongoose (*Herpestes ichneumon*). The European rabbit's increased frequency of presence promoted an abundant number of different predator markings near the rabbit burrows throughout the burnt area, particularly by red fox, emphasizing how relevant predator-prey relations and competition among predators in the area are. Micromammal availability throughout the whole study area also seems to have remained unchanged, and it can be related to the abundance of pine nuts available on the ground, soon after the wildfire and until the end of the studied period. Post-fire habitats at early successional stages can generate large availability of seeds (Ne’eman *et al*., 1993), being especially important in the burnt area where other feeding resources become limited at the beginning of the post-fire period. This availability of prey early since the post-fire period seems to have had a crucial role in the frequency of presence of the predator species found in the study area.

Red fox was the only species on which the recorded frequency of presence throughout the studied period was identical in both areas and across most checkpoints. The wildfire seems to have not affected this species frequency of presence, maintaining a high frequency of presence pattern for the entire duration of the study. Red foxes are highly adaptable animals, giving this species a greater natural resilience to overcome negative effects associated with wildfires in the short after-fire period, particularly when not limited by prey availability, as other studies also showed (Fisher & Wilkinson, 2005; Díaz-Ruiz *et al*., 2013; Hradsky *et al*., 2017; Puig-Gironès & Pons, 2020). Red fox is the terrestrial apex predator in the study area, which also benefits its frequency of presence at the expense of other predators since in other burnt areas with different apex predators, foxes can have their prey selection and availability limited (Zuñiga *et al*., 2020). Frequency of presence on European badger (*Meles meles*) and stone marten (*Martes foina*) remained identical throughout the study area for the entire study, with a slight increase recorded in the burnt area during the second sampling period. These two species are mustelids, with different behaviors and distinct ecological requirements, but as the red fox, both share diverse and adaptable diets that are dependent on the food sources available throughout each season

(Carvalho & Gomes, 2004; Papakosta *et al*., 2014; Hipólito *et al*., 2016; Puig-Gironès & Pons, 2020). Their adaptability, the availability of prey, and the regrowth of vegetation promoted a stable frequency of presence pattern for both species throughout the duration of the study when compared to other predators. Common genet (*Genetta genetta*) and Egyptian mongoose can also adopt a more diverse diet but are generally more specialized predators that mostly hunt small vertebrates (Rosalino & Santos-Reis, 2002; Carvalho & Gomes, 2004), and in the latter case, also lagomorphs (Rosalino *et al*., 2009). The frequency of presence of these two species was lower in the burnt areas, especially in the first sampling period, probably to the absence of forested paths that these species could use as refuge and pathways (Torre & Díaz, 2004), and because of direct competition from other predators that started to use the burnt area earlier. Despite this, a low number of presences by checkpoint was recorded in the burnt area for common genet, and later during the second sampling period for Egyptian mongoose when vegetation recovery had already begun. In the latter case, the decrease in European rabbit frequency of presence in the unburnt area may have also promoted the displacement of Egyptian mongoose activity to the burnt area, where the registered frequency of presence of its primary prey had increased earlier.

For the rest of the species detected, that include common weasel (*Mustela nivalis*), European hedgehog (*Erinaceus europaeus*), and Iberian mole (*Talpa occidentalis*), the information about their frequency of presence patterns is scarce and concentrates in the unburnt area. All three species are relatively small, which partly hinders the detection of their activity signs, especially in the unburnt area. Therefore, the information collected is expected to be underestimated, but it still allows some inferences about their post-fire frequency of presence. Common weasel's small size can be an activity inhibitor at earlier successional stages, in areas with almost no vegetation cover (Macdonald *et al*., 2004; Fisher & Wilkinson, 2005), making them vulnerable to larger predators, as it may have happened in the burnt area. Common weasel's frequency of presence may have concentrated in safer zones within the limit of the unburnt area, adapting their diet to a stable source of prey, as observed before by Erlinge (1975) and Elmeros (2006). European hedgehog and Iberian mole’s frequency of presence patterns also concentrated in boundary zones between the burnt and unburnt areas and sampling squares near rural areas. Both species are more related to rural than forested areas. European hedgehogs are increasingly associated with the human environment, particularly in rural areas (Rautio *et al*., 2015; Pettett *et al*., 2017), while moles avoid pine monocultures because of the low soil pH created by pine leaves that limits the distribution and species composition of soil invertebrates

(Zurawska-Seta & Barczak, 2012). Our results suggest that the recolonization of the burnt areas by these three *taxa* occurs later and from neighbouring rural areas, where their ecological requirements are more favourable, and predation is lower than in forested areas.

The use of presence/absence indicators at each checkpoint has proven efficient in assessing the overall activity of the community and as a quantification tool of the mammal diversity found throughout the study area. Overall, the joint number of indicators defined for each species enabled the validation of their frequency of presence in both areas and were particularly useful for less common and less conspicuous *taxa*, allowing to validate their presence through a broader array of signs and behaviors. Some indicators, however, were redundant in assessing the frequency of presence of more common or conspicuous *taxa*, e.g., the high number of footprints and droppings found per checkpoint rendered the rest of indicators redundant for red fox, or as the high number of droppings for European rabbit.

Footprints and droppings were the most common indicators found for *taxa* with the higher contribution among all used indicators, while gnawed pinions were crucial to identify the presence of arboreal species such as the red squirrel. Ground markings and burrows were less abundant but relevant identification indicators for wild boar, European badger, and European rabbit. The remaining indicators added relevant ecological information but are not viable individual options to future research that wishes to adopt the methodology described in this study. For less conspicuous and less common taxa, additional methods of monitoring their presence are recommended to improve efficiency. The same is true for post-fire studies in non-sandy soil areas, where this methodology and sampling period will be less efficient.

5.5 Conclusions

The main conclusions of this study into the immediate and short-term effects of a large and high-to-extreme-severity wildfire on the occurrence of medium-sized mammal species in one of the coastal maritime pine woodland areas of Central Portugal were as follows:

(i) both the number of species and their frequency of presence were lower in burnt than unburnt areas during the first two winters after the fire; however, only the differences during the first winter were significant, especially due to changes with time-since-fire in the unburnt areas;

(ii) the occurrence of six of the 11 species was significantly affected by the wildfire, in five cases negatively and in one case positively, and in particular during the first post-fire winter; the negatively-affected species covered all diet preferences (1 herbivore, 1 omnivore and 2 carnivores);

(iii) a significant role of time-since-fire was restricted to two of the 11 species but involved opposite senses, with European rabbit (*Oryctolagus cuniculus*) decreasing in frequency between the first and second post-fire winter as opposed to wild boar (*Sus scrofa*);

(iv) dropping and footprints were the most frequently observed indicators, but their relative importance differed markedly between the two sampling occasions.

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Chapter 6

6 Final considerations

This chapter provides an overview of the main results obtained in the previous sections (Chapters 2 to 5) considering the main purposes of the thesis.

Wildfires are natural events that are part of Mediterranean ecosystems, shaping fauna and flora for millennia (Pausas *et al*., 2008). With the development of human society, native ecosystems were progressively transformed, and with them, also the role of wildfires in the Mediterranean region. Nowadays, wildfires are still a crucial part of Mediterranean ecosystems, but as native ecosystems decline and are transformed into plantation forests, wildfires frequency, severity, and extent increase to a level never seen before (Stephens *et al*., 2014; San-Miguel-Ayanz *et al*., 2020). The slow response of human society towards climate change and nature conservation worldwide allowed significant changes in natural cycles and an increase of extreme natural events, including wildfires (Bowman *et al*., 2009; San-Miguel-Ayanz *et al*., 2020). Among several other adverse effects, wildfires currently threaten the survival of native fauna and flora in a growing number of regions of the world, contributing to the global loss of biodiversity (CBD, 2001). In recent decades, to better understand and counter the adverse wildfire effects, the scientific community has been progressively increasing the number of studies related to wildfires in several fields of expertise. However, the current scientific knowledge is still limited in several topics related to fire, especially regarding the effects of fire on animals (Fontaine & Kennedy, 2012; Titley *et al*., 2017; Pausas & Parr, 2018; Saunders *et al*., 2021). Considering the full extent of studies related to animal ecology and wildfires, information is particularly scarce regarding their immediate and short-term effects due to constraints such as the characteristics of the affected area, and the lack of well-established methodologies that allow the comparability of results between different studies and regions of the world (Griffiths and Brook, 2014). The unpredictability of the locations where the wildfire will occur, previous knowledge of the communities in the affected area, and the logistical obstacles in preparing the study area immediately after the fire add an increased difficulty to these studies. Also, the short-term fire effects commonly described on animals involve mortality and injury, escape to other areas, and an attraction effect for foraging species in the burnt area (Moreira *et al*., 2010), but how these processes occur and to what extent, the *taxa* involved and how they respond to these post-fire processes, or the interactions between *taxa* and natural structures or microhabitats, are largely unknown (Pausas, 2019). Addressing these knowledge gaps, chapters 2 and 3 of this thesis focused on the immediate and short-term effects of fire on ground-dwelling macroinvertebrates communities and the relevance of natural structures in their survivability during wildfires. These studies were conducted on maritime pine (*Pinus* *pinaster*) and blue gum eucalypt (*Eucalyptus globulus*) forest plantations, which are the dominant trees in the country (Silva, 2007). Known as fire-prone forests, they are devasted every year by wildfires (IFN6, 2015; San-Miguel-Ayanz *et al*., 2021). These characteristics, allied to the few existing studies, reinforce the need to generate new knowledge on the impacts of wildfire on fauna, providing information that can be used as a basis for the sustainable management of the wildlife resources present in these production forests. Beside these planted forests, native oak forests were also included, allowing to compare the same community but in a less common ecosystem with a lower degree of disturbance (IFN6, 2015). With this information, was possible to identify the differences in the macroinvertebrate community in burnt *versus* unburnt forests and between native forests *versus* production forests. Findings of these studies (chapter 2 and 3) revealed that stones, regardless of the forest type, do safeguard part of their associated macroinvertebrate community from fire, and that different land management characteristics promote different rates of mortality under the same wildfire conditions. The list of the *taxa* associated with stones in each forest type, in unburnt and burnt areas, and each *taxa* corresponding mortality rates showed that ground-dwelling macroinvertebrate community richness and abundance are highest in oak forests and lowest in eucalypt plantations. Comparison between the *taxa* identified in each unburnt forest type (pine *vs* eucalypt *vs* oak) shows that most *taxa* found in the plantations also exists in the oak forests, meaning that less disturbed native ecosystems maintain higher ground-dwelling macroinvertebrate diversity. Positive correlations found between larger and deep buried stones with higher ground-dwelling macroinvertebrate abundance and richness can in part explain this result, as stones become smaller and less deeply buried in plantation forests due to more intensive management practices. Overall, data collected in Chapters 2 and 3 showed a high resilience level of the ground-dwelling macroinvertebrate community to wildfires, confirming the importance of natural structures in the post-fire resilience of animal communities. The adopted methodology allowed the accomplishment of all main objectives, but some questions remain. The mortality observed in smaller animals may have been underestimated as these may have been consumed by the fire. Besides the size and depth of the stones, other stone and soil characteristics (*e.g*., structure, water retention, density, consistency, nutrients, pH) may also have influence the studied community. Local variations in the wildfire characteristics may also have influenced mortality, particularly on less severely burnt patches (Kwok & Eldridge, 2015; Blomdahl *et al*., 2018). Finally, despite the extent of the burnt area and the fact that fieldwork began just days after the fire, we cannot completely exclude that some individuals may have come from the adjacent unburnt areas.

Addressing these methodological limitations is recommended in future studies, starting immediately after the wildfire event, and extending the study in the middle and long-term, making it possible to evaluate the subsequent recolonization processes.

In Chapter 4, after confirming that ground-dwelling macroinvertebrate communities are resilient to wildfires and rapidly respond to post-fire changes in the ecosystem, mulching application, as a post-fire mitigation measure, was tested for potential adverse effects on the community in the short-term. Mulching is a common practice used after wildfires that consists of applying a layer of material (organic or inorganic) to prevent soil erosion and diminish runoff (Girona-Garcia *et al.*, 2021), being applied few months after the fire, before the first rains. This study was conducted in a eucalypt plantation, and the mulching consisted of eucalyptus logging residues applied at different rates. The results confirmed that the use of mulch as an erosion prevention mitigation measure, regardless of the application rate used, does not significantly affect the ground-dwelling macroinvertebrate community in the short-term, further reinforcing the resilience of these communities to changes in the ecosystem. However, additional studies should be conducted in different forests and with other mulching materials, including other common sources of potential cumulative impacts in the community related to forest management activities such as post-fire logging.

In addition to the work already mentioned in chapters 2 and 3, the large extension of the burned area in 2017 also allowed the opportunity to study the impacts of wildfire in mammals (Chapter 5), providing sufficiently large and isolated burnt areas close to unburnt counterparts suitable for monitoring. Terrestrial-medium-sized mammals were selected as the target community, due to their ecological and diversity relevance, but also based on the assumption that because of their ability to live in areas subjected to recurrent fires, their resilience to adverse fire effects is potentially high (Pausas & Parr, 2018). The post-fire response of several mammal species in coastal pine forests was identified, with arboreal herbivores being the most affected, while predators and omnivores maintaining or recovering their frequency of presence patterns in the burnt area shortly after or up to a year after the fire. The frequency of post-fire presence patterns varied by species, ranging from permanent avoidance of the burnt area in some species to fast expansion into those areas from others. Significant differences in the mammal diversity between burnt and unburnt areas were identified, but not between monitoring occasions. The rapid increase of frequency of presence of European rabbit (*Oryctolagus cuniculus*) in the burnt area showed that this species benefited with the wildfire, and at the same time, together with micromammals populations as available prey, was the most relevant factor for the overall maintenance of carnivore species presence in the burnt area, but further research is needed to verify this. On the other hand, wild boar (*Sus scrofa*) and red squirrel (*Sciurus vulgaris*) were the species most affected by the wildfire. Wild boar decreased the frequency of presence in the burnt area immediately after the fire but came close to the values obtained in the unburnt area after one year. The red squirrel is a heavily habitat-dependent species that became absent in burnt areas after the fire. In cases like this, the destruction of habitat and the resulting burnt area avoidance by a particular species can become a conservation issue unless careful planning is taken by stakeholders. Overall, the results obtained in this study have provided novel information about how mammal communities respond to wildfires and showed that the methodology developed can be used as a tool for the rapid post-fire assessment of several mammal species in burnt areas. For estimating population densities, and studying rare or less conspicuous species, additional methods, such as camera trapping, should be adopted. Several questions arise from this study that should be addressed in the future to understand how the survival of each species and their respective recolonization process of the burnt area occurs. Issues such as predator-prey relations, the seasonal diet adopted by each species during the first year after the wildfire, and the understanding of burrowing behavior and interaction with natural structures that may provide shelter from the wildfire should be a priority.

The several studies conducted in this thesis showed that the two animal communities studied (ground-dwelling invertebrates and mammals) are somehow resilient to wildfires and wildfire-related changes. The new information presented discloses some of their strategies and abiotic associations to overcome the immediate and short-term periods after a wildfire. The methodological procedures adopted, and the new data generated will be helpful and hopefully improved in the future to increase the knowledge regarding the animal communities addressed, as well as other faunal groups. The research concept adopted, more focused on relationships at a smaller scale and shorter periods, rather than on more generalist views and trends, is crucial to understand the impacts of wildfires in animals because of the complexity of interactions between biotic and abiotic factors, rapid changes, and its spatial variation due to distinct human interference. Hence, we believe that this thesis is a valid contribution to better understand the impacts of wildfires in the ecosystems, which is of crucial importance to adopt preventive and post-fire measures to promote and protect the forest associated biodiversity.

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Supplementary Material

Annex 1 Occurrence, dominant feeding behavior and habitat associations of each *taxa* in oak unburnt (U) and burnt (B) areas.

Order	Family	U	B*	Dominant feeding behavior	Dominant habitat association		
					Ground	Underground	Plants
Araneae	Agelenidae	•	•	Predator	•		•
	Amaurobiidae	•		Predator	•		
	Anyphaenidae	•		Predator			•
	Araneidae	•		Predator			•
	Corinnidae	•		Predator	•		
	Dictynidae	•		Predator	•		•
	Dysderidae	•	•	Predator	•		
	Gnaphosidae	•	•	Predator	•		
	Hahniidae	•	•	Predator	•		•
	Linyphiidae	•		Predator	•		
	Liocranidae	•		Predator	•		
	Lycosidae	•		Predator	•		
	Mimetidae	•		Predator			•
	Oecobiidae	•		Predator	•		
	Oonopidae	•		Predator	•		
	Oxyopidae		•	Predator			•
	Philodromidae		•	Predator			•
	Pisauridae	•	•	Predator	•		•
	Salticidae	•		Predator	•		•
	Scytodidae		•	Predator	•		
	Segestriidae	•		Predator	•		
	Sparassidae	•		Predator			•
	Theridiidae	•		Predator	•		
	Thomiisidae	•		Predator			•
	Zodariidae		•	Predator	•		
	Zoridae	•	•	Predator	•		
Blattodea	Ectobiidae	•	•	Omnivore	•		•
	Rhinotermitidae	•		Detritivore	•	•	
Chilopoda	Lithobiidae	•	•	Predator	•		
	Geophilidae	•		Predator		•	
	Scutigeridae	•		Predator	•		
Coleoptera	Anthribidae	•		Fungivore	•		
	Carabidae	•	•	Predator	•		•
	Chrysomelidae	•	•	Herbivore			•
	Cucujidae		•	Predator			•
	Curculionidae	•		Herbivore			•
	Elateridae	•		Herbivore	•	•	
	Endomychidae	•		Fungivore	•		
	Meloidae	•		Herbivore			•
	Scarabidae	•		Detritivore	•	•	
	Staphylinidae	•		Predator	•		
	Tenebridae	•	•	Omnivore	•		
Diplopoda	Julidae	•	•	Detritivore	•	•	
Hemiptera	Acanthosomidae	•		Herbivore			•
	Anthocoridae	•		Predator			•
	Aphidoidea	•		Herbivore			•
	Lygaeidae		•	Herbivore			•
	Miridae	•		Herbivore			•
	Nabidae	•		Predator			•
Hymenoptera	*Aphaenogaster*	•	•	Predator	•	•	
	Crematogaster	•		Predator	•		•
	Messor	•		Herbivore	•		
	Temnothorax	•	•	Herbivore	•		•
	Tetramorium	•	•	Predator	•		
	Camponotus	•	•	Omnivore	•		
	Formica	•	•	Predator	•		
	Lasius	•		Omnivore	•		
	Plagiolepis	•		Omnivore	•		
	Ichneumonidae	•		Herbivore			•
Isopoda	Cylisticidae	•	•	Detritivore	•	•	
	Porcellionidae	•	•	Detritivore	•	•	
	Stenoniscidae	•	•	Detritivore	•	•	
	Trichoniscidae		•	Detritivore	•	•	
Lepidoptera	Gelechiidae	•		Herbivore			•
Microcoryphia	Meinertellidae	•	•	Detritivore	•	•	
Zygentoma	Nicoletiidae	•		Detritivore	•	•	
Neuroptera	Myrmeleontidae	•		Predator	•		
Orthoptera	Gryllidae	•	•	Omnivore	•	•	
Pseudoscorpionida	Chthoniidae	•		Predator	•	•	
	Garypidea	•		Predator	•	•	
Thysanoptera	Thripidae	•		Herbivore			•
Pulmonata		•		Herbivore	•		•

*Only alive specimens. Bibliography used: Harde & Severa, 1984; Barrientos, 1988; Goulet et Huber, 1993; Buddle *et al*., 2000; Czechowski *et al*., 2002; Collet, 2003: Moretti *et al*., 2006; Andersen *et al*., 2009; Gongalsky *et al*., 2012; Kim & Holt, 2012; New, 2014; Lissner, 2014; Zaitsev *et al*., 2014; Barton *et al*., 2017; Nentwig *et al*., 2021; Oger, 2021.

Annex 2 Distribution, dominant feeding behavior and habitat associations of each *taxa* in unburnt (U) and burnt (B) areas of pine (P) and eucalypt (E).

Order	Family	PU	PB*	EU	EB*	Dominant feeding behaviour	Dominant habitat association		
							Ground	Underground	Plants
Araneae	Agelenidae	•	•	•	•	Predator	•		•
	Anyphaenidae	•	•			Predator			•
	Araneidae	•				Predator			•
	Corinnidae	•		•		Predator	•		
	Dysderidae	•	•	•		Predator	•		
	Gnaphosidae	•	•	•	•	Predator	•		
	Hahniidae	•	•	•		Predator	•		•
	Linyphiidae	•				Predator	•		
	Liocranidae	•		•	•	Predator	•		
	Lycosidae	•			•	Predator	•		
	Mimetidae			•		Predator			•
	Oecobiidae			•		Predator	•		
	Oonopidae	•	•	•		Predator	•		
	Salticidae	•		•	•	Predator	•		•
	Sparassidae	•				Predator			•
	Thomiisidae			•		Predator			•
	Zodariidae			•		Predator	•		
	Zoridae	•				Predator	•		
Blattodea	Ectobiidae	•	•	•	•	Omnivore	•		•
	Rhinotermitidae	•		•		Detritivore	•	•	
Chilopoda	Lithobiidae	•	•	•	•	Predator	•		
	Geophilidae	•	•			Predator		•	
	Scolopendridae			•		Predator	•		
Coleoptera	Carabidae	•	•			Predator	•		•
	Cucujidae			•	•	Predator			•
	Curculionidae	•	•	•		Herbivore			•
	Elateridae			•		Herbivore	•	•	
	Scolytidae	•				Herbivore			•
Dermaptera	Forficulidae		•	•		Omnivore	•	•	
Hemiptera	Anthocaridae			•		Herbivore			•
	Cicadidae	•				Herbivore			•
	Reduviidae			•		Predator			•
Hymenoptera	*Aphaenogaster*	•		•	•	Predator	•	•	
	Crematogaster	•				Predator	•		•
	Messor			•		Herbivore	•		
	Temnothorax	•	•	•	•	Herbivore	•		•
	Tetramorium	•				Predator	•		
	Camponotus	•		•		Omnivore	•		
	Formica	•		•	•	Predator	•		
	Plagiolepis	•		•		Omnivore	•		
	Diapriidae	•				Herbivore			•
	Mutillidae				•	Omnivore	•		•
Isopoda	Cylisticidae			•		Detritivore	•	•	
	Porcellionidae	•	•	•		Detritivore	•	•	
	Stenoniscidae	•		•		Detritivore	•	•	
Lepidoptera	Gelechiidae		•			Herbivore			•
Microcoryphia	Meinertellidae	•	•	•	•	Detritivore	•	•	
Opiliones	Phalangiidae	•	•		•	Predator	•		•
Orthoptera	Gryllidae	•		•		Omnivore	•	•	
Pseudoscorpionida	Chthoniidae	•				Predator	•	•	
Pulmonata				•		Herbivore	•		•

*Only alive specimens. Bibliography used: Harde & Severa, 1984; Barrientos, 1988; Goulet et Huber, 1993; Buddle *et al*., 2000; Czechowski *et al*., 2002; Collet, 2003: Moretti *et al*., 2006; Andersen *et al*., 2009; Gongalsky *et al*., 2012; Kim & Holt, 2012; New, 2014; Lissner, 2014; Zaitsev *et al*., 2014; Barton *et al*., 2017; Nentwig *et al*., 2021; Oger, 2021.