Universidade de Évora - Instituto de Investigação e Formação Avançada

Programa de Doutoramento em Biologia

Tese de Doutoramento

Post-fire regeneration of pollination processes: an ecological network approach

Paula Virgínia Serra Parreira Banza

Orientador(es) | Anabela Dias Belo
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A tese de doutoramento foi objeto de apreciação e discussão pública pelo seguinte júri nomeado pelo Diretor do Instituto de Investigação e Formação Avançada:

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Évora 2021


Wow! Check out that butterfly!
That's just a moth bud.
JUST A MOTH!
Moths are super important to the ecosystem!
I work all night pollinating plants and flowers!
Plus I'm food for lots of larger creatures!
Moths kind of rule!
I had no idea.
www.lunarbaboon.com

in memory of Penny Wolf

a great supporter of A Rocha Portugal scientific work

Acknowledgements

It is a common thing to say that a PhD thesis is the product of the work of many people and rarely the work of just one individual. In my case this has been absolutely true.

There are so many people that I would like to thank – I apologise beforehand to the ones I will probably forget to mention…

First of all, I would like to thank my supervisors, Anabela Belo and Darren Evans. Their constant support and encouragement are the reason I never gave up; they helped me immensely with everything related to the thesis – for this I am truly grateful.

To Callum and Renata, for their huge help with the statistics.

To Lieske, for sharing my many adventures during the fieldwork (particularly in the beginning when we had to select the sites).

To Todd, for working alongside me for 9 months, and to Penny Wolf, for providing the funds for his work; to Sandro, for helping me in the fieldwork while I supervised his Master in Conservation Biology; and to the many volunteers of A Rocha Portugal, for helping me in diverse tasks such as field and laboratory work (there are too many to mention by name, but know that I am deeply grateful…).

To Jens and Dra. Amália Oliveira, for their help with insect identification.

To Bert and Rúben, for some very helpful advices; and to Professor António Pereira Coutinho, for information on pollen.

To Luís and Isabel, for offering me (well, in truth I was the one who asked for…) their home to stay at during the fieldwork.

To Bébé, for "always being there for me"; and to Filipa and Gui (team members of A Rocha Portugal), for all the encouragement they gave me.

To so many good friends, near and far; their support, encouragement and prayers made all the difference.

In memory of my mother and to my parents-in-law, for their encouragement and financial support.

To my husband Marcial, my daughter Beatriz and my son Zé, for really believing their wife and mother, respectively, is a bright and clever person.

And to God, for performing so many miracles that enabled this thesis to become a reality.

Institutional Acknowledgments

This work was partially funded by National Funds through Fundação para a Ciência e Tecnologia (FCT, Portugal) - Foundation for Science and Technology under the Project UID/AGR/00115/2019.

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ENVIRONMENT AND DEVELOPMENT

With support from the Natural Environment Research Council and Butterfly Conservation (NERC, UK) - Industrial CASE studentship awarded to C.J.M. (Project Reference: NE/ K007394/1 and K007394/2).

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Summary

Post-fire regeneration of pollination processes: an ecological network approach

Wildfires shape ecological and evolutionary processes but are increasing in frequency as a result of climate change and other factors, particularly in the Mediterranean countries, with implications for biodiversity and ecosystem functioning. Whilst the recovery of plant communities after a wildfire is generally understood, the impacts on ecological processes, such as plant-pollinator interactions, have received little attention. Moths are a major group of insects contributing to global biodiversity and play an important role in food webs, with recent research suggesting they could be important, but overlooked, pollinators. However, it is unclear the extent to which wildfires affect insects, and nocturnal moths in particular, and how they disrupt their potential role as pollinators.

The aim of this study was to to examine the recovery of plant-pollinator interaction networks, considering diurnal insects, on one hand, and nocturnal moths on the other, to fire disturbance by comparing interacting plant and animal communities in post-fire burned and unburned areas. We investigated the effects of wildfire on diurnal and nocturnal pollen-transport networks over two years following a large fire that occurred in Southern Portugal. By comparing plants and insects carrying pollen collected at three burned sites and three adjacent unburned sites, we analysed the effects of wildfire on: a) abundance and species richness of diurnal insects, moths and flowers across seasons; b) the pollen transported by diurnal insects and moths; and c) the structure of nocturnal (moth) pollen-transport networks.

Burned sites had a significantly higher abundance of flowers, but nocturnal moths were less abundant and species-rich. Overall, 70% of individual moths carried pollen, and they transported pollen from 83% of the flower species present. The total pollen transport by the moth assemblage at burned sites was just 20% of that at unburned sites. Nocturnal pollen-transport networks had lower complexity and robustness following the fire than at nearby unburned sites.

For diurnal insects, wildfire had significant, interacting effects on the abundance but not on species richness; with time, they increased in abundance and species richness, most notably each spring. Pollen loads and species richness on individual diurnal insects were significantly higher in burned sites in the first spring only, but generally increased with time after the wildfire.

These results suggest negative effects of fire upon nocturnal moths that will likely permeate to other taxa through loss of ecological interactions. However, diurnal insects, and the pollen they transported, returned to the pre-fire, unburned state within a relatively short period. Understanding the responses of ecological networks to wildfire can inform management that promotes resilience and facilitates whole-ecosystem conservation.

Keywords: pollination, ecological networks, fire, Mediterranean, moths

Resumo

Recuperação pós-fogo de processos de polinização – uma abordagem às redes ecológicas

Os incêndios florestais moldam processos ecológicos e evolutivos, e espera-se que aumentem de frequência e intensidade como resultado de alterações climáticas, entre outros fatores, em particular na zona do Mediterrâneo, com implicações para a biodiversidade e funcionamento dos ecossistemas. Embora a recuperação das comunidades vegetais após um incêndio florestal seja geralmente compreendida, os impactos nos processos ecológicos, como as interações entre plantas e polinizadores, têm recebido pouca atenção. Os lepidópteros noturnos são um grande grupo de insetos que contribuem para a biodiversidade global e desempenham um papel importante nas teias alimentares; estudos recentes sugerem que podem ser importantes polinizadores, embora um pouco negligenciados. Contudo não existe informação suficiente sobre até que ponto os incêndios florestais afetam os insetos de uma maneira geral, e os lepidópteros noturnos em particular, e, em consequência, sobre como podem comprometer o seu papel potencial de polinizadores.

O objetivo deste estudo foi examinar a recuperação das redes de interação entre plantas e polinizadores após perturbação causada pelo fogo; consideraram-se insetos diurnos e lepidópteros noturnos e comparou-se a interação entre as comunidades de plantas e insetos em áreas ardidas e não ardidas.

Investigámos os efeitos do fogo nas redes diurnas e noturnas de transporte de pólen ao longo de dois anos, após um grande incêndio ocorrido no sul de Portugal. Ao comparar as plantas e os insetos vetores de pólen recolhido em três locais ardidos e três locais adjacentes não ardidos, analisámos os efeitos do fogo: a) na abundância e riqueza de espécies de insetos diurnos, lepidópteros noturnos e flores ao longo do tempo; b) no pólen transportado por insetos diurnos; e c) na estrutura das redes noturnas (lepidópteros noturnos) de transporte de pólen.

Nas áreas ardidas a abundância de flores foi significativamente maior, contrariamente aos lepidópteros noturnos, menos abundantes e com menor riqueza específica. Cerca de 70% dos lepidópteros noturnos transportaram pólen de aproximadamente 83% das espécies das flores presentes. O transporte total de pólen pela totalidade de lepidópteros noturnos foi de apenas 20% nos locais afetados pelo fogo, por comparação com os locais não ardidos. As redes noturnas de transporte de pólen apresentavam menor complexidade e robustez após o incêndio do que as de locais não ardidos.

No caso dos insetos diurnos, o fogo teve efeitos significativos no número de indivíduos, mas não na riqueza específica; ao longo do tempo, os insetos foram aumentando em número e riqueza de espécies, mais expressivamente em cada primavera. O total de pólen transportado e abundância de insetos diurnos foram significativamente maiores em locais afetados pelo fogo, mas apenas na primeira primavera; de uma forma geral foram aumentando após o incêndio, ao longo do tempo.

Os resultados do estudo sugerem efeitos negativos do fogo sobre os lepidópteros noturnos que provavelmente irão refletir-se noutros taxa através da perda de interações ecológicas. No entanto, no caso dos insetos diurnos e do pólen por eles transportado, assistiu-se a um retorno ao estado pré-incêndio após um período relativamente curto.

O conhecimento dos efeitos que o fogo tem sobre as redes ecológicas e, sobretudo, da forma como se dá a recuperação dessas redes após a ocorrência do fogo, pode ser muito útil para o estabelecimento de uma gestão adequada que promova a resiliência dos ecossistemas, facilitando desse modo a sua conservação de um ponto de vista global.

Palavras-chave: polinização, redes ecológicas, fogo, Mediterrâneo, lepidópteros noturnos

CHAPTER 1

General Introduction

General Introduction

In recent years, the frequency of wildfires in northern Mediterranean countries has increased mainly due to socioeconomic factors such as an increase in fuel accumulation at the landscape level due to land abandonment and/or an increase in exotic forestry plantations (Moreno & Oechel, 1994; Moreira, Rego and Ferreira, 2001; Naveh & Carmel, 2004; Pausas & Fernández-Muñoz 2012). Although humans have adapted fires for their own use, they can become more destructive than originally intended and, currently, it is an important tool for balancing land-management with protection of natural ecosystems. Species are not adapted to fire *per se*, rather they adapt to fire regimes and if this change dramatically, species that are adapted to a particular fire regime can be threatened and lost when that changes (Pausas & Keeley 2009; Keeley et al. 2011). The interaction between fires and anthropogenic actions, in the face of climate change, may potentially drive further increases in the frequency and severity of fires, particularly in situations of drought or extreme rainfall (Flannigan et al. 2013; Morán-Ordóñez et al. 2020a).

Global environmental changes are threatening Mediterranean forests and the services and goods they provide but, more important than climate change, better management policies can maximize the provision of those services and prevent extreme drought-events or wildfires (Morán-Ordóñez et al. 2020b). In Europe, Portugal has the highest number of burned areas *per* year since 2013; in 2017 more than 540,000 ha of forest were destroyed as a result of fires (EEA 2019). By the end of the 19th century, portuguese native forests were reduced to less than 7% of the territory (Mather & Pereira 2006; Louro et al. 2010) and they never recovered. One of the main reasons for this was the great increase in the extent of wildfires (Fernandes et al. 2014), especially since the 1970s. Frequent fires can affect forest regeneration in a way that compromises it in the long-term (Carrión et al, 2010). They can exhaust below-ground storage reserves preventing further resprouting and kill saplings that have not yet stored enough reserves for resprouting (Clarke et al. 2013); they can also recurrently burn shrublands (Nunes et al. 2005) and delay the progression of ecological succession.

To date, most studies on post-fire regeneration in Mediterranean ecosystems have focused on plants (Guo 2001; Mitchell et al. 2009; Schaffhauser et al. 2012; Marzano et al. 2012; Francos et al. 2019). However, whilst the recovery of plant communities to fire is generally understood, the recovery of important ecological processes, such as pollination, has received little attention. The pollination of flowering plants by animals is a crucial ecosystem service of great value to humanity because without it most flowering plants would not reproduce sexually and humans would lose food and other plant origin products (Buchmann & Nabhan 1996; Klein et al. 2007; Ollerton et a. 2010; Breeze et al. 2011; Potts et al. 2016). The number of flower-visiting species worldwide may total nearly 300,000 (Nabhan & Buchmann 1997). The importance of pollination to wild plants and as an ecosystem service, as well as a range of other ecological processes provided by pollinating insects, highlights that pollination systems should be a high priority for conservation (Kearns et al. 1998; Vanbergen 2013). Few studies have examined the response of interacting communities of flowering plants and insects to fire (Potts et al. 2003; Swengel & Swengel 2007; Domínguez et al. 2011; Driessen & Kirkpatrick 2016). Depending on several variables such as the mobility of insects and degree of exposure to the flames (Swengel & Swengel 2007), or environmental variables, like vegetation density (Driessen & Kirkpatrick 2016), the conclusions are that fire has an impact on the communities of insects. It may either have a significant effect on the plant-pollinator community, with insect abundance declining markedly after a fire (Potts et al. 2003), or a limited impact in the case of low–moderate-intensity fires (Driessen & Kirkpatrick 2016). Ecosystem changes affect the distribution, abundance, and effectiveness of pollinators. We can only consider restoration successful when the pollinators of plants have recovered and have themselves sufficient plant food sources. Thus, successful ecological restoration means that pollination services must be reinstated. Therefore, pollination can be a very useful functional bio-indicator for comparing restored communities to reference and to disturbed communities and it can provide information for a better assessment of future natural and human ecosystem disturbance (Forup et al. 2008).

Moths play a crucial role in communities because they are important primary consumers as plant herbivores (particularly during larval stages) and food providers for higher organisms in the food web (Fox 2013; Macgregor et al. 2015). They are part of Lepidoptera, the 4th most important Order of insects with approximately 150 000 species described; of those, 18 000 are butterflies and the rest are moths. This interaction between Lepidoptera and plants led to their inclusion on the main groups of pollinators in natural ecosystems (Weiss 2001; Wilmer 2011). Recent research suggested that moths are potentially pollinators of global importance (Devoto et al. 2011; Banza et al. 2015; Macgregor et al. 2015; Banza et al. 2019; Macgregor et al. 2019a; Walton et al. 2020) based on evidence of moths transporting pollen (using similar technics that allowed to remove pollen from the body of moths); although the proportion of pollen being carried by moths varies according to the type of habitat/ecosystem, moths play a significant role as pollen vectors and may be especially important in the Mediterranean (Banza et al. 2015).

There is a global concern about the decline of insects (Blanchet et al. 2020) and several studies show a decline of insect pollinators (Cane & Tepedino 2001; Biesmeijer et al. 2006; Potts et al. 2010; Carvalheiro et al. 2013; Dirzo et al. 2014; Hallmann et al. 2017). Although there are studies on the drivers affecting butterfly declines (Thomas et al. 2004; Van Strien et al. 2009; Nilsson et al. 2013; Melero et al. 2016) there is little information on what affects moth populations (Conrad et al. 2006; Fox 2013; Fox et al. 2014; Banza et al. 2019). Studies in some European countries showed that moths are in decline in Europe (Conrad et al. 2006; Groenendijk & Ellis 2011; Macgregor et al. 2019a) and other parts of the world (Sutrisno 2010; Young et al. 2017). A range of environmental causes for this decline include land use change and climate change (Stefanescu et al. 2011; Fox et al. 2014;), artificial light at night (Macgregor et al. 2017a; Van Langevelde et al. 2017) and wildfires (Banza et al. 2019); of the few studies of the effects of wildfire upon Lepidoptera, most find negative impacts (Kral et al. 2017). Fire can lead to mortality of larvae through host plant destruction (Fowles et al. 2004), subterranean pupae (Schmid et al. 1981), and even adults (Gerson & Kelsey, 1997).

Fires can shape plant–pollinator communities (Brown et al. 2017; Ponisio et al. 2016), leading to reduced abundance of pollinators and flowers (Potts et al. 2001) and reductions in plant reproductive success (Ne'eman et al. 2000), or increased floral resources through a flush of secondary succession (Capitanio & Carcaillet 2008; Potts et al. 2003). By altering community composition, fire may have secondary effects on moths and their pollen-transport interactions at community level. However, no study has investigated the direct effects of fire on plant–pollinator network properties (see review of Brown et al. 2017). Ecological network metrics are increasingly used as tools for biodiversity monitoring and assessment of environmental change (Derocles et al. 2018), because they can describe important changes in the structure and function of whole ecosystems that might not be detected by measuring species abundance and diversity.

Studying the effects of fire using a network approach will bring a better knowledge on how entire communities respond to that disturbance. And moths can play a crucial role on how to assess the restoration of plant-pollinator interaction networks after fire disturbance, specially in a Mediterranean context.

Objectives and structure of the Thesis

This thesis aims to assess the recovery of plant-pollinator interaction networks after a wildfire perturbation. The main objectives are: 1) to review the scientific literature about the role of moths in natural systems; 2) to compare the structure, complexity and robustness (Evans et al. 2013) of complete plant-pollinator and pollen transfer networks between fire-damaged and reference forest habitats, using an Ecological Network Analysis (ENA); 3) to determine the importance of both diurnal and nocturnal insects as plant pollinators within the networks, whilst examining spatial and temporal variation within and between quantitative networks.

To answer objective 1) we conducted an exhaustive bibliographic review about the importance of moths as pollen vectors and the main threats they face in the Mediterranean context (Chapter 2).

To answer objectives 2) and 3), we conceived, implemented and carried out a project in the Serra do Caldeirão region near Faro, Portugal, from April 2013 to May 2015. The study followed a large fire that occurred in July 2012 and affected approximately 225 km^2 of the area. This is a mountainous shrubland ecosystem (maximum altitude 575 m) containing semi-natural cork oak woodland of high conservation value. We established three 40 × 40 m study sites each in the burned area and three others in a nearby unburned area; all sites had intermediate densities of oak trees and shrubs at a similar successional stage. All sites contained a similar range of aspects and altitude and within the same treatment were separated by >500m. Sites were sampled approximately every 2 months by moth sampling and floral transects. Each site was sampled on 13–15 occasions in total.

Moths were sampled using Heath-style light traps (Heath, 1965) baited with 6 W actinic tubes (Philips TL6W/05, Philips) powered by 12 V batteries. Traps were situated at the centre of the site and operated between sunset and sunrise. Captured moths were retained in individual tubes for subsequent pollen analysis. Floral surveys were done by establishing two parallel 10-m transects, 10 m apart, at the centre of each plot. A 1 × 1 m quadrat was placed every two metres along each transect line (n = 10). For each quadrat, percentage cover of all plant species currently in flower was recorded. A pollen reference collection was prepared to assist with pollen analysis. For diurnal insects we conducted one transect of 15 m at each plot and all insects observed visiting plants in flower were captured using a hand net or directly into killing tubes with a drop of ethyl acetate for later identification and pollen analysis. Pollen sampling from all species of flowers and all individuals of moths and diurnal insects, was performed by using a small cube of Fuchsin-glycerin jelly to swab pollen from flowers and insects and fixing it on microscope slides, as described by Beattie (1971). We constructed quantitative and qualitative ecological networks and analysed them for unburned and burned areas. We used generalized linear models (GLMs) and generalized linear mixed-effects models (GLMMs) to test the effects of burning, season, sampling period and their two-way interactions on abundance and estimated species richness of moths, flowers and diurnal insects.

In Chapter 3 we examine the impacts of wildfire on plants, diurnal insects and pollen transport following the wildfire mentioned above. We attempted to answer the following questions: 1) Is there an effect of burning on the abundance, community and species richness of diurnal insects and how does this vary across seasons? 2) Does burning affect the amount and diversity of pollen being transported by insects and how does this vary across seasons? 3) Which specific insect species have an important effect on the system and how? 4) Is there any evidence of a negative impact of pollination service in the region, for example an adverse effect on honeybees? In burned areas we expected potentially more flower-visiting insects due to the flush of flowers after the fire. We also expected seasonal effects on insect abundance, with higher numbers in the spring and autumn compared with summer and winter, consistent with Mediterranean trends; but no differences in community composition and species richness as a result of burning, as these are more likely to be driven by seasonal variation. We also expected higher pollen loads being carried by diurnal insects in burned areas due to the flush of flowers after the fire and more pollen transported in the spring and autumn.

In Chapter 4 we examined the response of nocturnal moth–plant interaction networks to the same fire mentioned above. By assessing the abundance and diversity of moths, flowers and their networks of pollen-transport we tested four hypotheses about the effects of wildfire on nocturnal pollen transport systems: (a) that burned sites would have more flowers than unburned sites, because some species would respond to fire by flowering; (b) that burned sites would have fewer moths than unburned sites, because of damage to larval host plants; (c) that pollen-transport networks at burned sites would be less interaction-rich (because under hypothesis (b), the abundance and species richness of moths would be lower) and have lower complexity than at unburned sites; and (d) that pollen-transport networks at burned sites would have lower robustness than at unburned sites, because generalist species play important roles in maintaining network stability, but the loss of larval host plants might drive random local extinctions of generalist flower-visiting moths.

Chapter 5 is dedicated to the general discussion and conclusions and in the last part of the thesis there is extra information in the Annexes.

CHAPTER 2

A review of the threats to moths and nocturnal pollination in the Mediterranean region

In preparation for submission as:

Banza, P., Evans, D. M. & Belo, A. D.F. "A review of the threats to moths and nocturnal pollination in the Mediterranean region".

Abstract

Moths are an insect group of major importance for global biodiversity, but relatively poorly studied. In addition to playing a crucial role in food webs, recent research has suggested they are important, but overlooked, pollinators. There is a clear evidence of recent insect declines across the world, particularly of pollinators, leading to a significant negative impact on ecosystem processes and services, ultimately impacting human wellbeing. In parts of Europe, there is evidence of significant moth population declines, but little is known in the Mediterranean context, where data is scant. Here we review the major threats causing moth population decline in Europe's Biodiversity Hotspot, with a focus on land-use change (habitat loss and fragmentation, agriculture intensification), climate change, artificial light at night and wildfires. We then state the consequences for natural systems and relate those threats to the way they affect moth ecology, such as their movements, life cycle and plant pollination. Finally, we address some mitigation and conservation measures and identify the main questions for moth conservation in the Mediterranean context.

Keywords: Moths; pollination; land-use change; ALAN; wildfires; climate change

Introduction

Moths play a crucial role in communities because they are important primary consumers as plant herbivores (particularly during larval stages) and food providers for higher organisms in the food web (Fox 2012; Macgregor et al. 2015). They belong to Lepidoptera, the 4th most important Order of insects with approximately 150,000 species described; 18,000 of those are butterflies and the remaining are moths. The number of Lepidoptera in the Iberian Peninsula is around 5,000 species: 226 species of butterflies and the rest are moths (García-Barros et al. 2013).

The diversification of Lepidoptera started in Cretaceous, along with Angiosperms evolution (Grimaldi & Engel 2005). Lepidoptera and Angiosperms developed a close co-evolutionary relationship due to larval dependence on plants (Wahlberg et al. 2013; Edger et al. 2015) and the use of nectar from flowers as the main source of food for most of them (Erhardt & Mevi-Schütz 2010). Most Lepidoptera larvae feed on plant material using biting-chewing mouthparts but most adults use their proboscis to drink nectar from the flowers (Krenn 2010) and other liquid substances. This interaction between Lepidoptera and plants led to their inclusion into the main groups of pollinators in natural ecosystems (Weiss 2001; Wilmer 2011). Recent research, however, has suggested that, although overlooked, moths are important pollinators (Devoto et al. 2011; Banza et al. 2015; Macgregor et al. 2015; Banza et al. 2019; Walton et al. 2020). This research is based on evidence of moths transporting pollen and, although the proportion of pollen being carried by moths varies according to the type of habitat/ecosystem, moths play a significant role as pollen vectors. Furthermore, moths are influential components within ecological networks and may provide additional resilience by counter-balancing diurnal pollinator decline (Walton et al. 2020).

There is a global concern about the decline of insects (Blanchet et al. 2020) and several studies show a decline of insect pollinators (Cane & Tepedino 2001; Biesmeijer et al. 2006; Potts et al. 2010; Carvalheiro et al. 2013; Dirzo et al. 2014; Hallmann et al. 2017). Although there are studies on the drivers affecting butterfly declines (Thomas et al. 2004; Van Strien et al. 2009; Nilsson et al. 2013; Melero et al. 2016) there is little information on what affects moth populations (Conrad et al. 2006; Fox 2013; Fox et al.

2014; Banza et al. 2019). Studies in some European countries indicate that moths are in decline in Europe (Conrad et al. 2006; Groenendijk & Ellis 2011; Fox 2013; Macgregor et al. 2019a) and other parts of the world (Sutrisno 2010; Young et al. 2017). In Great Britain the abundance of individual macro moths decreased by 31% over 35 years (Conrad et al. 2006) and in the Netherlands 71% of the macro moths decreased in abundance (Groenendijk & Ellis 2011).

A range of environmental causes for this decline include habitat loss and fragmentation, agriculture intensification, climate change (Fox 2013; Fox et al. 2014; Stefanescu et al. 2011), artificial light at night (Macgregor et al. 2017a; Van Langevelde et al. 2017) and wildfires (Banza et al. 2019). All of them, acting together or isolated, will globally influence natural systems and agrosystems causing biodiversity loss with serious social and economic implications (figure 1). Most of these studies tend to focus on North European countries or other parts of the world and little is known about the Mediterranean Basin. It is essential for the conservation of moths and organisms that depend on them, to understand the major threats they face, particularly in biodiversity hotspots, such as the Mediterranean Basin.

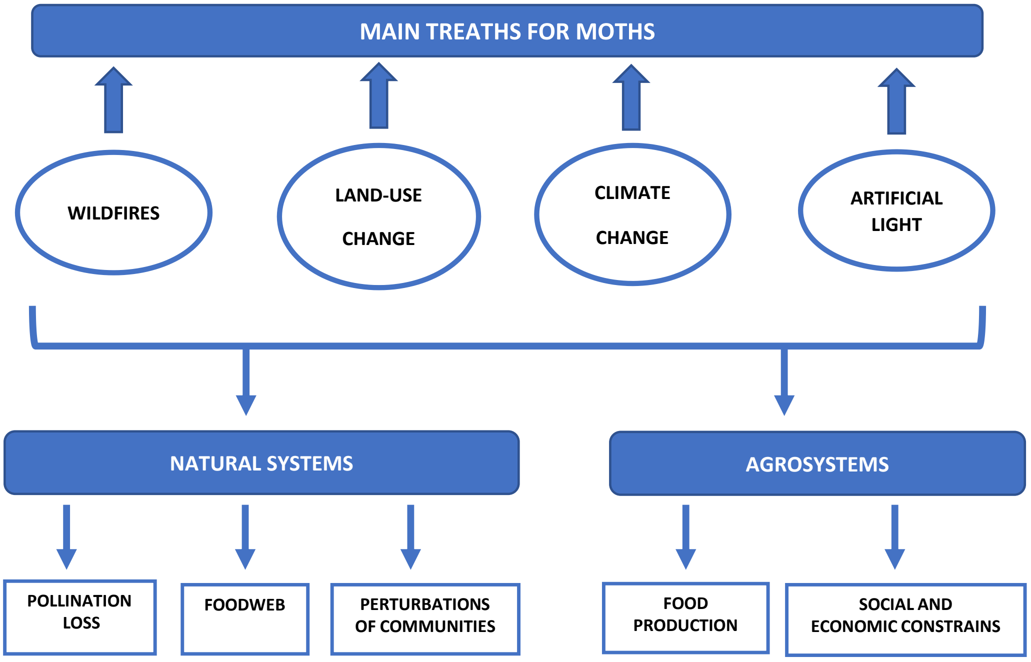


Figure 1. Main threats for moths and their effects on Natural ecosystem and Agrosystems.

In this review, we aim to assess the status of moth populations in the Mediterranean region and identify the major drivers that might disrupt them and their role as pollinators and in in the food web. We also identify some mitigation and conservation measures that could help prevent moth declines and promote a better understanding on how to protect this important group. Search was conducted using Google Scholar. The key words used in this search were: moths AND conservation AND Mediterranean; Lepidoptera AND conservation AND Mediterranean; pollinators AND conservation AND Mediterranean; moths AND conservation AND Mediterranean AND ("climate change" OR "land use change" OR "artificial light at night" OR wildfires). Many records were aimed at forest or agricultural pest problems, which was not our objective and therefore they were discarded. Occasionally, relevant publications retrieved from the reference list cited in the considered papers was also used.

Importance of moths as pollinators

Wild flowers and crops depend largely on wild pollinators for their reproduction (Klein et al. 2007; Ollerton et al. 2010). In Europe, around 84% of crop species and 78% of wild flowering plants depend, at least in part, on animal pollinators (EU – Pollinators Initiative 2018). They are essential for ecosystems diversity and stability and for almost 10% of world food production (Gallai et al. 2009). The taxonomic diversity of organisms acting as pollinators it´s very important to their ecologic function and it has increased, in parallel with flowering plants, since mid-Mesozoic until present time (Ollerton 2017).

Overall, wild insects pollinate crops effectively and enhance fruit set in crop systems worldwide (Herrera 1988; Garibaldi et al. 2013). Although, non-bees are least effective pollinators than bees on a *per* flower visit basis, they made more visits in the overall and contribute to seed production and, in general, they are less affected by land-use change, including agriculture intensification (Garibaldi et al. 2013; Rader et al. 2016).

Despite the close relationship between Lepidoptera and flowers, their role as pollinators has been the subject of intense debate. Some researchers consider Lepidoptera as "nectar thieves" instead of true pollinators, establishing a parasitic and not a mutualistic relationship (Wiklund et al. 1979, 1982). This view has been favoured by a general pattern in the use of floral resources (Stefanescu & Traveset 2009). This generalist behaviour is associated with the heteroespecific transfer of pollen and, therefore, with a low pollination efficiency (Morales & Traveset 2008). However, it is necessary to emphasize that even in very generalist pollinator species, some individuals may show a strong record of floral visits and, ultimately, act as pollinators. On the contrary, some studies clearly demonstrate otherwise and show the fundamental role of Lepidoptera (both diurnal and nocturnal) as pollinators of some plants, not only in the tropical or subtropical regions (Cruden & Hermann-Parker 1979; Johnson & Bond 1992; Oliveira et al. 2004; Martins 2014) but also in the temperate regions (Bloch et al. 2006; Devoto et al. 2011; Epps et al. 2015) and Mediterranean regions (Banza et al. 2015; Banza et al. 2020 *submitted*). For instance, Hahn & Bruhl (2016) identified 227 pollination interactions between moths and flowers in various natural ecosystems and some agrosystems in Europe and North America. According to this study moth pollination contribution to non-crop plants pollination can be crucial to maintain biodiversity in these ecosystems, pointing to the fact that there´s a need for more research on moth temporal fluctuations, abundance and community composition because their role as pollinators is underestimated, mainly because available studies are scarce. Macgregor et al. (2019b) detected pollen on individual moths, and even more multiple pollen types, by using DNA metabarcoding rather than microscopy; they also detected more interactions *per* moth species, proving the potential of DNA metabarcoding for studying plant-pollinator interaction networks, particularly for moths (Macgregor et al. 2019b). A recent study (Walton et al. 2020) suggested that nocturnal moths are influential components of wild plant-pollinator networks in agroecosystems and they may provide additional resilience to those pollination networks.

Evidences of declines in pollinators, highlighting butterflies and moths

Most studies on Lepidotera showed an alarming decline in populations of many species, both butterflies and moths (Maes & Van Dyck 2001; Conrad et al. 2006; Van Strien et al. 2009; Nilsson et al. 2013; Melero et al. 2016). The European Grassland Butterfly Indicator shows that since 1990 grassland butterfly abundance has declined by 30% and, in parts of Western Europe, butterfly numbers outside reserves have come to an absolute minimum (Van Swaay et al. 2016). According to The Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services report (IPBES, 2016) all wild insects have declined in occurrence, diversity and abundance for some species, at local and regional scales in North West Europe and North America; despite the absence of a global Red List assessments specifically for insect pollinators, regional and national assessments indicate high levels of threat for some bees and butterflies. IPBES reports a 9% of bee and butterfly species threatened, 37% of bees and 31% of butterfly populations decline in some European Countries. The example from Catalonia, where there is a Butterfly Monitoring Scheme network (BMS) from the beginning of the 90's, show that the decreases affect 70% of the 66 species analysed and, on average, are responsible for a population reduction of 20% *per* decade (Melero et al. 2016). While habitat specialists are those who show greater reductions, an important number of common generalist species is also suffering notable regressions (Melero et al. 2016). These declines are apparently related with anthropogenic global changes such as climate change, land-use change and habitat loss.

Unlike most insects, butterfly trends are reasonably well known in many European countries. This is possible thanks to programmes of citizen science-based monitoring – BMS – on a large scale, in some cases for several decades (Munguira et al. 2014). In contrast, moth populations trends are mainly unknown, with notable exceptions (e.g. Fox et al. 2014); the few known studies are mainly from North European countries and all of them showed evidences of national-scale declines in this species-rich insect taxon (Groenendijk & Van der Meulen 2004; Conrad et al. 2006; Mattila et al. 2006, 2008; Franzén & Johannesson 2007; Fox 2013; Stojanović et al. 2013). The lack of data on moth population trends is even bigger in the Mediterranean countries, with very few studies: e.g. Merckx (2015) presented a study of macro-moth diversity in a landscape undergoing abandonment in the Peneda mountain range (Portugal) and concluded that agricultural intensification has a negative impact on national declining and priority moth species; Scalercio (2009) studied moths populations in the Pollino Massif (South of Italy) in the context of climate change and found a decline on mountain moths species as a result of a decrease in the area of subalpine prairie. Bosch (2009) did a study in a Mediterranean scrubland community in NE Spain where he analysed pollen loads carried by pollinators, moths included, and detected a significant number of interactions between pollinators and plants, suggesting that ecological specialization is often overestimated in plant-pollinator networks.

Declines on butterfly populations and risk of extinction can be related with biological and ecological traits (Koh et al. 2004; Melero et al. 2016). Amongst those traits are habitat specialization, number of generations and larval host plant specificity. Some of the conclusions are that habitat specialists and multivoltine species are more likely to suffer severe fluctuations in population abundance; also, higher resilience to environmental changes was found in generalist species, even if many of these species are also declining (Melero et al. 2016). The declines of moth species can also be related with biological and ecological traits including body size, larval specificity, length of the flight period, and overwintering stage (Mattila et al. 2006). Normally a single trait may not be enough to allow prediction of the risk of extinction and there are interacting effects between them, including host plant distribution in monophagous species (Mattila et al. 2008).

Pollinator decline is a global phenomenon with potentially severe impacts on natural systems and agrosystems (Powney et al. 2019). The major drivers of pollinator decline are land-use change (including habitat loss and fragmentation, mainly related with agricultural practices), climate change, alien species and light pollution (Cane & Tepedino 2001; Dunn 2005; Potts et al. 2010; Carvalheiro et al. 2013; Fox 2013; Hallmann et al. 2017). A recent review by Macgregor & Scott-Brown (2020) giving evidence of pollination services done by nocturnal species also concluded that insect pollen vectors are vulnerable to anthropogenic drivers of environmental change (Macgregor & Scott-Brown 2020).

Although the information on Mediterranean countries is scarce, from the literature review we can state some of the major drivers of moth decline, including land use change, climate change and artificial light at night; we also added wildfires due to the results of the study by Banza et al (2019) and because wildfires are increasing in frequency, they can be counted amongst the major causes of moth declines. All these threats are affecting the role of moth as pollinators, in the food web and causing general disturbances to communities.

Major drivers of moths decline

Although we are focusing in the Mediterranean countries, some of the studies and conclusions presented below are from other parts of the world mainly due to the lack of information on particular aspects.

Land use and climate change

Land-use change and climate change, acting independently or in combination, are important drivers of Lepidoptera biodiversity, with some species increasing and other species decreasing according to different sensitivities to their distribution, host plant and environmental variables although the frequency of occurrence showed an overall decline (Groenendijk & Ellis 2011; Fox et al. 2014; Dennis et al. 2019). They can also have opposing effects on populations; according to Warren et al. (2001), in environments where the influence of agricultural intensification is least, climate warming could increase abundance and insect diversity. Some specific examples on how climate change affect Lepidoptera are given in Box 1.

Effect of land use change on Lepidoptera

- *Species abundance and richness*

Modifications to the landscape have influenced community composition and caused the decline of some butterflies and moths (Wenzel et al. 2006; Nilsson et al. 2013). According to Wenzel et al. (2006), the species mostly affected by the decline are species with special requirements (e.g. structured habitats, or habitat of certain sizes, poor dispersers, monophagous species, K strategists) and Red Data Book species. Also, many typical butterfly grassland species – species associated with open woodlands and wetlands – that were numerous in former times have declined severely (Nilsson et al. 2013). Another example of how habitat loss and fragmentation is associated with moth decline comes from a study by Rickman & Connor (2003); their results indicate that the density of the community of leaf-mining moths on *Quercus agrifolia* is higher on large patches as was found by Connor et al. (2000) for many other insect species.

- *Pollination service*

In general, regardless of the land-use type, changes whose intensity increases the availability of resources and the heterogeneity of microhabitats tend to have positive effects on the abundance and diversity of pollinators, whereas changes that reduce resource availability usually have negative effects. The response of pollinators to external factors depends on their specific traits (specialization, mobility, sociability, nesting site, phenology) and thus some species or groups may be favoured while others disadvantaged by different land-uses (Lázaro & Tur 2018).

- *Community compositon (generalists versus specialists)*

Studies from different countries (Warren et al. 2001; Stefanescu et al. 2009**;** Betzholtz & Franzén 2011; Slade et al. 2013) showed that climate and land-use changes are affecting specialist and generalist species, altering the composition of communities. According to Warren et al (2001), habitat modification and climate change are likely to cause habitat specialists to decline and be replaced by mobile and widespread generalists; a decline of three-quarters of the butterflies in Britain might be explained by the fact that negative responses to habitat loss have outweighed positive responses to climate warming and half of the species that were mobile and habitat generalists increased their distribution sites (consistent with a climate change explanation), whereas the other generalists and 89% of the habitat specialists declined in distribution size (consistent with habitat limitation) (Warren et al. 2001).

Again, little is known in Mediterranean countries, but a study on butterfly monitoring from a series of abandoned grasslands in northeast Spain showed that grassland abandonment had immediate strong effects on butterflies by substitution of grassland specialists for common butterflies, less important for conservation purposes (Stefanescu et al. 2009). A follow up study on butterfly monitoring in this same site (Colom et al. 2020a) concluded that land abandonment caused a replacement of multivoltine by monovoltine species; restoration combining mowing and grazing promoted a quick return to the pre-abandonment situation in the butterfly community, and increased generality and nestedness, which contributed to community stability. These results can probably be applied for moths as land abandonment might influence moth abundance and richness, which in turn might influence the whole community.

Effect of climate change on Lepidoptera

- *Population Dynamics*

Some studies done in Mediterranean countries related butterfly population dynamics with climate change, specifically precipitation (Carnicer at al. 2019; Herrando et al. 2019) and temperature (Zografou et al. 2014). Regarding precipitation, the most important conclusions from those studies were that butterfly populations are vulnerable to long-term drought periods, mainly because of their effect on host plants and subsequent impact on larval growing, which in turn may explain butterfly population declines (Carnicer at al. 2019). Herrando et al. (2019) also showed that birds and butterflies population trends were related with precipitation preferences, for instance, butterflies from arid environments, where there´s less availability of water, and bird species from humid habitats were decreasing the most. Water availability is also an important driver of change in the Mediterranean region, so butterfly species from arid environments are decreasing the most (Herrando et al. 2019). As for temperature, Zografou et al. (2014) found a marked change in butterfly community composition over a 13 year period (1998–2011/2012), concomitant with an increase of annual average temperature of 0.95°C; they observed an increase in the abundance of low-elevation species and a decline of species mainly occurring at higher elevations suggesting that this was a consequence of long-term climate warming.

- *Life cycle (Phenological changes and voltinism)*

Because of their diversity and abundance, Lepidoptera can be a good model to study the phenological adaptations. Reasonably well studied and understood, they are important in terrestrial ecosystems as primary consumers. Results of several studies (Roy & Sparks 2000; Stefanescu, et al. 2003; Valtonen et al. 2011; Karlsson 2014) showed that advanced phenology is related with climate change. Climate warming affects timing of flight (both first and peak abundance), duration of flight, timing and duration of larval feeding and pupation (Roy & Sparks 2000; Valtonen et al. 2011).

There are increasingly more examples of phenological disruptions of populations, communities, and ecosystems (Visser & Holleman 2001; Willis et al. 2008; Post et al.

2009). Normally species and populations can adapt their phenology to interannual climatic variation, but some species will tend to have a better ability for adaptation than others e. g., Salama et al. (2007) showed a highly significant increase in the diversity of the macro-moth community related with the rise in mean annual temperature.

Voltinism on moths and butterflies can be affected by land use and climate change, as studied by various researchers (Visser & Holleman 2001; Davies et al. 2006; Altermatt 2010; Martín-Vertedor et al. 2010; Casner et al. 2014). According to Casner et al. (2014), changes in land-use patterns and a changing climate affected butterfly species richness. A decline in the area of farmland and ranch land, an increase in minimum temperatures during the summer and maximum temperatures in the fall negatively affected net species richness, whereas increased minimum temperatures in the spring and greater precipitation in the previous summer positively affected species richness (Casner et al. 2014). A study from the Balearic Islands, Spain, showed that rising temperatures are having a great impact (positive and negative) on butterflies' population dynamics and that species responded differently according to season and habitat (Colom et al. 2020a). Altermatt et al (2010), listed a number of consequences of climate change on the voltinism of butterflies and moths of Central Europe. These included prolonged flight season, additional number of generations, changes on larva growth and development, replacement of uni or bi-voltine populations from Central Europe by immigrants from Southern European populations, which may be bi-or polyvoltine. Some of the main consequences of multi-voltinism can be: a) insect outbreaks and increased abundance of herbivorous pests to agriculture and forestry; b) disruption of the synchrony between insects and host plant phenology, which might reduce fitness in species of conservation concern; c) ability of species to adapt evolutionarily to environmental; d) negative influence on plant communities, especially since many long-lived plants (e.g. bushes, trees) may be unable to speed up their life cycle (Altermatt 2010). Amongst the consequences of advanced phenology is the pest control, as studied by Williams & Liebhold (1995). They investigated potential changes in spatial distribution of outbreaks of moth defoliators under different climate scenarios. Their conclusion was that climatic change may alter defoliation through both direct effects on an insect population and indirect effects on its habitat; it potentially may alter the geographical distribution of defoliation through changing the range of a defoliator species and the ranges of its host tree species (Williams & Liebhold 1995).

- *Distribution (latitude and altitude)*

With climate change, the geographic ranges of many butterfly species have shifted pole wards and uphill in elevation, leading to increases in species richness at high latitudes and elevations (Warren et al. 2001; Wilson et al. 2007; Hardy et al. 2014). Other studies suggested that the global warming and habitat loss might lead to significant losses of diversity, in particular of mountain species and in the regions where the species are found on the southern edge of its distribution area (Parmesan et al. 1999; Hill et al. 2002; Konvicka et al. 2003; Wilson et al. 2005, 2007; Obeso & Herrera, 2018). According to Hardy et al. (2014) the expectation is that butterfly species whose abundances are adversely affected by warming will have contracting geography and those whose populations benefit from warming will have expanding geography; the strong inference is that whereas many species are gaining northwards and at higher altitude, they are losing out at lower altitude and at the core of their distributions (Hardy et al. 2014). Another study on European butterfly populations done by Mills et al. (2017) concluded that range-edge populations appear more sensitive to changes in weather than those nearer the centre of species' distributions.

In the Iberian Peninsula the butterfly diversity is less at low latitudes (Hawkins & Porter, 2003) and elevations (Stefanescu et al. 2004) and therefore it´s more difficult for colonizing species to replace those lost to higher latitudes or elevations because of climate change (Ibáñez et al. 2006). The results from a study done in the Sierra de Guadarrama (central Spain) showed changes in species richness and composition in butterflies at a regional level suggesting that climate warming, combined with habitat loss and other drivers of biological change, could lead to significant losses in ecological diversity in mountains and other regions where species encounter their lower latitudinal-range margins due to the increasing dominance of dispersive or generalist species (Wilson et al. 2007). Merril et al. (2008), that studied the black-veined white butterfly, *Aporia crataegi*, in Central Spain found that climatic limitation is the most likely explanation for the low elevation range margin of *A. crataegi*, whereas the absence of host plants from high elevations sets the upper limit. This contrasts with the frequent assumption that biotic interactions typically determine warm range margins, and thermal cool limitation margins. A study on moths done by Scalercio (2009) suggests an increasing competition between high altitude and low altitude species as temperatures increase in Mediterranean countries. As a consequence, the upward shift in the range of low altitude species will strongly increase the risk of extinction of species specialized to live at high altitudes. And because the predicted extinction rate is higher than the colonization rate, biodiversity of Mediterranean areas of Europe will be impoverished by climate change (Scalercio 2009).

- *Mobility*

Mobility is often associated with species traits and characteristics of the landscape. Under the land-use change and climate change scenario it is important to understand how species survive in fragmented landscapes and with increasing rates of habitat and climate changes. Some studies focused on how Lepidoptera mobility is affected by climate and land-use changes (Sparks et al. 2005; Betzholtz & Franzén 2011; Slade et al. 2013). Sparks et al. (2005) studied migrating moths and butterflies in relation to temperatures on the migration route, to temperatures in England, and to the North Atlantic oscillation (NAO). Their study reveals that higher temperatures are associated with increased migrant numbers, suggesting that Britain and other northern temperate countries may expect to receive larger numbers of migrant Lepidoptera, if climate warms as predicted. Slade et al. (2013) showed that traits like wingspan, wing shape, adult feeding, and larval feeding guild predicted macro-moth mobility but those traits depended on the species affinity to the forest and the degree of fragmentation and connectivity; their conclusions were that, in general, species with weak forest affinity moved larger distances than species with strong and medium forest affinity. Their results also showed that species with a strong forest affinity and with large, pointed wings have a greater ability to disperse than similarly shaped species with medium to weak forest affinity. Betzholtz & Franzén (2011) also highlighted the relation between mobility and species traits, e.g. noctuid moths with certain traits, like adult feeding and wingspan move over longer distances than earlier known and widely distributed host-plant generalists were more mobile than host-plant specialists with more restricted distribution.

- *Pollination service*

Obeso & Herrera (2018) reviewed how global climate changes affect pollinators. They concluded that the most important mechanisms are the displacements in the areas of species distribution and changes in the phenology of plants and activity of the pollinators; they also concluded that climate change may act synergistically with other factors such as pesticides, parasites and other pest diseases, invasive species, and land-use changes, which might have a multiplicative effect, including changing pollinators size and colour (Obeso & Herrera, 2018). A recent review by Gérard et al. (2020) summarised the mismatches between plants and their pollinators caused by global warming; they provide evidences of phenological and spatial shifts due to morphological modifications and disruptions to host attraction and foraging behaviours, creating new community assemblages. Another recent study by Macgregor & Scott-Brown (2020) on the effects of global warming on plant-pollinator interactions concluded that under elevated temperatures insects using infrared radiation to seek out thermogenic flowers may have more difficulties, which might negatively impact plant-pollinator interactions at community level.

Box 1. Effects of climate change on Lepidoptera

Increase the availability of thermally suitable habitat for *Hesperia comma* and the **influence** of temperature variation **in the phenology and body size** (Davies et al. 2006). Climate warming has increased the availability of thermally suitable habitat for *Hesperia comma* at the cool, northern edge of the species' distribution, therefore increasing: (a) egg-laying rate and potentially the realized rate of population increase; (b) effective area of habitat patches as more microhabitats within a given vegetation fragment are now suitable for egg-laying; (c) buffering of populations against environmental variation, as eggs are laid within a wider range of microhabitats and (d) the number of habitat patches in the landscape that are currently available for colonization. Another study (Fenberg et al. 2016) on *H. comma* used temperature variation with particular life stages to predict changes in body size and phenology. Different temperatures in different months of the year predict adult body size – a positive relationship for males – and the timing of adult emergence – a negative relationship.

Changes on the timing of reproduction and growth of the winter moth *Operophtera brumata*. Visser & Holleman (2001) reported how climate change affects the timing of reproduction and growth of the winter moth *Operophtera brumata* by altering the synchrony between the oak bud burst and the egg hatching of the winter moth**.** In the recent warm springs, winter moth eggs were predicted to hatch up to three weeks before oak bud burst. Because newly hatched caterpillars may only survive 3 or 4 days (maximum 10) without food, this will lead to either mortality or dispersal of these small caterpillars. Climate change may disrupt those relationships and, as selection on response mechanisms may be slow, this results in maladaptive behaviour have profound effects on the population dynamics.

Polyvoltinism on *Lobesia botrana*. Martín-Vertedor et al. (2010) reported that the moth *Lobesia botrana* Den. & Schiff. (Lepidoptera: Tortricidae), a key vine pest that is usually trivoltine in Mediterranean latitudes, tends to advance spring emergence, displaying a partial fourth additional flight, a fact that is potentially attributable to global warming. Therefore, *L. botrana* phenology has significantly advanced by more than 12 days. Moreover, the phenological advance contributed to increased moth voltinism by promoting a complete fourth additional flight in 2006.

Artificial light at night (ALAN)

Artificial light at night (ALAN) is a well-studied phenomenon because it affects all kinds of living organisms in various ecosystems all over the world. Insects are one of the most important group with nocturnal habits. They adapted their eyes to use vision for all aspects of daily lives, including avoiding obstacles during locomotion, identifying mates, food and predators, and for orientation (Warrant 2017). For that they have different compound eyes more sensitive to light and specialized neural adaptations within the retina and optic lobe (Warrant 2017). As for moths, attraction to light is correlated with morphological characteristics, like eye size and body mass (Van Langevelde et al. 2011).

Moths with on average larger body mass, larger wing dimensions and larger eyes were attracted to lamps that emit light with smaller wavelengths (higher species richness and abundances of moths was found in traps with lamps that emit light with smaller wavelengths according Van Langevelde et al. (2011). Because most species of moth exhibit flight-to-light behaviour, they can be trapped in buildings, diverted into vehicular traffic, and burned or desiccated by lamp housings (Frank 2006) and that can weaken or eliminate small populations threatened by other disturbances, particularly habitats fragmented by urban development (Frank 2006).

Pollination is an ecosystem service that may be disrupted by increasing ecological light pollution (Macgregor et al. 2015; Macgregor et al. 2017; Knop et al. 2017). Macgregor et al. (2015) found that a high proportion of moths within an agroecosystem transported pollen of a substantial range of plant species. ALAN disrupts nocturnal pollination networks and has negative consequences for plant reproductive success. The presence of bright sources of ALAN may impact the vision of nocturnal pollinators by reducing ocular sensitivity and inhibiting night-time vision (Macgregor et al. 2015); it may also impact how flowers stand out for the insects (Macgregor et al. 2015; Macgregor et al. 2019a). Bright light may impact floral scent cues for nocturnal pollinators because of disruption of circadian rhythms by ALAN in timing the release of floral volatiles in some nocturnally pollinated plants (Prieto-Benítez et al. 2016; Bennie et al. 2016). This in turn might increase or decrease the visitation rates and unbalance plant pollinator interactions at the community level

(Macgregor et al, 2019a). Knop et al. (2017) showed that in artificially illuminated plant-pollinator communities, nocturnal visits to plants were reduced by 62% compared to dark areas which in turn resulted in an overall 13% reduction in fruit set of a focal plant, even though the plant also received numerous visits by diurnal pollinators. They also showed that the structure of combined networks of diurnal and nocturnal pollinators tends to facilitate the spread of the negative consequences of disrupted nocturnal pollination to daytime pollinator communities. Macgregor et al. (2019a) compared pollination success and quality of *Silene latifolia* at experimentally lit and unlit plots, testing lamp type and lighting regime; their results confirm that street lighting could affect plant reproduction through indirect effects mediated by nocturnal insects and further highlight the possibility for novel lighting technologies to mitigate the effects of ALAN on ecosystems. The review of Macgregor & Scott-Brown (2020) focus on nocturnal species as providers of pollination services and the mechanisms involved in night-time communication between plants and insect pollen vectors, highlighting the fact that they are vulnerable to anthropogenic drivers of environmental change, including ALAN.

ALAN interferes with population dynamics by affecting insect movement (Degen et al. 2016), foraging behaviour (van Langevelde 2017), reproduction and life cycle (Gaston et al. 2017; Van Geffen et al. 2014, 2015) and physiological pathways (Gaston et al. 2015; Desouhant et al. 2019) and it affects also community composition and interactions like pollination systems (Frank, 2006; Owens & Lewis 2018; Grubisic et al. 2018; Desouhant et al. 2019). Plants from different ecosystems benefit from pollination by moths and, in many cases, their importance to individual species and to communities is crucial. The review of MacGregor et al. (2015) identified several studies with numerous examples of moths as pollinators and they also identified ALAN as a driver of environmental change explaining the effects of artificial light on moths as individuals on reproduction, predation and vision; they refer the importance of a network approach because declines may potentially change the composition of moth assemblages and the nature and frequency of species interactions between moths and other taxa.

Fires

Despite of lack of studies on how wildfires affect moth populations, they are amongst the biggest threats to plant communities in the Mediterranean context (Guo 2001; Mitchell et al. 2009; Schaffhauser et al. 2012; Marzano et al. 2012; Francos et al. 2019), and certainly affect pollination, food chain and herbivory in natural systems and crop production in agrosystems (loss of pollination, pests).

A good number of studies on effects of fire upon insects are from North America (Hartley et al. 2007; Vogel et al. 2007; Chaundy-Smart et al. 2012; Moranz et al. 2012); most of these studies are related with prescribed fire for insect pest control (Schmid et al. 1981, McCullough et al. 1998). There are also some studies from other regions (Choi, 2018; Elias-Paiva 2018; New et al. 2000; Ricouart 2013; Teasdale et al. 2013) but not many from the Mediterranean-type ecosystems (Elia et al. 2012; Pryke & Samways 2012).

The majority of studies on insects, particularly Lepidoptera, found negative impacts (Kral et al. 2017) through increased mortality of larvae (NFowles et al. 2004), subterranean pupae (Schmid et al. 1981), and adult moths (Gerson & Kelsey 1997); the flight-to-light behaviour exhibited by many nocturnal moths can lead to high levels of direct mortality in adults, if fires occur at night (Gerson & Kelsey 1997). Mobility, life stage and feeding guild are very important for survivability after a fire (Kral et al. 2017). Adults with sufficient mobility are more likely to survive fire since they can escape direct mortality and recolonize burned areas. Eggs and larvae are considered the most vulnerable to fire because they lack mobility to avoid or escape fire (Anderson et al. 1989; Huebschman & Bragg 2000). Conversely, inactivity may be beneficial if food resources are low immediately following fire, and individuals emerge after plant regrowth (Menke et al. 2015).

Studies on the effect of fires upon butterflies reveal different results according to fire regime characteristics such as frequency, seasonality, intensity or ecological and climatic factors. Some studies show an increase in butterfly diversity (Panzer & Schwartz, 2000; Vogel et al. 2007; Scandurra et al. 2014; Baum & Sharber 2012), a decrease (Swengel 1996) or no difference (Nowicki & Kajzer-Bonk 2014; Ricouart 2013). However, a recent review (Carbone et al. 2019) on pollinators responses to fire suggested that fire regime determines the dynamics of pollinator communities; high fire frequency contributes to the decrease of pollinators and affect specially Lepidopterans. Previous studies showing an increase or no difference in butterfly diversity after fire, also mention a less balanced butterfly community and lower evenness after fire (Scandurra et al. 2014), and a clear reduction of the numbers of endemic/specialized species in favour of generalist ones (Ricouart 2013). Swengel (1996) found that prairie butterfly specialists showed a strong and significant decline after fire, and this effect persists for 3-5 or more years; according to Vogel et al. (2010), recovery time for butterfly population may potentially be longer. Also Kaynas & Gurkan (2007) found that although the species richness and species diversity of butterflies were higher in middle successional stages after the fire, abundance decreased with successional age.

Moranz et al. (2012) tested the effects of prescribed fire and grazing in an economically productive grassland landscape and concluded that butterfly species richness was not affected by the different treatments but was positively associated with pre-treatment proportion of native plant cover and with its density; the results vary according depending on the species being a specialist or a generalist; for example in case of the generalist *Danaus plexippus*, population density was highest in the burn-only treatment. This result was also confirmed by Baum and Sharber (2012) when they evaluated the effects of summer prescribed fire on *Asclepia viridis* and their use by *Danaus plexippus*. The Summer prescribed fire generated a newly emergent population of *A. viridis* and allowed for a new generation of adult monarchs to emerge and migrate south to their overwintering grounds; thus, fire might provide host plant patches and/or corridors for pre-migrant monarchs when host plant availability may be limited in other areas. Another conclusion by Moranz et al. (2012) was that historic land uses have reduced native plant cover and permitted exotic plant invasion; butterfly density was more influenced by this than by management treatments and conservation measures might require native plant restoration and not just restoration of disturbance processes.

In different parts of the world, studies on how wildfires affect moths showed declines in species richness and abundance at the burned sites compared with unburned neighbouring sites (Schaeffer 2013; Choi 2018). Fire produced immediate major shifts in moth distribution in burned areas compared with unburned areas although, according to Choi (2018), the recovery of moth assemblage was quick, within 3 years of fire. However, when compared, moth assemblages, were different after wildfires, mainly due to vegetation composition (Evans et al. 2013). These conclusions were also supported by Chaundy-Smart et al. (2012). They compared the effects wildfires and clear-cut on moths' assemblages and concluded that vegetation seemed to be particularly influential for nocturnal macro lepidoptera communities and plants are a prime determinant of moth diversity. When comparing moth communities between burned and unburned sites across seasons, the conclusion is that moths were much less abundant and less species rich after the wildfire (Schaeffer 2013; Banza et al. 2019).

Fire prevention management is becoming very important to regulate fire of natural or human origin, particularly in Mediterranean countries. Ricouart et al. (2013) tested the effects of fire prevention on butterfly community composition using different clear-cut/grazing treatments; their conclusion was that fire prevention management favoured some generalist species but damaged populations of specialist/endemic species. Conservation of specialist/endemic species is favoured by the use of grazing as a management proxy for fire, mainly for species naturally living in shrub/forest biota. These results are consistent with the study done by Scandurra et al. (2014) where they analysed the effect of fire on butterfly diversity by comparing the flight activity of butterflies before and after a fire incident in the Mediterranean maquis. Their results showed that after the fire occurred a rapid change in the butterfly community structure, increasing both species and individual abundance. These results are consistent with other studies (Verdasca et al. 2012; Elia et al. 2012) suggesting that occasional fires can have positive effects on richness and abundance of butterflies. Nevertheless, they found a lower evenness indicating a less balanced butterfly community after fire, despite the increase in richness and abundance, thus suggesting that fire should not be considered as a potentially suitable management practice in Mediterranean maquis. They gave the example of *Melanargia arge*, a rare endemism of southern Italy, that was not observed after the fire. On the contrary, *Cacyreus marshalli* an invasive species in many parts of Europe and the Mediterranean area, appeared after the fire. In conclusion, it seems that a given management practice can never be applicable to all sites, and conservation management should always be considered at a very local scale (Scandurra et al. 2014).

Mitigation and conservation management

There is a general awareness amongst conservationists and policy makers that despite increasing conservation efforts we have failed to halt the decline of biodiversity. The many international conventions and treaties for conservation efforts (Convention on Biological Diversity – CBD, 1992; Convention on International Trade in Endangered Species of Wild Flora and Fauna – CITES, 1973; Convention on Migratory Species of Wild Animals, 2002; policy tools such as European Habitats Directives and Natura 2000 Network), have been insufficient to stop the decline of biodiversity. There is a new agreement for a long-term (2050) vision and mid-term (2030) headline target for biodiversity in the EU with the aim to "put Europe's biodiversity on a path to recovery by 2030 with benefits for the people, the climate and the planet (EUBS 2020).

Moths tend to be overlooked because of the severe depredations they cause on crops and ornamental plants, or stored products and also because of their nocturnal habit; many people think they should be exterminated as pests and not worthy of conservation. However, there are increasing evidences on the importance of moths in nature, not just in the food chain, but as pollinators/pollen vectors (Devoto et al. 2011; Mcgregor et al. 2015; Banza et al. 2019), their evolutionary interest (Majerus 2002; Cook 2003) and aesthetic value (Kearns et al. 1998). New (2004) consider that moths are increasingly both targets for individual conservation management and tools in conservation assessment, for signalling wider environmental changes in response to anthropogenic activities, particularly those involving changes to vegetation (New 2004). Merckx et al. (2013) gave a global perspective on conservation of butterflies and moths and refer two main approaches: a) the single-species approach and b) landscape-scale conservation of multi-species assemblages. The importance of the first approach was that it succeeded on the conservation of several declining butterflies and raised public awareness and political interest in some European iconic species, which created opportunities for funding; on the negative side, it is impossible to provide targeted conservation programmes for more than a small number of highly valued species. So, they favoured the second approach, suggesting it can be used to conserving/protecting 'pristine' landscapes or restoring human-altered landscapes and they believe it can be done by both active management (i.e. restoration of semi-natural biotopes) and passive abandonment (i.e. rewilding).

Some of the suggested management measures for the recovery of threatened Lepidoptera populations, focus on the reformation of agricultural and forestry systems, mitigation for climate change, implementation of monitoring schemes and long-term funding for conservation (Nilsson et al. 2013; Warren & Bourn, 2011)

Mitigate the effects of land-use and climate change

There is a belief that Agri-Environment Schemes (AES) are amongst the most promising conservation measures to counteract biodiversity loss in agricultural dominated landscapes (McDonald & Smith 1991; Donald et al. 2001; Green et al. 2005; Donald & Evans 2006). They started to be implemented in Europe since early 1900s and provided economic incentives for farmers to change or adapt to new ways of farming. They include restricting farming intensity; maintain low-input farming practices by preventing intensification or farmland abandonment; maintain or create landscape elements such as hedges, ponds or wildflower strips. Some studies stated that promoting this AES may help mitigate effects of agricultural intensification on macro-moths, and because a wide range of other taxa are dependent on macro-moths they may therefore benefit from these measures. (Kleijn et al. 2006; Fuentes-Montemayor et al. 2011; Merckx & Macdonald 2015). Kleijn et al. 2006 evaluated the effects of AES on biodiversity of five European countries and concluded that they had marginal to moderately positive effects on biodiversity in all countries; however uncommon species and species listed in Red Data Books rarely benefited from AES, there´s a need to differentiate between biodiversity of common species that can be enhanced with relatively simple modifications in farming practices and diversity or abundance of endangered species which require more elaborate conservation measures. According to Fuentes-Montemayor et al. (2011) the implementation of simple AES management prescriptions applied to relatively small areas can increase the species richness and abundance of moth populations in agricultural environments.

Additionally, the study done by Merckx et al. (2015), investigated whether implementing AES over larger, landscape-scale areas, affect macro-moth populations by comparing their numbers in targeted areas with conservation goals in mind and non-targeted areas. They explored the effects on macro-moth abundance and species diversity to field margins and hedgerow trees (as part of a wider landscape) and if they differed in those areas; their conclusion was that the presence of hedgerow trees and wide margins each significantly increased macro-moth species numbers locally from *c.* 90 to *c.* 105 species on average, or by around 15% (Merckx et al. 2009a, 2013). These results suggest that the implementation of AES on promoting wide field margins and hedgerow trees may help mitigate negative effects of agricultural intensification not only on macro-moths but also on a wide range of other taxa that are dependent on macro-moths.

Different types of habitat and the way they are managed influence abundance and diversity of Lepidoptera, both butterflies (Balmer & Erhardt 2000; Brereton et al. 2008; Thomas et al. 2009; Kral et al. 2018) and moths (Groenendijk & Van der Meulen 2004; Mattila et al. 2006; Broome et al. 2011; Merckx et al. 2013). According to Kaiser-Bunbury et al. (2017) land degradation results in declining biodiversity and the disruption of ecosystem. Vegetation restoration is a common tool used to mitigate these impacts and increasingly aims to restore ecosystem functions rather than species diversity functioning worldwide. Pollination is an important ecosystem function and the global decline in pollinators aggravates the resistance of natural areas and agro-environment to disturbances. By analysing plant-pollinator networks on restored and unrestored land, Kaiser-Bunbury et al. (2017) concluded that ecosystem restoration increased pollinator species, visits to flowers and interaction diversity; more precisely, they suggested that vegetation restoration can improve pollination and pollination networks may be an important tool in environmental management. Evans et al. (2016) combined metabarcoding with a network approach to provide indicators to monitor and assess management effectiveness and validate conservation goals to support forest managers and conservation practitioners.

Merckx et al. (2013) consider the need to manage unsustainable land use and massively restored degraded natural habitats in order to preserve and restore natural areas. They propose using Lepidoptera to monitor conservation efforts worldwide and stress the importance of adopting a landscape scale allied to a resource-based view, both for single-species and for biotope/community conservation. Fox et al. 2014 suggested habitat protection, management and restoration as a way to mitigate the combined impacts of land use change and climate change; because different species of moths respond in different ways to the diverse patterns of change, they suggest the maintenance of existing high-quality habitats and creation of new areas. These efforts would minimize declines and maximize increases for moth species, regardless of whether they are responding most strongly, or in combination, to land use or climatic changes. For most species of moths there´s not enough ecological knowledge to be able to provide specific conservation measures. However, Veraghtert & Merckx (2014) mentioned management guidelines and general rules of thumb for forests, grasslands and heathlands: a) habitat resource quality and quantity and landscape connectivity in between patches of habitat; b) forest conservation management; c) sufficiently varied provision of nectar resources and spatial and temporal heterogeneity for other essential habitat resources.

The review of Wilson & Maclean (2010) draws some conclusions about the priorities for insect conservation and research in a changing climate. Some of their priorities included: a) management of networks of habitat, or of landscapes for species to be able to expand their distributions; b) conservation interventions in the form of assisted colonization for species which are likely to suffer the most severe reductions in distribution size, and which have the least chance of reaching locations which become climatically suitable; c) research into the habitat requirements and dispersal capacity for threatened species with high climate change sensitivity, or reduced capacity for adaptation; d) research on distribution patterns as a way to predict suitable locations and habitats for species; e) research on how climate conditions influence resource availability for threatened species; f) Monitoring of habitat use and population responses to habitat management.

Other management measures for the recovery of threatened Lepidoptera populations, include the implementation and integration of existing national and local monitoring schemes and the establishment of a global programme to help direct policy decisions (Potts et al. 2010). Dennis et al. (2017) stressed the importance of citizen science because it appears to offer opportunities for largescale, cost-effective biodiversity monitoring and, at the same time, contribute to public engagement as well as serving to reconnect an increasingly urban human population with nature.

Mitigating the effects of ALAN

Artificial light effects on moths include a wide range of factors, and one of the most important is spectral composition of light. Moth eyes are highly sensitive to short wavelength radiation, and less sensitive to longer wavelengths (Agee 1973). To mitigate potential negative effects of artificial light on moths it has been suggested the use of lights with short wavelengths (Frank 1988; Van Langevelde et al*.* 2011). But Van Geffen (2015) demonstrated the stronger effects of short wavelength radiation on moth development, reproduction and interactions with other trophic levels in contrast with the milder effects (in most cases) of red light; compared to green and white light, effects of red light are less pronounced but not fully mitigate negative effects (Van Geffen et al. 2014, 2015). This conclusion is supported by Van Langevelde et al. (2017) who found that effects on moth feeding behaviour are strongest under green light, although feeding frequency is also severely reduced under white and red light; their results suggest that the use of longer wavelengths as a conservation tool are not as effective as previously reported (Longcore & Rich 2004; Van Langevelde et al. 2011; Gaston et al. 2012).

When we compare light bulbs, it is generally thought that bulbs with broader spectrum (e.g. LEDs) can potential cause greater ecological impacts than bulbs with narrow spectrum (e.g. LPS), as the wider range of wavelengths emitted have the potential to affect a greater range of taxa and biological processes (Davies et al., 2013; Longcore et al., 2018). In the particular case of LEDs this means that a more feasible mitigation strategy could be adjusting the spectral composition of LEDs to reduce the intensity of the most biologically disruptive wavelengths and, at the same time, maintaining their benefits to people (Gaston et al. 2012). This is in line with the overview of Grubisic et al. (2018) on the recent research into ecological effects of artificial light at night. They mentioned the large potential to mitigate ecological effects of light pollution by developing technological solutions and lighting schemes and strategies. Some of the examples mentioned include: a) replacement of traditional light sources to white LED lamps; b) adjusting the intensity of light sources and avoid illumination when it is not needed applying shields and adequate lamp designs to direct light and minimize glare, light trespass and skyglow; c) adjusting the spectral composition of LED´s light by reducing the output of harmful blue wavelengths; d) use of dimmers, timers and motion sensors to reduce nocturnal illumination levels and unnecessary illumination in rural and sub-urban areas; e) calculate and predict the impacts of new light sources based on their spectral composition by applying novel indices based on ecological, physiological and astronomical effects. The review of Boyes et al. (2020) refers to some mitigation measures for outdoor lighting mentioned above. They used a life cycle approach on moths to test the effects of ALAN on moth behaviour and physiology, not only in adult moths, where mercury vapour, metal halide, and compact fluorescent bulbs induce flight-to-light behaviour more than LED and sodium lamps, but also disruption of reproduction, larval development, and pupal diapause, with likely negative impacts on individuals but indirectly affecting entire communities.

Apart from light type, lighting regime and distance from the light may significantly affect nocturnal wildlife, and some studies (Azam et al. 2015) suggested part-night (PN) lighting regime could be a solution to mitigate the effects of ALAN on ecosystems. However, a study by Macgregor et al. (2019), that compared pollination success and quality on *Silene latifolia*, using different types of street lights – high-pressure sodium lamps (HPS) and light-emitting diodes (LEDs) – and lighting regime, from full night (FN) to part-night (PN) lighting, appears to suggest that plants may benefit from being situated under FN lighting; these results demonstrate the potential for ALAN to disrupt pollination systems across the community of plants, disproportionately strengthening some interactions and weakening others as previously mentioned by Macgregor et al. (2015).

According to Desouhant et al. (2019), insects are relevant biological models to investigate the impact of ALAN. They are at the base of the trophic chain and should be considered sentinel species to calibrate lighting and to establish dose-responses to wavelengths and intensity. Insects provide an excellent opportunity to understand evolutionary processes that could help stakeholders consider darkness as a resource to preserve biodiversity as well as numerous ecosystem services in which insects are involved (Desouhant et al. 2019).

Mitigating the effects of Fires

In several parts of the world, fires are used as a management tool for conservation of species and habitats (e.g. species of invertebrates). The use of fires may be beneficial because they maintain a mosaic of patchy unburnt habitats and stimulate vegetative growth (York 1996). Hartley et al. (2007) studied the responses of prairie arthropod communities to fire and concluded that the same fire regime that minimized woody plant invasion also maximized arthropod diversity; the negative effects of fire on the arthropod community were minor compared to the strong positive indirect effects of small-scale burning on arthropod diversity. Moretti et al. (2006) also corroborate previous observations that for all functional groups of invertebrates at burned sites, particularly after repeated fires, fire disturbance promotes species richness in forests. At the same time, they concluded that, although these forest communities appear to be quite resilient to fire, prescribed burning cannot be advocated as a preventative measure to avoid intensive crown fires. Even though fire may be a valuable management tool to maintain the quality of habitat for some butterfly species (New et al. 2000; Mjadwesch & Nally 2008; Baum & Sharber 2012) it can be potentially destructive to other butterfly habitats (Sands & New 2002). Therefore, using fire as a management tool may have severe impacts on butterflies (Friend 1996; Sands & New 2002; Thom et. al 2015), namely to their early stages of development by direct effects of fire (Thom et al. 2015) or due to the burning of food plants. Friend (1996) reported that after high intensity fires butterflies showed a decrease in abundance, and for endangered species, it may be a risky strategy due to the threat of mortality. The impacts of fire can also be exacerbated by other disturbances such as drought (Relf & New 2008). Swengel & Swengel (2007) recommended the creation of permanent non-fire refugia for Lepidoptera conservation in fire-managed and fire prone sites, particularly targeting specialist species; their indication was to give more emphasis on unintensively mechanical management (e.g. mowing) than fire management, as this benefit Lepidoptera listed species as well as co-occurring unlisted specialist. Swengel (2001) compared insect responses to fire with other conservation managements of open habitat and stated the importance of careful thinking on the long-term strategy of using recurrent fires for the conservation of open-habitat insects. Fires will cause the regrowing of the vegetation, which will attract recolonizing grassland insects but these tend to be widespread and abundant species, even pests, while localized and rare species, the insects at which conservation efforts are typically aimed, tend to require longer periods to rebuild numbers post-fire, if they do at all. Thus, the control of pest insects by fire will likely be short-term, but the inadvertent control of non-target non-pest species by the same fires may be much longer term. Even before (Swengel 1996), the same author suggested that habitat maintenance with haying, grazing and brush-cutting is much more favourable for prairie-specialist butterflies than burning. So, the land area and various habitat types should be distributed as evenly among management units as possible; large, uniform treatments should be avoided, and instead small, scattered, patchy treatments used (Swengel 1996). These conclusions are supported by the management suggestions for conservation of butterflies made by Thom et al. (2015): within an area, it should be created smaller units that are burned on a multi-year rotation to provide refugia for organisms across all life stages; the units should be designated by the patches of larval host plants that are used by the given species using multiyear monitoring efforts; multi-year rotational burn cycle would also increase habitat heterogeneity. Pryke & Samways (2012) suggested a multi-taxa approach for monitoring arthropod recovery after fire and in management conservation programmes and monitoring should also take into consideration annual variation in unburned areas. Kral et al. (2017) conducted a literature review to synthesize research on arthropod responses to fire in the Great Plains to offer more insights to land managers, policy makers, and researchers. They suggested several management recommendations: a) maximize heterogeneity on the landscape; b) burning may be an appropriate conservation strategy when considering all arthropods collectively; c) more research in order to make species-specific recommendations (current arthropod and plant communities, previous land use, and desired outcomes).

In the Mediterranean region, fires are a common feature, either of natural origin or sometimes caused by human activities. In some cases, fire prevention management is becoming very important to regulate those disturbances caused by fires. Ricouart et al.

(2013) investigated the butterfly community composition using three different types of fire prevention management approaches in the Eastern Pyrenees. Their results indicate that the best conservation management approach should only be of placing grazing animals in the area. A pilot study done by Scandura et al. (2014) analysed the effect of fire on butterfly diversity at a protected site in southern Italy and concluded that fire was beneficial for some community traits such as abundance or richness but negative for evenness, and consequently their suggestion was that management practices and conservation should always be considered at a very local scale. The lack of studies done in the Mediterranean regarding Lepidoptera and fire disturbance is one of the biggest threats for conservation management and more research is needed to overcome this problem.

Box 2. Cases of conservation success

There´s some cases of conservation success amongst species of butterflies related with habitat management: Thomas et al. (2009) related the case of Large Blue, *Maculinea arion*, which became extinct in 1979. *M. arion* is an extreme specialist that switches from feeding on a plant to living as a social parasite inside *Myrmica* ant colonies during a 10-month larval instar and 3-week pupal period. This ant species requires short turf on freely drained soils, making it highly vulnerable to droughts. It is thought that the extreme drought of 1976 knocked the last remaining colony down so far that it could not recover. The recovery programme aimed to restore the butterfly to a wide range of sites, with a variety of different aspects and topographical conditions to maximise its chances of survival. Since the start it has been successfully re-established the species at over 30 sites.
The butterfly *Maculinea alcon* has been used by the Dutch Butterfly Conservation as a flagship species to conserve wet heathlands in The Netherlands (Wallis DeVries, 2004). Butterflies and moths can be of great use in nature conservation because of their potential significance as indicator species in endangered habitats.

Another example comes from the Chalkhill Blue *Polyommatus coridon*, a widespread butterfly of lowland calcareous grassland in southern Britain, considered a good indicator of habitat condition. *Polyommatus coridon* has been identified as a Species of Conservation Concern in the UK Biodiversity Action Plan due to a greater than 25% decline in range size since the 1950s, with losses due to the combined effects of habitat destruction, agricultural intensification and neglect. Analysis of annual butterfly monitoring data (transects) collected at 161 sites from 1981 to 2000 show a population recovery due to, amongst other factors, greater protection and management of sites, agri-environment schemes and warm, but wet summers associated with increases in abundance (Brereton, et al. 2008).

Moth Conservation in a Mediterranean Context

Studies on multiple drivers of Butterflies decline in the Mediterranean countries include information about the effects of land use and climate change on butterflies population dynamics (Stefanescu et al. 2003; Wilson et al. 2007; Stefanescu et al. 2011; Stefanescu et al. 2011; Colom et al. 2020a; Colom et al. 2020b); there is also information on fire prevention (Ricouart et al. 2013; Scandura et al. 2014) and some reviews on pollinators declines have some information on Lepidoptera (Obeso & Herrera, 2018; Lazaro & Tur, 2018). However, studies on moths are very few and mainly related to specific species (Scalercio et al. 2009; Martín-Vertedor et al. 2010), with the exception of a study on the effects of fire on pollination networks with moths as pollen vectors (Banza et al. 2019) and a case study on moth conservation in the context of rewilding landscapes (Merckx 2015).

When we consider moths conservation in the Mediterranean context, systematic monitoring is needed before we can tease out effects of multiple drivers on moth declines. Probably the main research priority is to develop a monitoring scheme for moths as it already exists for Butterflies (European Butterfly Monitoring Scheme EBMS) in most European countries though only recently implemented in Portugal. Beside population monitoring schemes, there are other ways to do moths assessment, like Red Data Books, Species Surveys and Mapping.

In Portugal there is a project to do a Red List of Invertebrates (LVI, 2020), which will include, for all targeted species, their extinction risk assessment, as well as specific data sheets for each species assessed as threatened.

Some of the main questions for moth conservation in the Mediterranean countries can be stated: 1) How are we going to assess and maping moths populations? 2) How important are moth as pollinators/pollen vectors? 3) How does climate change, land-use change and ALAN, affect moths and pollen-transport networks? 4) What are the functional consequences of those threats on Lepidoptera life-cycles and plant reproduction? 5) Can the impacts of those threats be mitigated? And how are we going to do it?

A decade ago, Warren & Bourn (2011) proposed ten challenges to conserve

Lepidoptera in Europe and try to meet the new target of halting biodiversity loss in Europe and in the Mediterranean. They were focused on agricultural and forestry systems, habitat management, mitigation for climate change, creation and maintenance of key sites, developing monitoring programmes, funding for nature conservation, and political and public awareness. Even though moths are not as attractive to people as butterflies, several studies reveal their importance in natural systems, and since they can be easily catch with a light trap, their use as an environmental educational tool is facilitated and can improve the engagement of people in monitoring living organisms (Pocock et al. 2018). Examples of monitoring schemes amongst butterflies (Bates et al. 2014; Dennis et al. 2017) and moths (Groenendijk & Van der Meulen 2004; Slade et al. 2013) and the data collected from citizen science has been used in various studies and contribute to increase our knowledge of this group and help the conservation efforts.

Conclusions

We stated that the main threats to moths in Mediterranean context are wildfires, climate change, land-use change and artificial light at night. Taking in consideration the evidence of declines in pollinators and the negative impacts on natural ecosystem processes and services, we stated the importance of preserving them because of their role as pollinators in the food-web and for the communities. Additionally, we wrote about some conservation measures and questions for future research. Probably the main priority is to develop a systematic monitoring scheme, species surveys and mapping in order to answer some of the main questions addressed above like species and populations studies, importance of moths as pollinators and the effects of the main threats to moth on pollination networks. Those studies will allow to understand and quantify disturbances in the food web, in pollination and will contribute to the preservation of moths and their communities.

CHAPTER 3

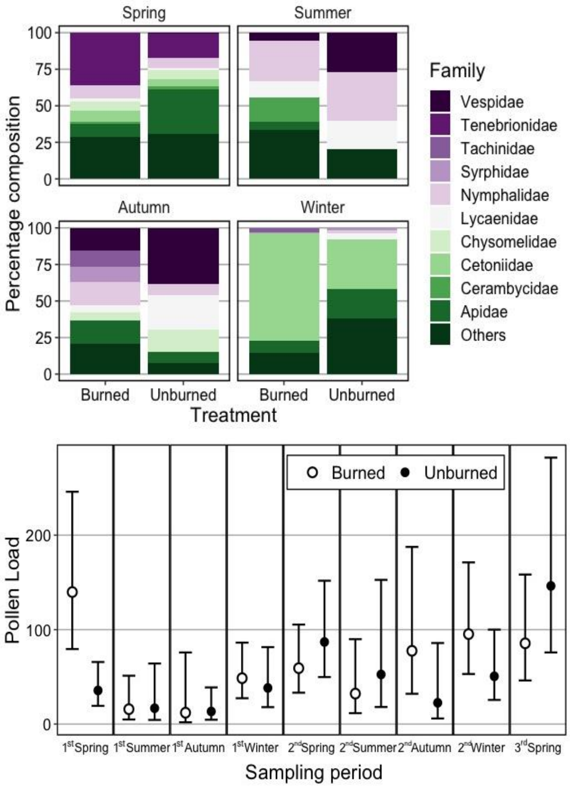
Wildfire disrupts pollen transport by diurnal insects in Southern Portugal

Submitted to Ecological Entomology as:

Banza, P., Evans, D. M., Medeiros R., Macgregor, C.J. & Belo, A. D.F. Wildfire disrupts pollen transport by diurnal insects in Southern Portugal.

Manuscript ID 20-0459-EEN
07-Oct-2020

Graphical Abstract



- A wildfire in Portugal had significant, interacting effects on the abundance of diurnal insects but not on species richness.
- Pollen loads and species richness on individual insects were significantly higher in burned sites in the first spring only.
- Overall, across most of the community metrics examined, our results suggest that diurnal insects, and the pollen they transported, returned to the pre-fire state within a relatively short period.

Abstract

1. Climate change is a key driver of increased wildfire activity globally. Whilst the recovery of plant communities after fire is generally understood, the impacts on ecological processes, such as pollen transport by insects, have received little attention.
2. We investigated the effects of wildfire on diurnal insects and pollen transport over two years following a large fire that occurred in Southern Portugal. By comparing samples collected at three burned sites and three adjacent unburned sites, we examined the effects of burning on: a) abundance and species richness of diurnal insects across seasons; b) the pollen being transported; and c) the three most abundant species: *Oxythyrea funesta*; *Heliothaurus ruficolis* and *Apis mellifera*.
3. Wildfire had significant, interacting effects on the abundance of insects but not on species richness. With time, insects increased in abundance and species richness, most notably each spring.
4. Pollen loads and species richness on individual insects were significantly higher in burned sites in the first spring only, but generally increased with time after the wildfire.
5. The abundance of *O. funesta* was similar between burned and unburned areas in the spring but, in the winter, was significantly higher in burned. The abundance of *H. ruficolis* was slightly higher in burned areas. Wildfire did not affect the abundance of *A. mellifera.*
6. Overall, across almost all of the community metrics used, our results suggest that diurnal insects, and the pollen they transported, returned to the pre-fire, unburned state within a relatively short period.

Keywords: pollination, plant-insect interactions, Mediterranean, mutualisms, ecosystem services

Introduction

Wildfires are a natural feature of ecosystem disturbance and their importance is recognized for vegetation dynamics (Moreno & Oechel 1994; Lloret et al. 1999; Paula & Pausas 2008, Velle et al. 2012). Globally, there is evidence that fires will increase in number, intensity and occur over extended periods of the year within the next decades (e.g. Flannigan et al. 2013). In the United States, increased forest fire activity has been attributed to anthropogenic climate change, among other factors (Abatzoglou & Williams 2016). In northern Mediterranean countries, the frequency of fires has also increased in recent years (Moreira et al. 2001; Naveh & Carmel 2004; Pausas & Fernández-Muñoz 2012) mainly related to socio-economic factors such as an increase in fuel accumulation at the landscape level due to land abandonment, and an increase in exotic forestry plantations (Pausas & Vallejo 1999). In Europe, Portugal has the highest number of burned areas per year since 2013; in 2017 more than 540,000 ha of forest were destroyed as a result of fires (EEA 2019).

To date, most studies on post-fire regeneration in Mediterranean ecosystems have focused on plants (Guo 2001; Mitchell et al. 2009; Schaffhauser et al. 2012; Marzano et al. 2012; Francos et al. 2019). These studies have shown that plants have numerous strategies to survive, regenerate and colonize after the fire (Lloret et al. 1999), which include fire-stimulated germination, or resprouting from stumps, lignotubers, or burls (James 1984). Indeed, for many plants fire is essential to create the bare ground necessary for colonization and to provoke the fire-stimulated germination required. However, whilst the recovery of plant communities to fire is generally understood, the recovery of important ecological processes, such as pollination, has received little attention. The pollination of flowering plants by animals is a crucial ecosystem service of great value to humanity because without it most flowering plants would not reproduce sexually and humans would lose food and other plant origin products (Buchmann & Nabhan 1996; Klein et al. 2007; Ollerton et al. 2010; Breeze et al. 2011; Potts et al. 2016). The number of flower-visiting species worldwide may total nearly 300,000 (Nabhan & Buchmann 1997). The importance of pollination to wild plants and as an ecosystem service, as well as a range of other ecological processes provided by pollinating insects, highlights that pollination systems should be a high priority for conservation (Kearns et al. 1998; Vanbergen 2013). However recent studies suggest that most pollination systems are dominated by a small number of common species and not so much by threatened species, so conservation of biological diversity requires more than arguments based only on ecosystem services (Kleijn et al. 2015).

Relatively few plant-pollinator interactions are absolutely obligate. Most are more generalized on the part of both plants and animals, and they also vary through time and space (Feinsinger 1987; Roubik 1992; Waser et al. 1996, Fuster et al. 2020). According to Brosi (2016) some degree of specialization is necessary for successful pollination when we consider individual pollinators.

Few studies have examined the response of interacting communities of flowering plants and insects to fire. Potts et al. (2003) found that fire has a significant effect on the plant-pollinator community, with insect abundance declining markedly after a fire. The magnitude of insect decline is mostly related to the degree of exposure to the flames as well as the mobility of insects (Swengel & Swengel 2007). But community recovery can be rapid. Potts et al. (2003) found a peak in insect diversity in the first two years post-fire, followed by a steady decline, with floral regeneration closely matched by that of the principal pollinators. However, Domínguez et al. (2011) found that communities tend to be less resilient after a fire, because fires produce a homogenized forest landscape. and an increase of shrub and herbaceous communities. Ecosystem changes affect the distribution, abundance, and effectiveness of pollinators, so successful post-fire regeneration depends upon the reinstatement of pollination services. Thus, pollination ecology can be a useful tool for examining the recovery of ecosystem functioning post-fire, by comparing disturbed communities with reference communities (Forup et al. 2008). Previously, we investigated the effects of fire on nocturnal pollen-transport networks following a large wildfire that occurred in Southern Portugal in 2012, and found they had lower complexity and robustness compared to unburned areas (Banza et al. 2019). Burned sites had significantly more abundant flowers, but less abundant and species rich moths, and total pollen transport by moths was just 20% of that at unburned sites. However, it is unclear whether these patterns are consistent for diurnal pollinating insects, and for the quantity and diversity of pollen they transport.

In this study, we examine the impacts of wildfire on plants, diurnal insects and pollen transport following the same wildfire mentioned above. We aim to answer the following questions: 1) Is there an effect of burning on the abundance, community and species richness of diurnal insects and how does this vary across seasons? 2) Does burning affect the amount and diversity of pollen being transported by insects and how does this vary across seasons? 3) Which specific insect species have an important effect on the system and how? 4) Is there any evidence of a negative impact of pollination service in the region, for example an adverse effect on honeybees?

In burned areas we expect potentially more flower-visiting insects due to the flush of flowers after the fire. We also expect seasonal effects on insect abundance, with higher numbers in the spring and autumn compared with summer and winter, consistent with Mediterranean trends. However, we do not anticipate differences in community composition and species richness as a result of burning, as these are more likely to be driven by seasonal variation. We expect higher pollen loads being carried by diurnal insects in burned areas due to the flush of flowers after the fire (with some plants potentially contributing with more pollen, and others less) and more pollen transported in the spring and autumn.

Materials and Methods

Field site

The study was conducted in an area of semi-natural cork oak-wood heathland of high conservation value dominated by *Cistus ladanifer* L. (gum cistus), *Lavandula stoechas* L. subsp. *stoechas* (lavender), *Arbutus unedo* L. (strawberry tree), *Erica arborea* (heather), and *Quercus suber* L. (cork oak) with some patches of *Pinus spp.* (pines). The sites (Table S1, Supporting Information) were selected in an area burned in July 2012 and in unburned adjacent areas located in Eastern Algarve (Serra-do-Caldeirão), Portugal (full details are in Banza et al. 2019). The study began in April 2013 and continued until May 2015. Within the area we selected three 40 x 40 m^2 post-fire plots and three unburned plots as a reference for potential ecological status before any fire damage. All plots had similar altitude, slope and exposure and they were at least 300 meters apart from each other. Each plot was visited approximately once every two months to sample insects and flowering plants in flower**.** During each visit, temperature, wind speed and weather conditions were also recorded.

Floristic surveys

To quantify floral resources for insect pollinators, two parallel 10 m transect lines were established, 10 m apart, at the centre of each plot. A 1 x 1 m^2 quadrat was placed every two meters along each transect line (n = 10). In each quadrat, percentage cover and height of all plant species currently in flower were recorded. Specimens of all plants in flower were collected and identified using the Iberian Flora (Castroviejo 1986-2014) and collections in the University of Évora Herbarium (HUEV). The flowering period for each plant was recorded, to help determine which species were on site during the fieldwork.

A pollen reference collection was also prepared to assist with subsequent pollen analysis, by sampling pollen from all flowering plants in flower present at the sites and fixing it on a microscope slide using fuchsin jelly (Beattie 1972). The reference collection contained pollen of 86 plant species from 34 families, including all species recorded on transects (Table S2, Supporting Information).

Diurnal insect surveys

Transects of 15 m were conducted at each plot and all insects observed visiting plants in flower were captured using a hand net or directly into killing tubes with a drop of ethyl acetate for later identification and pollen analysis. Each insect caught was transferred to a killing tube as quickly as possible to reduce stress and decrease the chance of any pollen loss. Sampling occurred between 10 am and 4 pm and the timing of plot visits was randomised during the sampling period to avoid any effect of daytime on potential pollinator activity. All samples collected were frozen at the end of each day to reduce decomposition. Insects were later identified using a binocular microscope and a selection of field guides (Delachaux 1990; Chinery 1979; Maravalhas 2000). Any insects that could not be identified were morphotyped.

Pollen analysis

All frozen insects were placed in a re-hydration box for 12 hours before processing them. The head, proboscis and legs of caught insects were swabbed using a small cube of Fuchsin-glycerin jelly (Beattie 1972) and a microscope slide was prepared and examined at 400x magnification (microscope: Leitz HM-Lux 3). Pollen was identified to the lowest possible taxonomic level using the pollen reference collection mentioned above.

Statistical methods - overview

Analyses were conducted in R version 3.6.3 (R Core Team 2018) with a diverse selection of packages, of which the most important was lme4 (Bates et al. 2015; for constructing the majority of generalised linear mixed-effects models). A full list of packages used, with their references, is given in Table S3 (Supporting Information).

We grouped our data according to sampling period and treatment (i.e. burned and unburned). Seasons were defined as follows: October-December ("autumn"), January-March ("winter"), April-June ("spring"), and July-September ("summer"). These reflected four clearly separable phases in the annual cycle of floral and insect abundance. As sampling took place between April 2013 and May 2015, this resulted in a total of 9 seasons being sampled. For clarity, "season" henceforth refers to a four-level variable (autumn, winter, spring and summer) and "sampling period" refers to a nine-level continuous variable (spring of year 1, etc) that describes the number of seasons since the study commenced. Species richness was extrapolated using the Chao2 estimator (Chao 1987) to estimate the true value.

Statistical testing

We used general and generalised linear mixed-effects models to test the effects of fire, sampling period and the interaction between the two variables on abundance and estimated species richness of diurnal insect samples (Poisson family and log link function) and on pollen count and pollen species richness (Gaussian family with log 10 transformation). If the interaction between fire and sampling period was not significant, the model was retested with the main effects only and a new model containing fire and season would then be fitted to test for the interaction between fire and season. We separately retested the effects of fire on insect abundance for three species of insects that dominated the sample: *Apis mellifera* (Linnaeus, 1758) (Order Hymenoptera, Family Apidae), *Heliothaurus ruficolis* (Fabricius, 1781) (Order

Coleoptera, Family Tenebrionidae) and *Oxythyrea funesta* Poda, 1761 (Order Coleoptera, Family Cetoniidae). To investigate effects on pollen transport, we first checked the proportion of insects found to be carrying pollen; using individual, pollen-carrying insects as replicates, we then tested for effects of fire and sampling period (or season) on the pollen transport metrics.

To account for spatial autocorrelation, we included site as a random effect in models for all analyses where we had multiple replicates per sampling period in each treatment; additionally, we included year as a random effect in the models containing season and not sampling period. Significance of fixed effects was tested using Likelihood Ratio Tests; as a consequence, where interaction terms were found to be significant and retained, we present χ^2 and P-values for the interaction term only (not independently for its constituent variables). Additionally, we tested for differences in community composition of insects at family level, comparing communities sampled in burned and unburned sites using Bray-Curtis dissimilarities tested by permutational multivariate analysis of variance, using the adonis function of Vegan (Oksanen et al. 2016).

Results

Overview

A total of 28 different families of flowering plant in flower were described in the study area. The most abundant plants (e.g. found in most transects) were Asteraceae, Cistaceae, Fabaceae and Lamiaceae; Asteraceae and Fabaceae were also the most represented in terms of number of different species (Banza et al. 2019). A total of 572 insects were caught and identified into 138 morphotypes. Of these, 47 were identified to species level representing 280 individuals; 13 to genus level (from 9 genera) corresponding to 21 individuals. 60 morphotypes (representing 148 individuals) were identified to family level only (44 families), 8 morphotypes (representing 13 individuals) were identified to order and 15 insects were not identified (representing 10 morphotypes) (Fig. 1 and Table S4, Supporting Information); all morphotypes were included in subsequent analyses, regardless of the level of identification. Therefore, our total sample contained at least 138 taxa of at least 44 families. The most abundant species found across all sampling periods were *Apis mellifera* with a total of 79 individuals, *Heliothaurus ruficolis* with a total of 82 individuals and *Oxythyrea funesta* with a total of 89 individuals.

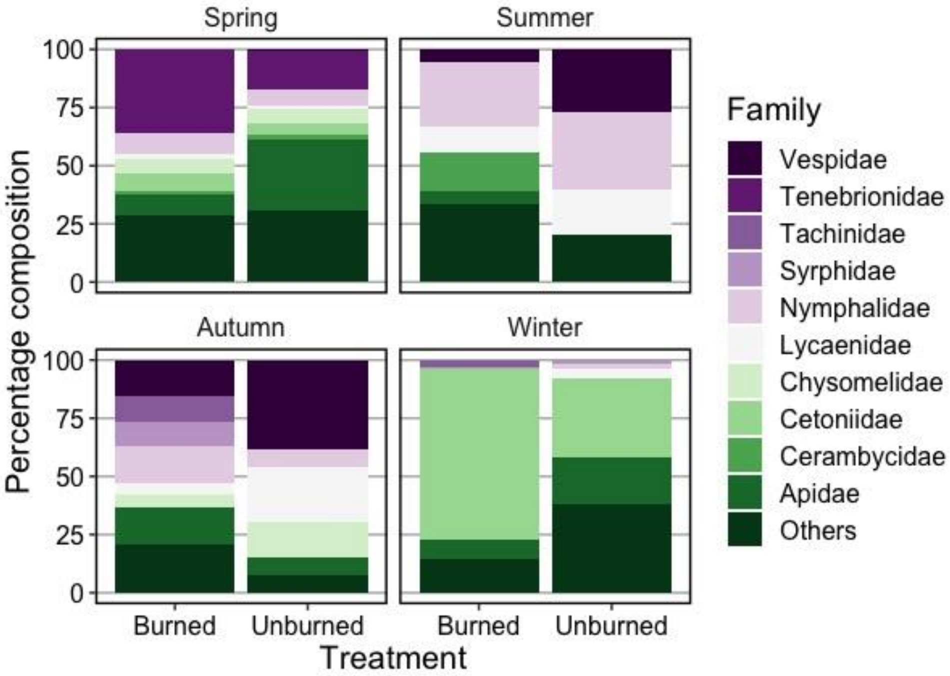


Figure 1. Assemblage composition by family of insects caught in burned and unburned areas of Serra-do-Caldeirão, Portugal, and across seasons. Families never comprising >10% of individuals in any combination of season and treatment are grouped as "Others", and all other families are shown independently.

Almost every caught insect was found to be carrying pollen. In total they carried 151,422 pollen grains from 62 plant pollen morphotypes. Overall, insects carried pollen from 57 plant species, representing 81.5% of the 70 plant species identified on floral surveys. The most representative plant families are represented in Figure 2 (and Table S5, Supporting Information). In the spring, pollen loads on insects were greatly dominated by *Tuberaria guttata* (L.) Fourr, followed by *Coleostephus myconis* (L.) Rchb.f. and *Lavandula stoechas* L.; in the summer, the most common pollen species carried by insects was *Ulex argenteus* Webb; in the winter, these were *Lithodora prostrata* (Loisel.) Griseb., *Ulex argenteus* Welw. ex Webb; and *Ulex eriocladus* C.Vicioso; and in the autumn pollen loads were dominated by *U. eriocladus*, followed by *C. myconis*. (Fig. 2 and Table S5; Supporting Information).

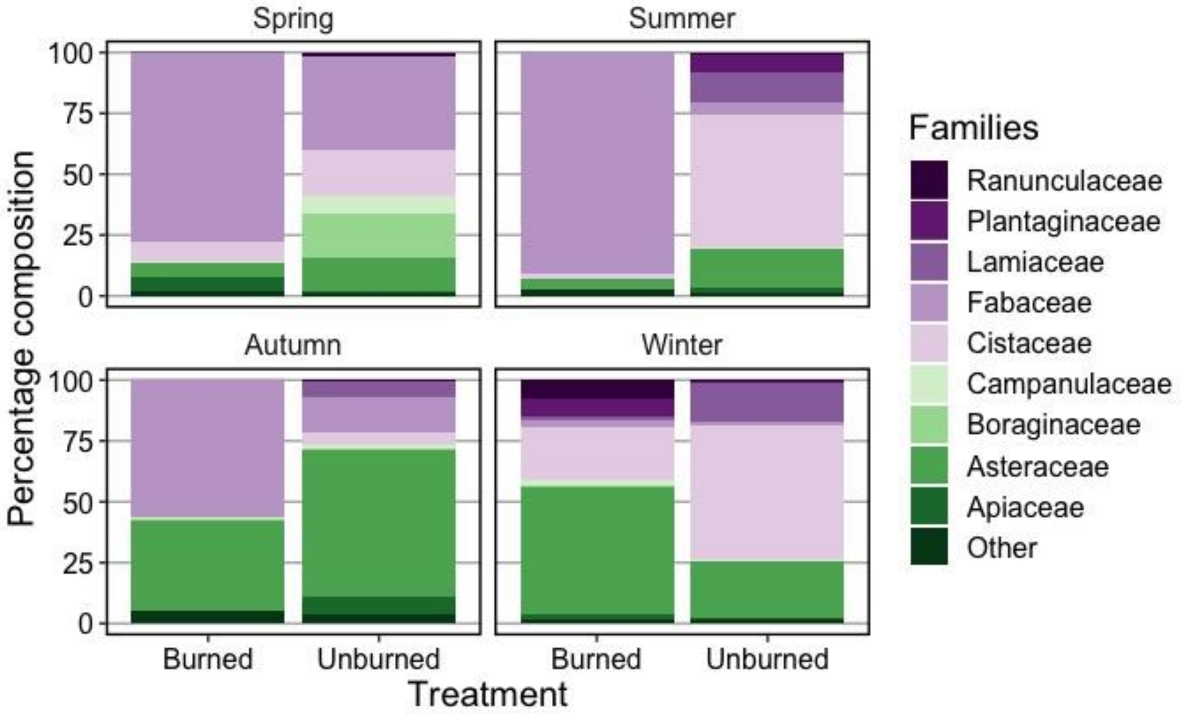


Figure 2. Assemblage composition by families of pollen carried by insects caught in burned and unburned plots of Serra-do-Caldeirão, Portugal, and across seasons. Families never comprising >7% of individuals in any combination of season and treatment are grouped as "Other", and all other families are shown independently.

Impacts of burning on insect abundance, species richness, and community composition

Fire and sampling period had significant, interacting effects on the abundance of insects ($\chi2$ / LRT = 23.645, d.f.= 8, p=0.0026), Fig. 3 (Table S4), but not on estimated species richness (LRT; $\chi2$ = 4.663, d.f.= 8, p= 0.7929). Once the interaction was removed, estimated species richness was not significantly affected by fire alone ($\chi2$ / LRT = 0.010, d.f. = 1, p = 0.9221) but there were significant differences among sampling periods ($\chi2$ / LRT = 71.439, d.f. = 8, p < 0.0001 - Fig. 4, Table S4). The interaction between fire and season was also not significant in explaining estimated species richness ($\chi2$ / LRT = 0.75336, d.f.= 3, p= 0.8606).

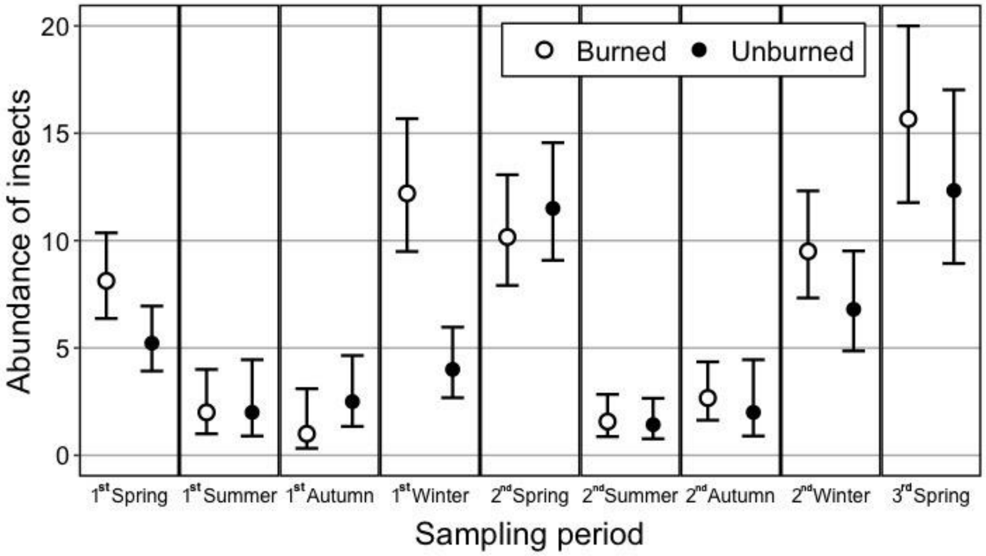


Figure 3. The effects of fire and season on the abundance of insects at burned plots (open circles) and unburned plots (closed circles) in Serra-do-Caldeirão, Portugal. Circles represent the model-predicted abundance. Error bars show 95% confidence intervals.

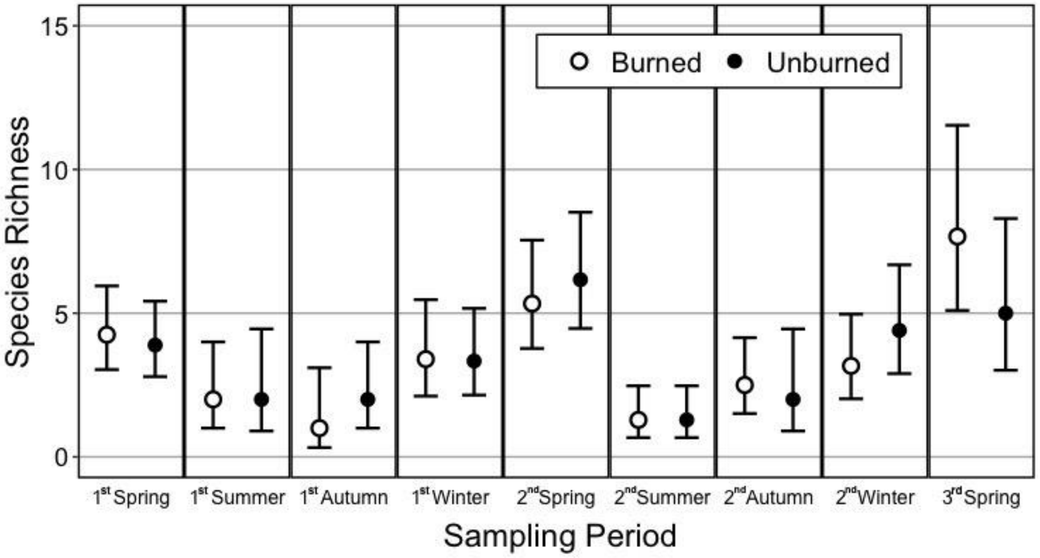


Figure 4. The effects of fire and season on the species richness of insects at burned plots (open circles) and unburned plots (closed circles) in Serra-do-Caldeirão, Portugal. Circles represent the model-predicted abundance. Error bars show 95% confidence intervals.

Insects were significantly more abundant in burned sites than unburned sites in the first spring and winter but there were no significant differences in abundance between burned and unburned sites in the summer and autumn or in the subsequent spring and winter seasons. Generally, both the abundance and species-richness of insects peaked in the spring; insect abundance was also significantly higher in the winter compared to summer or autumn, but species richness was not.

Across the duration of the experiment, insect abundance and species richness increased noticeably each spring (Figs. 3 & 4). However, there was no significant effect of burning on the insect community composition at family level *(Anosim Stat R=0.667, p=0.1,* Fig 1.*)*

Pollen transport

Burning and sampling period had significant interacting effects on the two pollen transport metrics that we tested (Table S6, Supporting Information): the total pollen load (Fig. 5) and number of pollens morphotypes (Fig. 6) *per* pollen-carrying individual insect. Specifically, the pollen loads and species richness of individual insects were significantly greater in burned sites in the first spring but no other significant differences or general patterns were found between burned and unburned sites in the remaining sampling periods. Overall pollen loads and species richness tend to be greater in spring compared to the other seasons; there is a general tendency for both pollen load and pollen species richness to increase across the whole sampling period (Fig. 5 & 6).

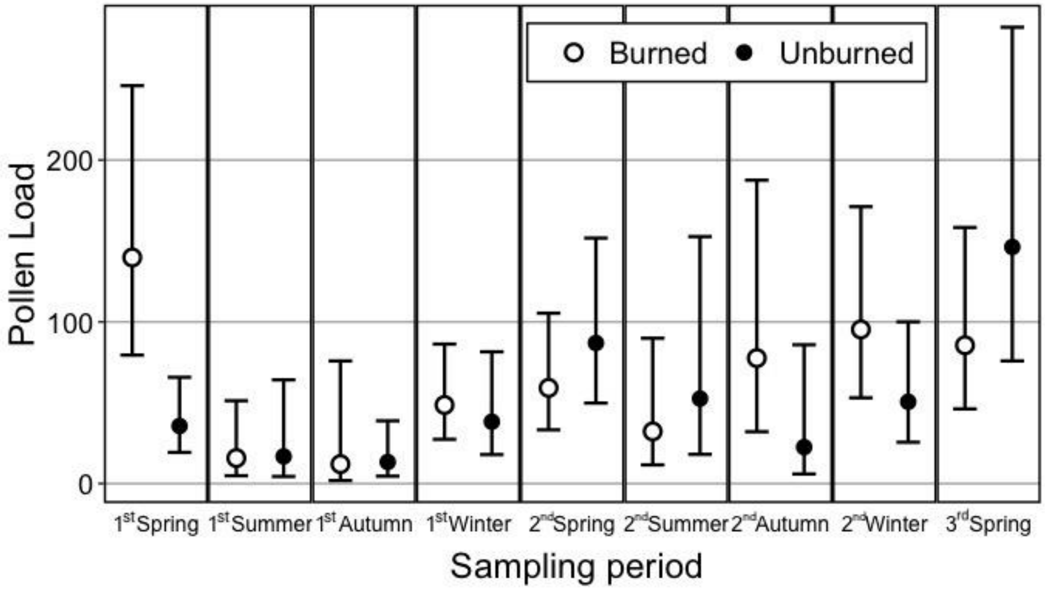


Figure 5. The effects of fire and season on the pollen load of insects per individual pollen-carrying insect at burned sites (open circles) and unburned sites (closed circles) in Serra-do-Caldeirão, Portugal. Error bars show 95% confidence intervals of the model-predicted pollen loads.

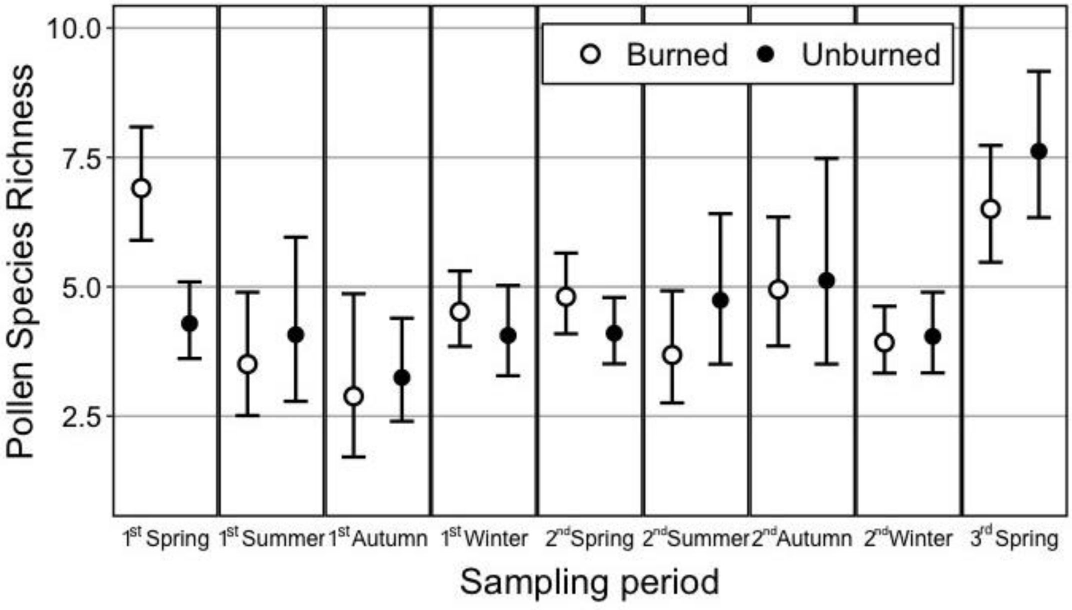


Figure 6. The effects of fire and season on the pollen species richness per individual pollen-carrying insect at burned sites (open circles) and unburned sites (closed circles) in Serra-do-Caldeirão, Portugal. Error bars show 95% confidence intervals of the model-predicted pollen loads.

Overall, across almost all the community metrics, the significant interaction between burning and sampling period (Tables S6-S7, Supporting Information) indicates that over this period of 1-3 years post-fire there was a return to the pre-fire, unburned state. Over 99% of insects carried pollen, so no statistical analyses were conducted regarding potential differences in the proportion of insects carrying pollen between burned and unburned sites (c.f. Banza et al. 2019) (Fig. 5 & 6).

Apis mellifera (Linnaeus, 1758)

This species was not recorded in any of the two summer sampling periods but was present in all other sampling periods. There was no significant interaction between burning and sampling period ($\chi2$ / LRT = 1.205, d.f.= 3, p=0.7517) or between burning and season ($\chi2$ / LRT = 0.9676, d.f.= 2, p= 0.6164) in the abundance of *Apis mellifera*. There was also no individual effect of burning ($\chi2$ / LRT = 3.4344, d.f.= 1, p=0.1161) but there was a positive marginally significant effect of sampling period ($\chi2$ / LRT = 12.607, d.f.= 6, p=0.0497) driven by a greater abundance of *Apis mellifera* in the second spring (a mean of 5.23 ± 1.37 SE) compared to the other sampling periods (highest mean = 3.02 ± 0.71 SE for the third spring).

Heliothaurus ruficolis (Fabricius, 1781)

This species was only found in the spring hence the interaction and effects of burning and year were tested, instead of sampling period or season. There was no significant interaction between burning and year in the abundance of *Heliothaurus ruficolis* ($\chi2$ / LRT = 1.2573, d.f.= 1, p= 0.2622) but there was a marginally non-significant effect of burning ($\chi2$ / LRT = 3.5781, d.f.= 1, p= 0.0585) and a significant effect of year ($\chi2$ / LRT = 10.1212, d.f.= 2, p= 0.0063). Although the interaction between burning and year is not significant, abundance of *Heliothaurus ruficolis* is slightly greater in burned areas (mean 6.13 ± 0.94SE) compared to unburned areas (mean 3.65 ± 0.78 SE) and it is greater in the first sampling year (mean 9.81 ± 2.32 SE), compared to the second (mean 3.64 ± 0.73SE) or the third (mean 5.25 ± 0.94 SE).

Oxythyrea funesta Poda, 1761

This species was only found in the spring and the winter. The interaction between treatment and sampling period was not tested because there was not enough data across all categories but there was a marginally non-significant effect of sampling period alone ($\chi 2$ / LRT = 9.2555, d.f.= 4, p= 0.055021), seemingly driven by the lower abundance of *O. funesta* in the third spring (mean 0.61 ± 0.80 SE), compared to the first and second springs (mean 1.76 ± 0.80 SE and 1.59 ± 0.49 SE, respectively). There was also a significant interaction between burning and season in the abundance of *Oxythyrea funesta* ($\chi 2$ / LRT = 4.5873, d.f.= 1, p= 0.03221). The abundance of *Oxythyrea funesta* was similar between burned and unburned areas in the spring (mean 1.40 ± 0.529 SE and mean 1.00 ± 0.577 SE, respectively) but in the winter, there was significantly greater abundance in burned areas (mean 17.75 ± 2.107 SE) compared to unburned areas (mean 2.00 ± 0.7071 SE).

Discussion

Wildfire had significant, interacting effects on the abundance of diurnal flower-visiting insects but not species richness, with some seasonal effects, but no significant effect on community composition at the family level. Individual insect pollen loads and species richness were significantly higher during the first spring post-fire, but not subsequently. However, there was a general tendency for both pollen load and species richness to increase during the study.

Within burned plots of the study area, there was evidence of secondary succession with a flush of flowering plants, like in other studies of Mediterranean plant community post-fire recovery (Capitanio & Carcaillet 2008) and accompanying diurnal pollinators (Potts et al. 2003; Van Nuland et al. 2013). We found an increase in winter floral abundance, primarily by annual flowers, whereas perennial flowers had reduced abundance at the burned sites. This is likely due to the fact that some plants may be stimulated by fire (Herranz, Ferrandis & Martínez-Sánchez 1998) or because of higher light levels associated with reduced shrub cover at burned sites. The secondary succession flush of flowering plants after the fire likely created more opportunities for insects to feed but species richness of insects did not change probably because the plant community remained the same for each season. Insects depend on plants for feeding, particularly at larval stages but as adults the flowers are a very important source of food, either for nectar or pollen or both. Insects were more abundant in the burned plots in the first spring and winter, in accordance with a higher availability of food.

The community composition of insects at the family level was not affected by burning but there were some seasonal effects. Abundance and species richness peaked in the spring and insects were more abundant at burned sites in the first spring and winter. Again, this was likely response to plant turnover in the spring and in the winter. However, there were no significant differences in abundance between burned and unburned plots in the summer, autumn or in the subsequent spring and winter seasons. As bushes and other perennial flowering plants recovered in the burned areas, the number of annual flowers stabilized and that likely affected the abundance of insects.

Examining pollen transport gives a good indication as to how important ecological processes recover after a fire. Our results showed that burning had a significant effect only in the first spring, insects carried more pollen and from more pollen types in burned sites than in unburned sites. However, no other significant differences or general patterns were found between burned and unburned sites in the remaining sampling period. This can be explained again by the fact that there were more flowers in the spring than the other seasons, and hence more possibilities for insects to carry pollen from them. Summer is usually very hot with less flowers, so pollen transport was relatively lower than in spring. Many plants flower after the first autumn rains, creating what is known locally as the “second spring”, and flowering continues into winter because temperatures are mild (e.g. February and March had a mean temperature of above around 20ºC or more; Table S8, Supporting Information).

However, there was a general tendency for both pollen load and species richness to increase during the study. This pattern was observed across both burned and unburned sites, so may have been driven by factors other than the fire. It is also possible that the fire may have caused regional-scale disturbance to pollination systems (affecting all sites, not just the burned sites) which then recovered over time. Such effects cannot be identified with the space-for-time experimental design that we used, and would require baseline data to have been collected at burned sites before a fire occurred.

Separate analyses of dominant diurnal flower-visiting species caught showed no significant interaction between burning and sampling period or between burning and season in their abundance. *Apis mellifera* was not recorded in the Summer months, perhaps due to the higher temperatures (average temperature in Summer 2013 – 32.4ºC; Summer 2014 – 31.9ºC), which makes insect mobility more difficult, or the scarcity of flowers, reducing the availability of pollen and nectar resources. This species collects pollen from a wide range of taxa (Schmalzel 1980), and is able to adapt to different vegetation characteristics. The most abundant bee species tend to be generalist flower visitors, what gives them numerous advantages because of the general higher availability of pollen and nectar resources in burned areas (Potts et al. 2003). However, we found a difference in the pollen they collected from burned areas compared to unburned areas, except in the Autumn, when the pollen was collected mainly from *Ulex eriocladus* in both cases. In the Spring pollen collected from burned areas was mostly from plants of the Asteraceae family and *Lavandula stoechas* and in unburned areas was collected mostly from two types of plant: *Lavandula stoechas* and *Tuberaria guttata*; the diversity of pollen types was higher in the burned areas compared to unburned areas. In the Winter pollen collected from burned areas was dominated by *Ulex eriocladus* with some *U. australis* but in unburned areas the pollen collected was mainly from *Lavandula stoechas* and *Cistus salviifolius* L. (Figure b, Supporting Information).

The abundance of *Heliothaurus ruficolis* was slightly higher in burned areas compared to unburned areas. It is a phytophagous beetle species that feeds from pollen, mainly on plants whose pollen structures are more accessible, such as those in the Asteraceae family (Figure c, Supporting Information). After the fire the number of these plants increased (1st Spring), what might explain the slightly higher abundance of these species in the burned areas.

Regarding *Oxythyrea funesta*, the abundance was similar between burned and unburned areas in the spring but in winter there was significantly higher abundance in burned areas compared to unburned areas. *O. funesta* is a phytophagous beetle species that feeds on pollen, but also raid floral organs, damaging especially colour flowers buds and flowers. Again, the differences in the winter between burned and unburned areas might be explained by the type of flowers present in those areas. In burned areas most of the pollen found in those insects was from *Cistus spp.* and *Ulex argenteus* and in unburned areas was from *Cistus salviifolius* (Figure d, Supporting Information).

The increase in abundance of *Heliothaurus ruficolis* and *Oxythyrea funesta* could also be attributed to the fact that the local populations survived the fire as eggs or larvae protected in the soil but also to the reduction of their predators as a result of the fire (Pausas et al. 2018).

Recently, we demonstrated the impacts of the same wildfire on nocturnal pollen-carrying moths, ultimately showing that pollen-transport networks in burned areas became less robust to perturbation and comprised a substantially changed set of interactions. In spite of increased floral abundance after burning, the total effect of burning on pollen transport was negative in all seasons, because moths were less abundant and species rich at burned sites (Banza et al. 2019). Furthermore, there was no evidence of a return to pre-fire state. This result contrasts with the present study of diurnal pollen-carrying insects, which shows some positive effects of fire in the diurnal plant-pollinator systems and some evidence of a return to pre-fire state. Both studies complement each other and show the importance of studying the entire plant-insect community in order to understand better how it recovers from the disturbance caused by fire.

Our work highlights a number of directions for future research on post-fire pollination function. Firstly, pollen transport does not necessarily translate to successful pollination in all cases (King et al. 2013), that is, the effects of the changes in pollinator abundance and pollen transport that we observed do not necessarily reflect the actual reproductive success and productivity of plants in burned and unburned areas. Secondly, recent studies have directly compared diurnal and nocturnal pollination (e.g. Knop et al. 2017; Walton et al. 2020), whereas we analysed data on diurnal pollination (this study) and nocturnal pollination (Banza et al. 2019) separately, due to different sampling methodologies. Future work merging diurnal and nocturnal pollination could be valuable to understand overall impacts of wildfire on pollination systems, since the individual effects on the different pollinator guilds were very different. Such studies might be facilitated by using standardized methods across diurnal and nocturnal surveys, by conducting nocturnal transects instead of light-trapping (e.g. Macgregor et al. 2017a). Finally, our findings are likely to be mainly a reflection of changing foraging habits of adult insects responding to the variation in the availability of floral resources. Understanding the population impacts of fires on insects may require study of the immediate impacts on other stages of the insect life-cycle.

Conclusions

We found evidence that wildfire disrupts pollen transport by diurnal insects, at least in the short term, with similar patterns to those insects in unburned areas returning to pre fire unburned state with time. There was a general tendency for both pollen load and insect species richness to increase during the study. However, there was no significant effect of burning on the insect community composition at the family level. Our study demonstrated that even a small sample of diurnal flower-visiting insects can carry a large amount of pollen and that overall pollen-transport can be disturbed by wildfire. However, more research is needed to better understand the functional consequences of wildfire on plants and insects. To achieve this, incorporating diurnal and nocturnal insects (together with information on their traits) into more complete ecological network analyses would provide numerous opportunities to understand and manage the resilience of fire-prone ecosystems.

CHAPTER 4

Wildfire alters the structure and seasonal dynamics of nocturnal pollen-transport networks

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Published as:

Banza P, Macgregor CJ, Belo ADF, Fox R, Pocock MJO, Evans DM. Wildfire alters the structure and seasonal dynamics of nocturnal pollen-transport networks. *Functional Ecology* 2019;33:1882–1892. Doi: 10.1111/1365-2435.13388

Abstract

1. Wildfires drive global biodiversity patterns and affect plant-pollinator interactions, and are expected to become more frequent and severe under climate change. Post-fire plant communities often have increased floral abundance and diversity, but the effects of wildfires on the ecological process of pollination are poorly understood. Nocturnal moths are globally important pollinators, but no previous study has examined the effects of wildfire on nocturnal pollination interactions.
2. We investigated the effects of wildfire on nocturnal pollen transport networks. We analysed the abundance and species richness of moths and flowers, and the structure of these networks, at three burned and three unburned sites in Portugal for two years, starting eight months after a large fire.
3. Nocturnal pollen-transport networks had lower complexity and robustness following the fire than at nearby unburned sites. Overall, 70% of individual moths carried pollen, and moths were found to be transporting pollen from 83% of the flower species present. Burned sites had significantly more abundant flowers, but less abundant and species-rich moths. Individual moths transported more pollen in summer at burned sites, but less in winter; however, total pollen-transport by the moth assemblage at burned sites was just 20% of that at unburned sites. Interaction turnover between burned and unburned networks was high.
4. Negative effects of fire upon moths will likely permeate to other taxa through loss of mutualisms. Therefore, if wildfires become more frequent under climate change, community resilience may be eroded. Understanding the responses of ecological networks to wildfire can inform management that promotes resilience and facilitates whole-ecosystem conservation.

Keywords: Disturbance, ecological networks, fire, flowering plants, Lepidoptera, Mediterranean, moths, pollination

Resumo Português (Second language abstract: Portuguese)

1. Os fogos florestais induzem padrões mundiais de biodiversidade, afetando as interações planta-polinizador, e é expectável que se tornem mais frequentes e severos num cenário de alterações climáticas. As comunidades vegetais do pós-fogo apresentam frequentemente maior abundância e diversidade florística. No entanto, os efeitos dos fogos florestais sobre o processo ecológico da polinização são pouco conhecidos. Os lepidópteros noturnos são polinizadores importantes a nível mundial, mas apesar disso nenhum estudo escrutinou, até à data, os efeitos dos fogos florestais sobre as interações produzidas entre as plantas e os polinizadores noturnos.
2. Investigámos os efeitos dos fogos florestais nas redes de transporte de pólen por polinizadores noturnos. Analisámos a abundância e riqueza específica de traças e plantas em flor, e a estrutura destas redes, em três áreas ardidas e três não ardidas em Portugal, durante dois anos, com início oito meses após um grande fogo.
3. As redes noturnas de transporte de pólen apresentaram menor complexidade e robustez após o fogo quando comparadas com áreas próximas não ardidas. Globalmente, 70% das traças transportavam pólen do qual 83% pertencia a plantas em flor presentes no local. Nas áreas ardidas a floração foi significativamente mais abundante, mas a abundância e a riqueza específica das traças foram menores. Nas áreas ardidas, cada traça individualmente transportou mais pólen no Verão, mas menos no Inverno; no entanto, o total de pólen transportado pelo conjunto das traças foi de apenas 20% do das áreas não ardidas. O *turnover* das interações entre áreas ardidas e não ardidas foi elevado.
4. Os efeitos negativos dos fogos sobre as traças irão provavelmente fazer-se sentir noutros taxa em consequência da perda de mutualismos. Portanto, se os fogos florestais se tornarem mais frequentes por causa das alterações climáticas, a resiliência das comunidades pode ser afetada. Compreender as respostas das redes ecológicas aos fogos florestais pode contribuir para uma gestão que promova a resiliência e facilite a conservação do ecossistema como um todo.

Introduction

Wildfire drives biodiversity patterns globally through heterogeneous disturbance regimes (Kelly & Brotons 2017). It is especially important within Mediterranean ecosystems (Faivre et al. 2011), where wildfires have become more frequent and severe since the 1970s because agricultural abandonment has caused fuel accumulation (Moreira et al. 2001; Pausas & Fernández-Muñoz 2011). Climate change is expected to drive further increases in frequency and severity of fires (Flannigan et al. 2013).

Fires can shape plant-pollinator communities (Brown et al. 2017; Ponisio et al. 2016), leading to reduced abundance of pollinators and flowers (Potts et al. 2001) and reductions in plant reproductive success (Ne'eman et al. 2000), or increased floral resources through a flush of secondary succession (Capitanio & Carcaillet 2008; Potts et al. 2003). By altering community composition, fire may have secondary effects on plant-pollinator networks (Welti & Joern 2017), but no study has investigated the direct effects of fire on plant-pollinator network properties (Brown et al. 2017). Ecological network metrics are increasingly used as tools for biodiversity monitoring and assessment of environmental change (Derocles et al. 2018), because they can describe important changes in the structure and function of whole ecosystems that might not be detected by measuring species abundance and diversity.

Moths are potentially pollinators of global importance (Macgregor et al. 2019a; Macgregor et al. 2015), and may be especially important in the Mediterranean (Banza et al. 2015). They are in decline (Conrad et al. 2006), with probable drivers of those declines including habitat fragmentation, climate change (Fox et al. 2014), and artificial light at night (Macgregor et al. 2017a; van Langevelde et al. 2018). Wildfire may also affect moths; of the few studies of the effects of wildfire upon Lepidoptera, most find negative impacts (Kral et al. 2017). Fire can lead to mortality of larvae through host plant destruction (Fowles et al. 2004), subterranean pupae (Schmid et al. 1981), and even adults (Gerson & Kelsey 1997). However, the effects of fire on moths and their pollen-transport interactions at community-level have not been studied.

Here, we examined the response of nocturnal moth-plant interaction networks to a large fire in southern Portugal. By assessing the abundance and diversity of moths, flowers, and their networks of pollen-transport interactions year-round at three burned and three unburned large sites for two years following the fire, we tested four hypotheses about the effects of wildfire on nocturnal pollen-transport systems: (i) that burned sites would have more flowers than unburned sites, because some species would respond to fire by flowering; (ii) that burned sites would have fewer moths than unburned sites, because of damage to larval host plants; (iii) that pollen-transport networks at burned sites would be less interaction-rich (because under hypothesis ii, the abundance and species richness of moths would be lower) and have lower complexity than at unburned sites; and (iv) that pollen-transport networks at burned sites would have lower robustness (a measure of the tolerance of networks to species extinctions (Memmott et al. 2004)) than at unburned sites, because generalist species play important roles in maintaining network stability (Tylianakis et al. 2010), but the loss of larval host plants might drive random local extinctions of generalist flower-visiting moths.

Materials and methods

Study system

The study followed a large fire in July 2012, affecting approximately 225 km^2 in the Serra do Caldeirão region near Faro, Portugal (see Fig. S1 in Supporting Information). This is a mountainous shrubland ecosystem (maximum altitude 575 m) containing semi-natural cork oak woodland with high conservation value.

Fieldwork took place from April 2013 to May 2015. We established three 40 x 40 m study sites each in the burned area and a nearby unburned area (Fig. S1). All sites had intermediate densities of oak trees and shrubs at a similar successional stage. The sets of burned sites and of unburned sites each contained a similar range of aspects and altitudes, and all were situated on slopes of > 10% gradient (Table S1). Sites within the same treatment were separated by > 300 m, and sites in different treatments by > 500 m. Throughout the study, sites were sampled approximately every two months by moth sampling and floral transects. Each site was sampled on 13-15 occasions in total.

Moth sampling

Moths were sampled using Heath-style light traps (Heath 1965) baited with 6 W actinic tubes (Philips TL6W/05, Philips, Amsterdam, Netherlands) powered by 12 V batteries. Traps were situated at the centre of the site and operated between sunset and sunrise; exact set-up and collection times varied throughout the year (Fig. S2). Captured moths were retained in individual tubes for subsequent pollen analysis. Moths were identified to the lowest possible taxonomic level, using a local reference collection and several UK field guides (Manley 2008; Sterling & Parsons 2012; Waring & Townsend 2009).

Floral transects

Two parallel 10 m transects were established, 10 m apart, at the centre of each plot. A 1 x 1 m quadrat was placed every two metres along each transect line (n = 10). For each quadrat, percentage cover of all plant species currently in flower (henceforth referred to as flowers) was recorded. Specimens of all flowers were collected and identified using the Iberian Flora (Castroviejo 1986-2014), Flora-On: Flora de Portugal Interactiva (http://flora-on.pt), and collections in the University of Évora herbarium (HUEV); nomenclature and family-level taxonomy were subsequently corrected to follow the Plant List (http://www.theplantlist.org). A pollen reference collection was prepared to assist with pollen analysis, by sampling pollen from each species of flower present at the sites and fixing it on microscope slides. The reference collection contained pollen of 86 plant species from 34 families, including all species recorded on transects.

Pollen identification

All sampled moths were examined for pollen. After relaxation for 12 hours, the head, proboscis and legs of each moth was swabbed with a small cube of fuchsin jelly (Beattie 1972), and a microscope slide prepared with the swab and examined at 400x magnification. Pollen was identified to the lowest possible taxonomic level using the pollen reference collection described above. Whilst pollen-transport by moths does not prove the existence of successful pollination of any plant (King et al. 2013), it is a commonly-used proxy in pollination networks (Banza et al. 2015), being less time-consuming to collect data on than alternative measures such as single-visit deposition.

Analytical methods

Analyses were conducted in R version 3.4.4 (R Core Team 2018), using a range of packages (Table S2).

Seasons were defined as follows: October-December ("autumn"), January-March ("winter"), April-June ("spring"), and July-September ("summer"). These represented clearly-separated phases in annual cycles of floral and moth abundance, with flushes in spring and autumn. Over the study period, we sampled for 9 seasons. Therefore, "season" henceforth refers to a four-level categorical variable (spring, summer, etc.), and "sampling period" refers to a nine-level continuous variable (spring of year 1, etc.) describing the number of seasons since the study commenced. For network analysis, we pooled interactions across sites and samples into distinct networks for each treatment (burned or unburned) and sampling period, to construct a total of 9 pairs of networks.

Sampling completeness

Detecting 90% of species and/or interactions comprising a network has previously been proposed as a balance between obtaining a representative sample of the network, and the diminishing returns of increasing sampling effort (Chao et a. 2009). For each of our networks we estimated sampling completeness of species and interactions. Sampling completeness of moth and flower species was calculated for each network as (100 × observed richness) ÷ (estimated richness), where the estimated species richness was calculated using the Chao2 estimator (Chao 1987). Sampling completeness of interactions was calculated following Macgregor et al. (2017b), using SCW2 and the Chao2 estimator. Interaction sampling completeness was estimated for each observed moth species as (100 × observed interactions) ÷ (estimated interactions), where the estimated interaction richness was calculated using Chao2, and the mean of all species' interaction sampling completeness was taken, weighted by each species' estimated interaction richness.

Pollen-transport networks

We constructed 9 pairs of bipartite pollen-transport networks using the pooled data from each sampling period and treatment, and calculated weighted descriptive metrics for analysis. We created quantitative, interaction frequency-weighted pollen-transport networks, weighting each interaction by the number of individual moths of a species carrying pollen of a plant species, because interaction frequency predicts the relative strength of pollination interactions well (Vázquez et al. 2005). Specifically, to test the effects of burning on network complexity and consumer-resource asymmetry, we analysed: linkage density (a measure of network complexity), generality of plants and of moths (measures of consumer-resource asymmetry; sometimes termed 'vulnerability' and 'generality' respectively), and niche overlap (a measure of the degree to which species share interaction partners). Additionally, we compared the 'robustness' (tolerance to species extinctions (Burgos et al. 2007)) of burned and unburned networks by simulating the random loss of moth species (taking the mean robustness across 1000 bootstrapped simulations). For comparison, we repeated these analyses with quantitative, pollen load-weighted pollen-transport networks, weighting interactions by the total number of pollen grains of a plant species carried by all individual moths of a species.

Statistical testing

We used generalised linear models (GLMs) and generalised linear mixed-effects models (GLMMs) to test the effects of burning, season, sampling period and their two-way interactions. We tested for effects on abundance and estimated species richness (using Chao2) of moths and flowers between samples, separately retesting the effects of fire on floral abundance and richness of annual and biennial plants only (henceforth 'annuals') and all other plant species (perennials, bulbs, shrubs and trees; henceforth 'perennials'). Additionally, we tested for differences in community composition of moths and flowers at family-level, and moths, flowers and interactions at species-level, between burned and unburned sites, using Bray-Curtis dissimilarities tested by permutational multivariate analysis of variance.

To investigate effects on pollen-transport, we first tested for effects on the proportion of moths carrying pollen. Using individual, pollen-carrying moths as replicates, we tested for effects on pollen count and species richness. We then pooled the pollen loads of all moths within each sample, and tested for effects at sample-level on the total quantity and species richness of pollen being transported by the entire moth assemblage. We examined the relative abundance of species recorded on floral transects and in winter, when a single plant species (*Ulex argenteus* Webb) dominated the assemblage, we separately retested the effects of burning on floral abundance, proportion of moths carrying pollen, and pollen count at individual- and sample-levels, both for *U. argenteus* alone and for all other plant species combined.

Finally, we tested for effects on the five network metrics described above. We used treatment and season in all models as fixed effects; an interaction term between the two was initially included, but if found to be non-significant, was removed and the model retested with the two variables included separately. For analyses with multiple replicates per sampling period (i.e. when replicates were individual moths (n = 3406), pollen-carrying moths (n = 2934), samples of moths (n = 73), or quadrats on floral transects (n = 1260), but not when replicates were networks (n = 18)), we also included sampling period as a fixed effect, and tested its two-way interactions with both treatment and season as above. To account for spatio-temporal autocorrelation, we included site as a random effect in all analyses with multiple replicated per sampling period, but no random effects were included when networks were replicates. For dependent variables, we selected between Poisson and log-transformed Gaussian error distributions on a case-by-case basis (selecting the best-fitting model by visual inspection of model residual plots). The exceptions to these were the proportion of moths carrying pollen, for which we used a binomial error distribution, and the five network metrics, for which we used untransformed Gaussian error distributions. Significance of fixed effects was tested in GLMs using F-tests and GLMMs using Likelihood Ratio Tests; consequently, where interaction terms were significant and retained, we present χ^2 and P-values for the interaction term only (not independently for its constituent variables).

Moths might have cross-contaminated each other with pollen whilst in moth-traps, so we repeated all relevant main analyses using only the individual-level pollen-transport interactions where ≥ 5 pollen grains of a plant species were sampled from a single moth. This approach has been used previously in similar studies (Banza et al. 2015; Devoto et al. 2011) to provide a conservative estimate of true flower-visitor interactions, and is likely to be sufficient to exclude all such contamination (Del Socorro & Gregg 2001), but might also lead to exclusion of some functional pollination interactions.

To test the effect of burning on species' degree (number of links formed per species), we also aggregated data from all sampling periods to form a single network for each treatment (n = 1 pair) and for each combination of treatment and season (n = 4 pairs). We tested the effect of burning on the frequency distribution of degree of each network for both moths and plants overall and in each season, using one-tailed Kolmogorov-Smirnov tests, with the null hypothesis that degree distribution was not higher for unburned sites than burned sites.

Interaction turnover

We examined the causes of spatial interaction turnover between burned and unburned networks within pairs. Interaction turnover can be driven by change in species presence (of plants, moths, or both), or change in interactions despite universal presence of both partners (interaction rewiring). All scenarios are plausible outcomes of burning, so we calculated the β-diversity of the pair of networks for each of the 9 sampling periods attributable to, respectively, change in moth and/or plant species presence, and network rewiring, following Kemp et al. (2017). This was the number of interactions present in one network but absent from the other for each reason, as a fraction of the total number of unique interactions across both networks. We also calculated the total Jaccard β-diversity of each pair of networks, which is the total number of interactions present in only one network divided by the total number of unique interactions, and was therefore equal to the sum of the β-diversity attributable to each cause of turnover. We inspected these results for seasonal trends in the causes of interaction turnover between burned and unburned networks.

Results

Overview

A total of 3406 moths of 327 morphotypes, representing at least 311 species in 31 families (Table S3), were caught in light-traps. Of these, 2394 individuals (70.3%), of 297 morphotypes (90.8%) representing at least 282 species of 31 families, carried pollen of 66 morphotypes. Of 70 plant species (representing 28 families; Table S4) identified on floral transects, at least 58 (82.9%) were also identified as pollen carried by moths. Applying a conservative threshold to remove potential cross-contamination of pollen within light-traps, the number of moths carrying at least 5 pollen grains of a given plant species was only 950 (27.9%) of 186 morphotypes (56.9%). 52 pollen morphotypes were found in quantities of at least 5 pollen grains on an individual moth.

Abundance, richness and composition

We found that burning and season had significant, interacting effects on the abundance of both moths (Table S5; $\chi^2 = 36.24$, $P < 0.001$) and of flowers ($\chi^2 = 34.81$, $P < 0.001$). There was no interaction between the effects of burning and season on estimated species richness of either moths or flowers, but estimated species richness of moths was significantly affected by both burning ($\chi^2 = 9.39$, $P = 0.002$) and season ($\chi^2 = 41.71$, $P < 0.001$), whilst estimated species richness of flowers was significantly affected by season ($\chi^2 = 17.96$, $P < 0.001$) but not by burning ($\chi^2 = 1.88$, $P = 0.170$). Specifically, moths were more abundant and species-rich in unburned sites, and peaked in abundance in summer (Fig. 1). Flowers peaked in abundance and richness in spring, but were less abundant in unburned sites in winter (Fig. 1): a pattern driven primarily by annual flowers, whereas perennial flowers had reduced abundance at burned sites (Fig. S3). Both burning and season significantly altered community composition at family level of both moths and flowers (Table S6), whilst at species level, community composition of moths, flowers and interactions was significantly altered by burning but not by season (Fig. S4).

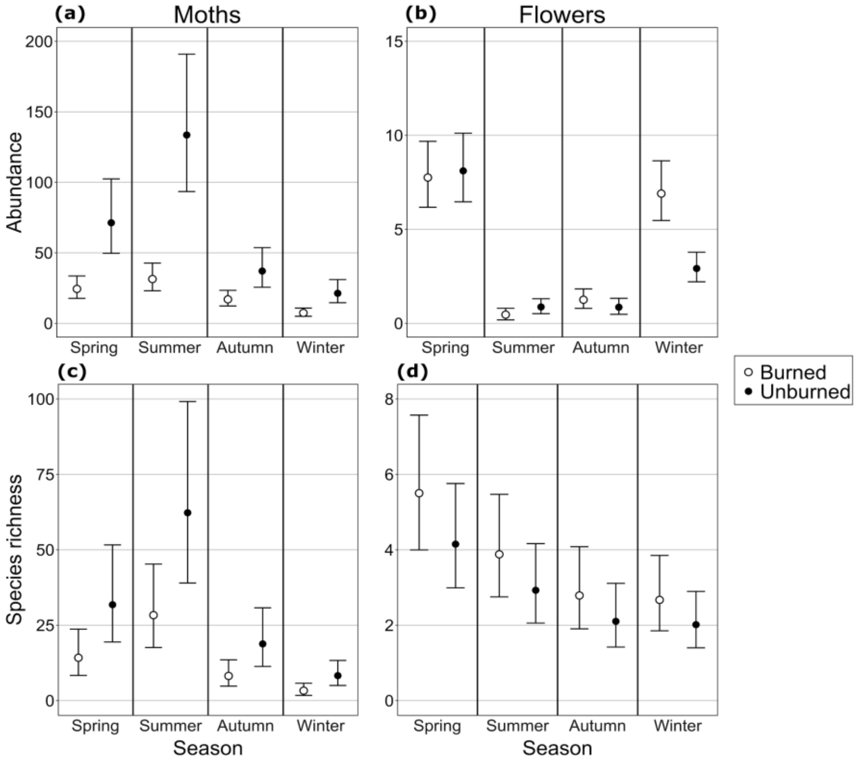

Figure 1. The effects of fire and season on the abundance and estimated species richness of moths and flowers at burned sites (open circles) and unburned sites (closed circles). For moths, circles represent the model-predicted abundance and species richness per trap; for plants in flower, circles represent the model-predicted percentage cover and species richness per transect. Error bars show 95% confidence intervals. Species richness was estimated using the Chao2 incidence-based estimator. Analyses of moth abundance and species richness were based on moth-trap samples (n = 73); analyses of floral abundance and species richness were based on 1 x 1 m quadrats (n = 1260).

Pollen-transport

Burning and season had significant, interacting effects on four pollen-transport metrics (Table S7): the proportion of moths carrying pollen (χ^2 = 33.21, $P < 0.001$), the total pollen load (χ^2 = 8.84, $P = 0.032$) and number of pollen types (χ^2 = 11.17, $P = 0.011$) per individual pollen-carrying moth, and the number of pollen types per sample of moths (χ^2 = 9.65, $P = 0.022$). The total pollen count per sample of moths was also affected by both burning (χ^2 = 11.82, $P < 0.001$) and season (χ^2 = 44.28, $P < 0.001$), but without interaction. Specifically, moths were most likely to carry pollen in spring, when over 95% of moths carried pollen at burned and unburned sites alike (Fig. 2).

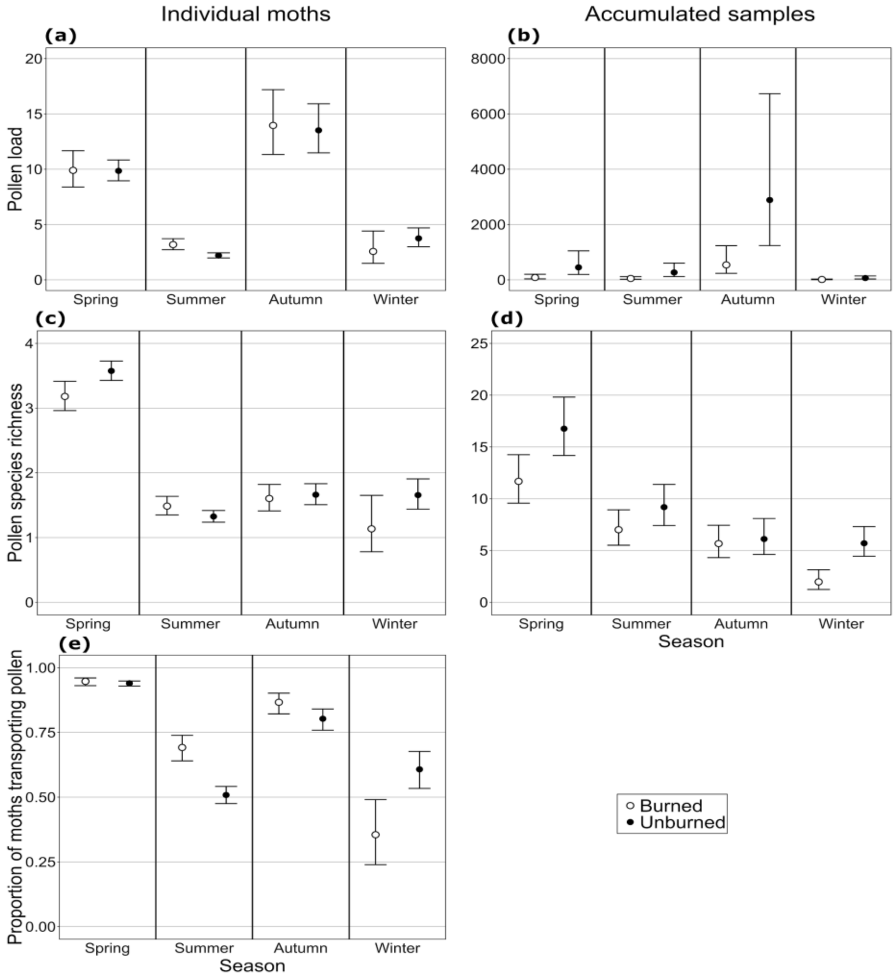

Figure 2. The effects of fire and season on the pollen loads of moths. Circles represent the model-predicted pollen load **(**a) and species richness (c) of pollen of individual moths, the cumulative pollen load (b) and richness (d) of all moths in a sample, and (e) the model-predicted proportion of moths found to be carrying pollen (open = burned sites, closed = unburned sites). Error bars show 95% confidence intervals. Analyses of the pollen loads of individual moths were based on pollen-carrying moths (n = 2394), analyses of accumulated samples of pollen were based on moth-trap samples (n = 73), and analysis of the proportion of moths carrying pollen was based on all individual moths (n = 3406).

However, individual moths were more likely to carry pollen, and had larger and more species-rich pollen loads, in burned sites than unburned sites during summer, and *vice versa* during winter (Fig. 2). In winter, moths were less likely to carry pollen of the dominant flower species, *Ulex argenteus*, at burned sites, but equally likely to carry pollen from other species; the abundance of *U. argenteus* was significantly reduced at burned sites whereas other flowers were more abundant (Fig. S5). The total quantity and species richness of pollen transported by the moth assemblage was lower at burned sites than unburned sites in all seasons, except that species richness did not differ between treatments in autumn (Fig. 2). Repeating these analyses with only interactions consisting of ≥ 5 pollen grains did not qualitatively change our findings (Table S7), except that there was no significant effect of burning on the species richness of individual moths' pollen loads.

Network analysis

We found that linkage density of pollen-transport networks was significantly affected by both burning ($\chi^2 = 4.77$, $P = 0.049$) and season ($\chi^2 = 6.83$, $P = 0.006$), without interaction. Linkage density was lower in burned networks across all seasons, and lower in autumn and winter than spring and summer (Fig. 3). Likewise, network robustness was significantly affected by both burning ($\chi^2 = 5.04$, $P = 0.044$) and season ($\chi^2 = 4.69$, $P = 0.022$), being lower in burned networks and in winter (Fig. 3). Generality (mean links per species) both of moths and of plants was significantly affected by season (plants: $\chi^2 = 7.10$, $P = 0.005$; moths: $\chi^2 = 13.13$, $P < 0.001$) but not by burning (plants: $\chi^2 = 4.10$, $P = 0.066$; moths: $\chi^2 = 0.97$, $P = 0.344$). Generality of plants was highest in summer, and of moths in spring (Fig. 3). Niche overlap was not affected by either variable (burning: $\chi^2 = 0.87$, $P = 0.370$; season: $\chi^2 = 2.44$, $P = 0.813$). Results were qualitatively similar when we weighted pollen-transport networks by pollen load, except linkage density was not significantly affected by burning (Table S8). Likewise, repeating analyses with only interactions consisting of ≥ 5 pollen grains, we found the same directional trends as described above (Table S9), but reductions in linkage density and robustness at burned sites were no longer significant. This is most likely because these networks contained many fewer interactions, increasing the error margins around metrics.

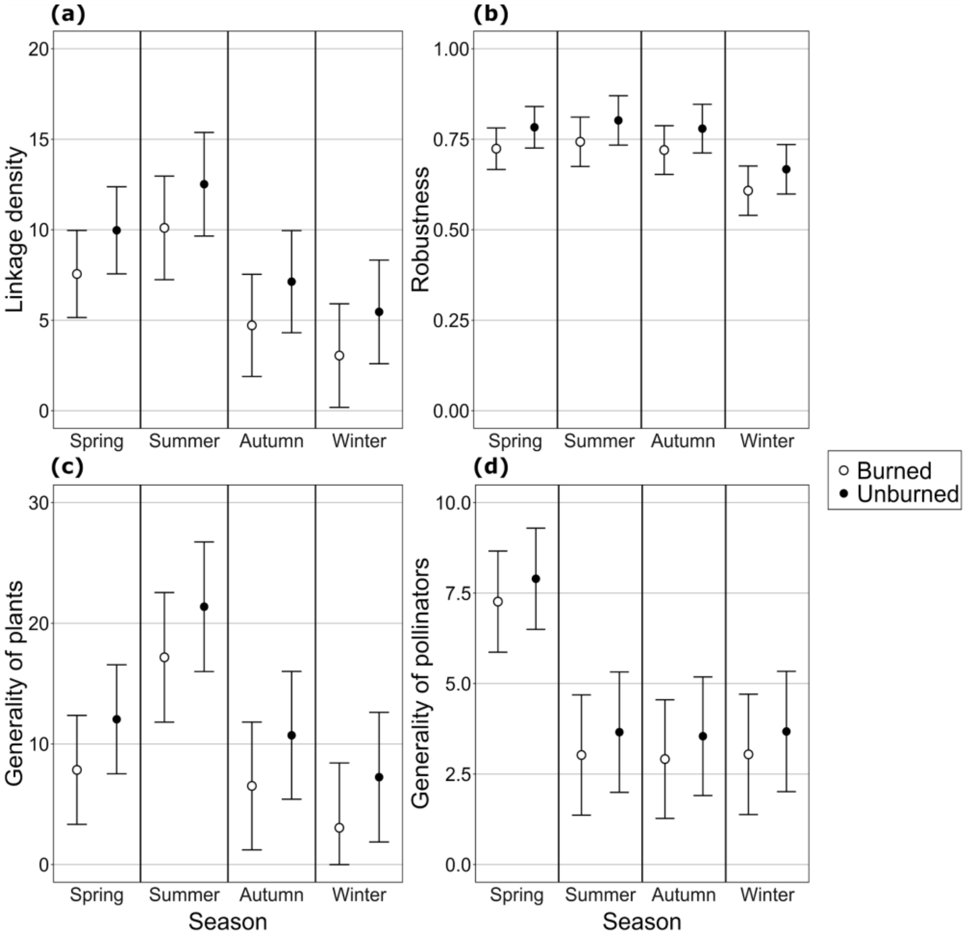

Figure 3. The effects of fire and season on a selection of network metrics (linkage density, robustness, generality of plants and generality of moths) calculated for quantitative, interaction frequency-weighted, pollen-transport networks. Points represent the model-predicted network metrics and error bars show 95% confidence intervals. Analyses were based on one burned network and one unburned network for each sampling period in the study (n = 18).

The frequency distribution of degree (no. links per species) was significantly lower at burned sites than unburned sites for both moths and plants (Fig. S6), indicating that species formed fewer interactions at burned sites. Testing seasons separately, degree distribution was significantly lower in burned networks for moths in winter only, and for plants in winter and spring.

Longevity of effects of fire

Overall, across almost all community and network metrics, we found no significant interaction between burning and sampling period, once season was taken into account (Tables S5-S10). This indicates that temporal trends over the duration of our study did not differ between burned and unburned sites.

Interaction turnover

In all sampling periods there was high spatial turnover of interactions between burned and unburned networks, indicating that few interactions were present in both (Fig. 4). From spring to autumn, the principal cause of this turnover was change in the moth species present in the network; however, in winter, there was comparatively high turnover attributable to change in both moths and flowers, indicating that winter-time interactions at burned and unburned sites involved very different assemblages of both flowers and moths.

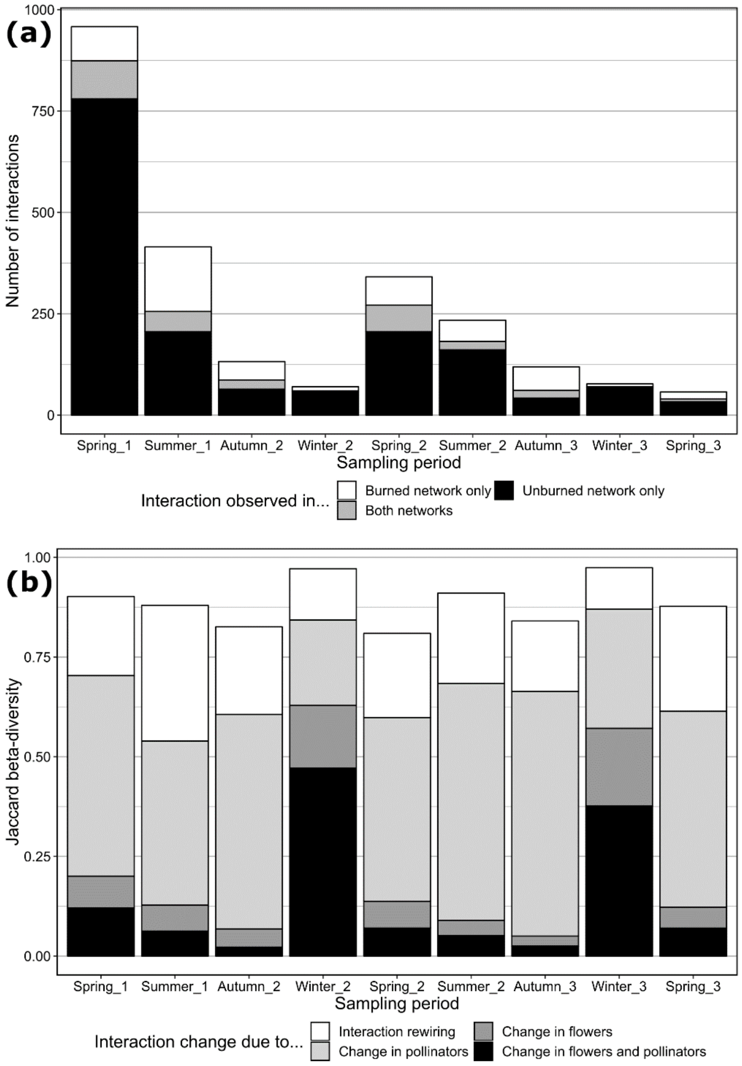

Figure 4. The quantity and causes of spatial interaction turnover between burned and unburned networks. In (a), bars show the total number of unique interactions observed in each sampling period, and coloured sections show the proportion of those interactions observed in the burned or unburned network only or in both networks. In (b), bars show the total Jaccard β-diversity value for spatial turnover of interactions in each sampling period, and coloured sections show the proportion of interaction turnover caused by change in flowers, moths or both, or by interaction turnover (Table S10).

Sampling completeness

On average, the sampling of our 18 networks was substantially less complete than the ideal threshold of 90% (Fig. S7), especially for moths (mean sampling completeness 48.3%), with plants (75.0%) and interactions (73.5%) being slightly better-sampled. Nevertheless, sampling completeness did not differ significantly between burned and unburned networks for moths (t = 1.93, d.f. = 13.17, $P = 0.076$), plants (t = 1.48, d.f. = 15.29, $P = 0.158$) or interactions (t = 0.52, d.f. = 14.20, $P = 0.613$), suggesting that any conclusions drawn from our comparisons between burned and unburned sites are robust.

Discussion

We show the disruptive effects of wildfire on moth communities and nocturnal pollen-transport networks, contrasting with positive effects of fire reported in some diurnal plant-pollinator systems (Capitanio & Carcaillet 2008; Potts et al. 2003). It may therefore be important to merge diurnal and nocturnal networks to gain an unbiased understanding of the effects of environmental change on pollination systems. After burning, nocturnal pollen-transport networks were less robust to perturbation and comprised a substantially-changed set of interactions. Moths provided abundant pollen-transport, with 70% of individuals carrying pollen, but the total effect of burning on pollen-transport was negative in all seasons, in spite of increased floral abundance after burning, because moths were less abundant and speciose at burned sites. These negative impacts could permeate to other taxa, but building resilience into ecosystems, especially those under managed burning, might be facilitated by understanding relationships between fire history and plant-pollinator network properties (Brown et al. 2017).

Fire as a driver of environmental change

Previous studies of the effects of fire on Mediterranean plant communities (Capitanio & Carcaillet 2008) and diurnal pollinators (Potts et al. 2003; Van Nuland et al. 2013) reported a flush of secondary succession, consistent with the increase in winter floral abundance at our burned sites. In fire-prone systems, some native plants may be stimulated to germinate by fire (Herranz et al. 1998) or assisted by increased light levels associated with reduced shrub cover at burned sites.

The negative effects of wildfire on moth populations over a period of 1-3 years after burning, with no detectable return to pre-fire states, can be interpreted in the light of demonstrated negative impacts of wildfire on moths (Fowles et al. 2004; Gerson & Kelsey 1997; Schmid et al. 1981). Whilst most abundant bee species are generalist flower-visitors and could capitalise on increased general availability of pollen and nectar resources in burned areas (Potts et al. 2003), many Lepidoptera are specialists as larvae (Bernays & Chapman 1994), and may be unable to breed in burned areas if host plants are destroyed by fire. We found that the moth community changed significantly at burned sites, indicating that the severity of the effects of fire may vary between different moths. Further research might reveal whether this variation is linked to life-history or functional traits in moths, or more directly to changes in the availability of each species' larval host plants. Whether ecological succession would, over a longer timescale, cause the burned sites to converge on the state of the unburned sites, or whether they would instead reach an alternative stable state, remains to be seen.

However, the long-term role of wildfires in driving moth population declines remains unclear. Wildfires are mostly of low importance in countries where moth declines have been most convincingly shown, e.g. in the UK (Conrad et al. 2006), but play a substantial role in shaping ecosystems in other regions (Flannigan et al. 2013; Kelly & Brotons 2017). Evaluating trends in moth populations in such regions at a large spatio-temporal scale would therefore be valuable. Potential interactions between wildfire and other drivers of environmental change also warrant further attention. Climate change and agricultural abandonment may be especially important since both drivers are of known importance to Lepidoptera (Parmesan et al. 1999; Uchida & Ushimaru 2014) and play a role in increasing fire frequency (Flannigan et al. 2013; Price & Rind 1994; Pausas & Fernández-Muñoz 2011), which might reduce the long-term ability of communities to recover (Oliver et al. 2015).

Finally, it should be noted that our results pertain to the effects of a single wildfire, due to the logistical challenges that would be posed by sampling after multiple fires. All burned sites were burned at the same time, by the same fire, and burned and unburned sites were spatially more clustered within treatments than between treatments. Therefore, further study of the effects of other wildfires, covering a wider range of conditions than was feasible in this study (e.g. fires on different continents, in different ecosystems and habitat types, of different sizes and intensities, with burning occurring at different times of year, in association with different weather conditions, and so forth), might unveil even greater complexity in the responses of moth and plant communities.

Moths as pollinators

Our findings add to the evidence that moths are previously undervalued providers of pollen-transport (Macgregor et al. 2019a; Macgregor et al. 2015); perhaps especially in Mediterranean systems (Banza et al. 2015), where we detected the highest proportion of moths carrying pollen in any study to date. The pollen of some 83% of locally-flowering plants was carried by moths. An important future research question is the functional importance of moths as pollinators of the plant species whose pollen they transport.

Pollen-transport by individual moths was increased at burned sites in summer, but reduced in winter, despite the increase in floral abundance and richness. In winter, moths mainly transported pollen of *Ulex argenteus* at unburned sites, but rarely did so at burned sites (Fig. S5). Potentially, more moths may have visited *U. argenteus* at unburned sites in search of nectar (Stokes et al. 2003) because there were fewer alternative floral resources (Fig. 1). Moths were less abundant at burned sites in summer but floral abundance was unchanged, potentially increasing the likelihood of pollen removal by making each moth more likely to be among the first visitors to any given flower (Young & Stanton 1990). Variation in diurnal visitation rates between burned and unburned sites could also have influenced pollen availability in all seasons. Finally, changes in community composition at burned sites could have made certain species with important roles in pollen-transport relatively more or less abundant.

When the pollen loads of all moths in a sample were aggregated, the overall effect of burning was a consistent reduction in nocturnal pollen-transport across all seasons. This reflected previous studies of other pollinator taxa, where flower-visitation was reduced after fire (Ne'eman et al. 2000), even for plant species that respond to fire by flowering (Geerts et al. 2011).

Networks

Ecological network approaches have considerable potential to help understand the effects of fire on the risk of cascading extinctions due to loss of mutualisms (Brown et al. 2017). We find significant structural differences between networks at burned and unburned sites. Reduced robustness at burned sites indicates that wildfire leads to nocturnal pollen-transport systems that are less tolerant of further perturbation, and at greater risk of cascading extinctions. There was high interaction turnover between networks at burned and unburned sites, driven by change in moth species presence (in all seasons) and plant species presence (in winter). The interactions comprising networks can vary spatio-temporally with little associated change in network structure (Kemp et al. 2017; Olesen et al. 2008); turnover is often demonstrated within seasons or over consecutive years. By gathering year-round data, we showed that the direction and significance of the effects of wildfire changed seasonally. Future ecological network studies could therefore run across seasons to avoid over-simplified conclusions.

Conclusions

Improving the understanding of the functional importance of nocturnal pollinators, especially in Mediterranean systems where very large proportions of moths carry pollen, is important. The effects of drivers of environmental change on nocturnal plant-pollinator networks have generally not been investigated (but see Knop et al. 2017). Given that our results contrasted with the positive effects of wildfire reported in some diurnal plant-pollinator systems, it is unsafe to assume that the effects of drivers of change on nocturnal pollination networks will be the same as their known effects on diurnal systems.

The negative impacts of wildfire on moth abundance and pollen-transport were likely driven by direct mortality of immature life stages and reduction in availability of larval resources. However, future mechanistic studies are required to understand the relative importance of these mechanisms at population- and community-level, and the impacts on co-evolutionary dynamics. Further study, over time as the burned ecosystem regenerates and across multiple fires at the same sites, could establish the influence of repeated pulse perturbations on ecosystem recovery, improving our understanding of the resilience of fire-prone systems and the potential importance of increasingly frequent fires under climate change. A deeper understanding of the responses of ecological networks to wildfire may facilitate whole-ecosystem conservation (Tylianakis et al. 2010) and restoration (Raimundo et al. 2018), allowing resilience to be built into fire-prone ecosystems (Evans et al. 2016).

CHAPTER 5

General Discussion

General Discussion

We studied the recovery of plant-pollinator interaction networks after a fire disturbance using diurnal and nocturnal insects. Our findings add to the evidence that moths were previously undervalued providers of pollen transport (Macgregor et al. 2015, 2019), especially in Mediterranean ecosystems, where we detected the highest proportion of moths carrying pollen in any study to date (Banza et al., 2015). Moths provided abundant pollen transport, with 70% of individuals carrying pollen. Furthermore, moth communities were significantly different at burned sites. Moths were less abundant and less species-rich, indicating that the severity of the effects of fire may vary between different moths. Because moths were less abundant and less species richness, the total effect of burning on pollen transport was negative in all seasons, in spite of increased floral abundance after burning. When the pollen loads of all moths in a sample were aggregated, the overall effect of burning was a consistent reduction in nocturnal pollen transport across all seasons. This reflected previous studies of other pollinator taxa, where flower visitation was reduced after fire (Ne'eman et al. 2000), even for plant species that respond to fire by flowering (Geerts et al. 2011) (Chapter 4).

There were significant structural differences between networks at burned and unburned sites. Reduced robustness at burned sites indicates that wildfire leads to nocturnal pollen-transport systems that are less tolerant of further perturbation, and at greater risk of cascading extinctions. There was high interaction turnover between networks at burned and unburned sites, driven by change in moth species presence (in all seasons) and plant species presence (in winter). The interactions comprising networks, can vary spatio-temporally with little associated change in network structure (Kemp et al., 2017; Olesen et al. 2008); turnover is often demonstrated within seasons or over consecutive years. By gathering year-round data, we showed that the direction and significance of the effects of wildfire changed seasonally (Chapter 4).

We found evidence that wildfire had significant interacting effects on the abundance of diurnal insects but not on species richness. Pollen loads and species richness on individual insects were significantly higher in burned sites in the first spring only. There was a general tendency for both pollen load and insect species richness to increase during the study. However, there was no significant effect of burning on the diurnal insect community composition at the family level. Wildfire disrupts pollen transport by diurnal insects, at least in the short term. Overall, across most of the community metrics examined, our results suggest that diurnal insects, and the pollen they transported, returned to the pre-fire state within a relatively short period. Our study demonstrated that even a small sample of diurnal flower-visiting insects can carry a large amount of pollen and that overall pollen-transport can be disturbed by wildfire (Chapter 3).

Within burned plots of the study area, there was evidence of secondary succession with an initial flush of flowering plants, like in other studies of Mediterranean plant community post-fire recovery (Capitanio & Carcaillet 2008) and accompanying diurnal pollinators (Potts et al. 2003; Van Nuland et al. 2013). We found an increase in winter floral abundance, primarily by annual flowers, whereas perennial flowers had reduced abundance at the burned sites. This is likely due to the fact that some plants may be stimulated by fire (Herranz et al. 1998) or because of higher light levels associated with reduced shrub cover at burned sites (Chapters 3 and 4).

We stated the importance of moths as pollinators, particularly in a scenario of declining of insect pollinator populations. We described the main threats to moths, namely land use change, climate change, artificial light at night and wildfires and the consequences of these for natural ecosystems and how they affect moth ecology (life cycle, movements and pollination). Finally, we address some mitigation and conservation measures and identify the main questions for moth conservation in the Mediterranean context (Chapter 2).

Future research

Our work highlights a number of directions for future research on post-fire pollination function. Firstly, pollen transport does not necessarily translate to successful pollination in all cases (King et al. 2013), that is, the effects of the changes in pollinator abundance and pollen transport that we observed do not necessarily reflect the actual reproductive success and productivity of plants in burned and unburned areas. Our work was mostly about pollen transport, but future work should test how that translates into real plant-pollinator interactions.

Secondly, recent studies have directly compared diurnal and nocturnal pollination (e.g. Knop et al. 2017; Walton et al. 2020), whereas we analysed data on diurnal pollination (Banza et al. 2020 *submitted*) and nocturnal pollination (Banza et al. 2019) separately, due to different sampling methodologies. Future work merging diurnal and nocturnal pollination could be valuable to understand overall impacts of wildfire on pollination systems, since the individual effects on the different pollinator guilds were very different. Such studies might be facilitated by using standardized methods across diurnal and nocturnal surveys, namely by conducting nocturnal transects instead of light-trapping (e.g. Macgregor et al. 2017). Future methods using DNA metabarcoding for studying plant-insect interactions provides promise (Evans et al. 2016). Putting these in more advanced networks (multilayer networks) will help us better understand biodiversity and ecosystem functioning.

Thirdly, our findings are likely to be mainly a reflection of changing foraging habits of adult insects and variation in the availability of floral resources, or because of direct mortality of immature life stages, responding to the reduction of larval resources, particularly in the case of moths. Understanding the impacts of fires on insects at population and community levels, including co-evolutionary dynamics, may require study of the immediate impacts on all stages of the insect life-cycle. The lack of data, generally, in the Mediterranean region makes long term trend studies very difficult and there is a need to co-ordinate systematic biomonitoring.

Finally, further study over time as the burned ecosystem regenerates, and across multiple fires at the same sites, could establish the influence of repeated perturbations on ecosystem recovery, improving our understanding of resilience of fire-prone systems and the potential importance of increasingly frequent fires under global change.

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Appendix 1 - Supporting Information associated with the manuscript:

Wildfire disrupts pollen transport by diurnal insects in Southern Portugal

Table S1 Locations of the six study sites. Latitude, longitude and altitude are given for the centre of each 40 x 40 m study plot. Latitude and longitude are given in decimal degrees to 5 decimal places, and therefore are accurate to within approximately 1 m. Aspect and slope were calculated from Intermap NEXTMap 5 data, accessed through Strava.

Site	Treatment	Latitude (°)	Longitude (°)	Altitude (m asl)	Aspect	Slope (%)
F1	Burned	37.17713	-7.86082	261	E	20
F2	Burned	37.19641	-7.86006	372	WNW	30
F3	Burned	37.19848	-7.85699	449	W	22.5
NF1	Unburned	37.16919	-7.86516	340	NE	14
NF2	Unburned	37.17334	-7.86506	317	W	12
NF3	Unburned	37.18149	-7.86588	245	NW	10

Table S2 Summary of the plant families identified on floristic surveys, with the number of species and the total number of 1 x 1 m quadrats in which members of each family was recorded. *Apiaceae* includes one flower type not identified beyond family level, and therefore was represented by *at least* four species.

Family	No. species	No. quadrats
Adoxaceae	1	4
Amaryllidaceae	2	17
Apiaceae	4*	29
Asparagaceae	2	3
Asteraceae	14	379
Boraginaceae	2	39
Brassicaceae	1	3
Campanulaceae	2	25
Caprifoliaceae	2	16
Caryophyllaceae	2	8
Cistaceae	4	167
Ericaceae	3	26
Euphorbiaceae	1	4
Fabaceae	10	285
Gentianaceae	1	36
Geraniaceae	1	2
Iridaceae	2	11
Lamiaceae	3	200
Linaceae	1	1
Orchidaceae	1	1
Papaveraceae	1	2
Plantaginaceae	1	30
Primulaceae	1	24
Ranunculaceae	2	30
Resedaceae	2	23
Rosaceae	2	12
Solanaceae	1	39
Thymelaeaceae	1	8

Table S3 R packages used during analysis. Packages were loaded into at least one script during the analytical process but may not have formed part of the final analysis.

Package	Citation
AICcmodavg	Mazerolle, M.J. (2016) AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 2.1-0. https://cran.r-project.org/package=AICcmodavg.
arm	Gelman, A. & and Su, Y.-S. (2015) arm: Data Analysis Using Regression and Multilevel/Hierarchical Models. R package version 1.8-6. https://CRAN.R-project.org/package=arm.
bipartite	Dormann, C.F., Gruber B. & Fruend, J. (2008) Introducing the bipartite Package: Analysing Ecological Networks. *R News*, **8**, 8–11.
car	Fox, J. & Weisberg, S. (2011) *An {R} Companion to Applied Regression.* Second Edition. Sage, Thousand Oaks, CA, USA.
data.table	Dowle, M., Srinivasan, A., Short, T., Lianoglou, S., Saporta, R. & Antonyan, E. (2015) data.table: Extension of Data.frame. R package version 1.9.6. https://CRAN.R-project.org/package=data.table.
effects	Fox, J. (2003) Effect Displays in R for Generalised Linear Models. *Journal of Statistical Software*, **8**, 1–27.
ggmap	Kahle, D. & Wickham, H. (2013) ggmap: Spatial Visualization with ggplot2. *The R Journal*, **5**, 144–161.
ggplot2	Wickham, H. (2009) *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag, New York, USA.
glmmADMB	Fournier, D.A., Skaug, H.J., Ancheta, J., Ianelli, J., Magnusson, A., Maunder, M., Nielsen, A. & Sibert, J. (2012) AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optimization Methods and Software*, **27**, 233–249.
gridExtra	Auguie, B. (2016) gridExtra: Miscellaneous Functions for "Grid" Graphics. R package version 2.2.1. https://CRAN.R-project.org/package=gridExtra.
lme4	Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, **67**, 1–48.
MASS	Venables, W. N. & Ripley, B. D. (2002) *Modern Applied Statistics with S*. Fourth Edition. Springer, New York, USA.
plyr	Wickham, H. (2011) The Split-Apply-Combine Strategy for Data Analysis. *Journal of Statistical Software*, **40**, 1–29.
RColorBrewer	Neuwirth, E. (2014) RColorBrewer: ColorBrewer Palettes. R package version 1.1-2. https://CRAN.R-project.org/package=RColorBrewer.

reshape2	Wickham, H. (2007) Reshaping Data with the reshape Package. *Journal of Statistical Software*, **21**, 1–20.
RVAideMemoire	Hervé, M. (2016) RVAideMemoire: Diverse Basic Statistical and Graphical Functions. R package version 0.9-56. https://CRAN.R-project.org/package=RVAideMemoire.
scales	Wickham, H. (2016) scales: Scale Functions for Visualization. R package version 0.4.1. https://CRAN.R-project.org/package=scales.
svglite	Wickham, H., Henry, L., Luciani, T.J., Decorde, M. & Lise, V. (2016) svglite: An ‘SVG’ Graphics Device. R package version 1.2.0. https://CRAN.R-project.org/package=svglite.
tidyr	Wickham, H. (2016) tidyr: Easily Tidy Data with `spread()` and `gather()` Functions. R package version 0.5.0. https://CRAN.R-project.org/package=tidyr.
vegan	Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H. & Wagner, H. (2016) vegan: Community Ecology Package. R package version 2.3-5. https://CRAN.R-project.org/package=vegan.

Table S4 Summary of captured insects according to Order, Family, Genus, number of identified species, number of morphotypes and the total number of individuals.

Order	Family	Genus	Identified species	No. morphotypes	No. individuals
Coleoptera				3	7
	Cerambycidae			4	8
			Nustera distigma		1
	Cetoniidae			3	10
		Protaetia		1	2
			Protaetia opaca		2
			Oxythyrea funesta		89
			Tropinota squalida		14
	Chrysomelidae			3	22
			Chrysolina americana		1
			Dicladispa testacea		1
			Lachnaia hirta		1
	Cleridae			1	1
			Trichodes octopunctatus		1
	Coccinellidae			1	4
			Coccinella septempunctata		5
	Curcurlionidae			1	3
	Dasytidae			2	17
	Elateridae			4	19
	Malachiidae			1	2
	Meloidae			4	9
	Mordellidae			1	3
	Oedemeridae			4	8
	Tenebrionidae			2	3
			Heliothaurus ruficolis		82
Diptera				1	2
	Bombyliidae			3	7
	Empididae			2	2
	Syrphidae			3	5
	Tachinidae			3	5
	Tipulidae			1	1
Hemiptera				2	2
	Acanthosomatidae			1	1
	Pentatomidae			1	1
			Graphosoma lineatum		2
Heteroptera	Miridae			1	1
Hymenoptera	Andrenidae			2	2

Order	**Family**	**Genus**	**Identified species**	**No. morphotypes**	**No. individuals**
		Andrena		3	9
		Panurgus		1	1
	Apidae			1	1
		Eucera		1	1
		Xylocopa		1	1
			Apis mellifera		79
			Bombus terrestris		2
			Bombus hortorum		2
	Crabronidae			1	1
	Halictidae			3	5
		Lasioglossum		3	4
	Colletidae	Hylaeus		1	1
	Ichneumonidae			1	1
	Megachilidae			1	1
		Rhodanthidium		1	1
	Sphecidae			1	1
	Vespidae			2	2
			Polistes dominula		4
			Vespa crabro		3
			Vespula vulgaris		5
Lepidoptera				1	1
	Erebidae		*Utetheisa pulchella*		1
	Gelechiidae		*Dichomeris lamprostoma*		1
	Geometridae		*Aspitates ochrearia*		1
			Scotopteryx peribolata		1
	Hesperiidae	Thymelicus		1	1
	Lasiocampidae		*Trichiura ilicis*		1
	Lycaenidae		*Aricia cramera*		2
			Lampides boeticus		1
			Leptotes pirithous		8
			Satyrium ilicis		2
			Plebejus argus		1
			Polyommatus icarus		2
	Noctuidae		*Autographa gamma*		1
			Synthimia fixa		1
	Nymphalidae		*Coenonympha pamphilus*		7
			Lasiommata megera		1
			Maniola jurtina		20
			Melanargia ines		5

Order	**Family**	**Genus**	**Identified species**	**No. morphotypes**	**No. individuals**
			Pararge aegeria		1
			Pyronia cecilia		2
			Pyronia tithonus		2
			Vanessa cardui		2
	Papilionidae		*Iphiclides feisthamelii*		1
			Papilio machaon		1
	Pieridae		*Colias croceus*		2
			Euchloe crameri		6
			Pieris brassicae		1
			Pieris rapae		4
			Pontia daplidice		1
	Pterophoridae		*Amblyptilia acanthadactyla*		1
	Pyralidae		*Endotricha flammealis*		1
	Tortricidae			1	1
Neuroptera				1	1
	Chrysopidae			1	1
Unidentified				10	15
Totals	**44**	**9**	**47**	**91**	**572**

Table S5 – Number of pollen grains found on insects, according to species and family, grouped by season and by treatment.

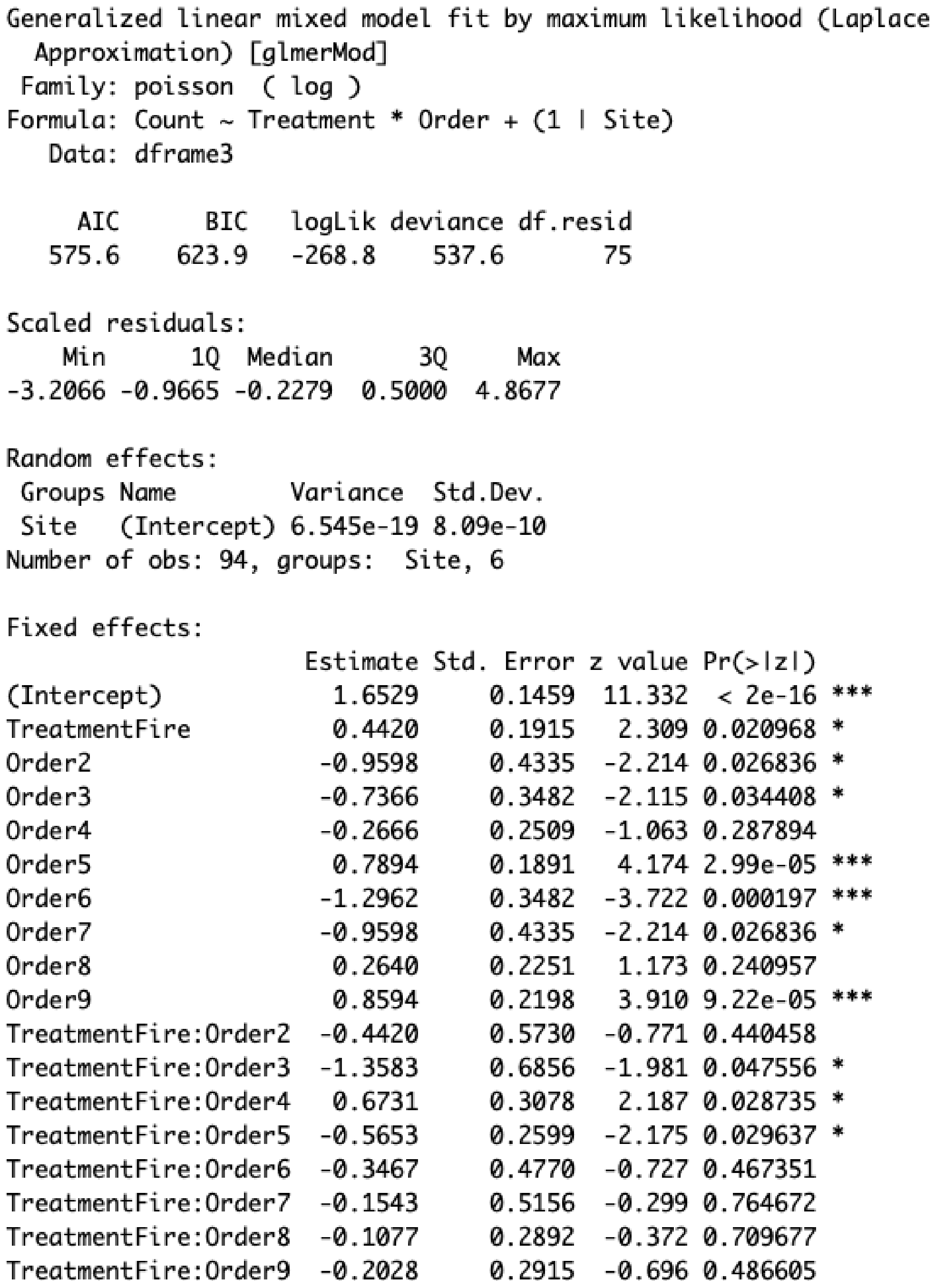
Pollen species	Family	Total Count	Spring Count	Summer count	Autumn count	Winter count	Burned count	Unburned count
Allium paniculatum	Amaryllidaceae	50	3	0	0	47	49	1
Anagallis arvensis	Primulaceae	536	434	11	12	79	407	129
Anarrhinum bellidifolium	Plantaginaceae	4746	4535	118	1	92	67	4679
Andryala integrifolia	Asteraceae	5756	4289	1	445	1021	5590	166
Anthyllis vulneraria	Fabaceae	611	529	39	12	31	224	387
Apiaceae	Apiaceae	4407	4296	29	14	68	3252	1155
Arbutus unedo	Ericaceae	81	0	1	79	1	79	2
Calluna vulgaris	Ericaceae	197	159	5	0	33	167	30
Campanula lusitanica	Campanulaceae	308	243	16	5	44	165	143
Carlina racemosa	Asteraceae	2	2	0	0	0	2	0
Centaurium erythraea	Gentianaceae	54	32	2	10	10	14	40
Centranthus calcitrapae	Valerianaceae	139	84	0	2	53	139	0
Chamaemelum mixtum	Asteraceae	1856	1676	6	1	173	49	1807
Cistus crispus	Cistaceae	4147	3982	1	36	128	279	3868
Cistus ladanifer	Cistaceae	1788	1082	3	25	678	1159	629
Cistus salviifolius	Cistaceae	7304	2319	4	28	4953	1792	5505
Cistus sp.	Cistaceae	4309	939	373	0	2997	3911	398
Coleostephus myconis	Asteraceae	15432	13592	5	1754	81	15034	398
Cynara cardunculus	Asteraceae	530	312	186	0	32	38	492
Daphne gnidium	Thymelaeaceae	200	142	12	17	29	53	147
Daucus carota	Apiaceae	377	70	233	9	65	312	65
Echium plantagineum	Boraginaceae	262	239	15	0	8	209	53
Erica arborea	Ericaceae	46	16	0	1	29	31	15
Eryngium campestre	Apiaceae	61	32	10	15	4	61	0
Euphorbia exigua	Euphorbiaceae	14	14	0	0	0	0	14
Euphorbia sp.	Euphorbiaceae	2	2	0	0	0	2	0
Galactites tomentosa	Asteraceae	3547	2717	1	6	823	3493	54
Genista triacanthos	Fabaceae	2015	467	0	768	780	2015	0
Gladiolus italicus	Iridaceae	2	0	0	0	2	2	0
Helychrysum stoechas	Asteraceae	4178	2295	360	109	1414	1790	2388
Jasione montana	Campanulaceae	2382	722	50	34	1576	2118	264
Lavandula stoechas	Lamiaceae	11574	10051	26	5	1492	2873	8701
Leontodon taraxacoides	Asteraceae	5512	3661	216	0	1635	2131	3381
Leucojum autumnale	Amaryllidaceae	134	22	5	102	5	121	13
Lithodora prostrata	Boraginaceae	4172	169	0	27	3976	4169	3
Logfia gallica	Asteraceae	32	31	0	0	1	13	19
Lotus parviflorus	Fabaceae	16	0	0	0	16	16	0
Lotus subbiflorus	Fabaceae	1073	1073	0	0	0	0	1073
Ornithogalum broteroi	Asparagaceae	265	253	5	4	3	245	20
Pulicaria odora	Asteraceae	9292	8744	263	232	53	5695	3597

Pollen species	**Family**	**Total Count**	**Spring Count**	**Summer count**	**Autumn count**	**Winter count**	**Burned count**	**Unburned count**
Pulicaria paludosa	Asteraceae	228	150	3	27	48	133	95
Ranunculus bullatus	Ranunculaceae	4	1	3	0	0	0	4
Ranunculus gramineus	Ranunculaceae	1125	633	124	0	368	660	465
Ranunculus sp.	Ranunculaceae	9	9	0	0	0	9	0
Reseda media	Resedaceae	726	615	20	62	29	647	79
Sanguisorba minor	Rosaceae	6	6	0	0	0	0	6
Sanguisorba verrucosa	Rosaceae	376	361	0	8	7	133	243
*Scilla autumnalis**	Asparagaceae	15	0	0	0	15	15	0
*Scilla monophyllos**	Asparagaceae	42	6	7	2	27	34	8
Silene gallica	Caryophyllaceae	80	60	1	7	12	42	38
Solanum nigrum	Solanaceae	252	127	12	90	23	107	145
Spergularia purpurea	Caryophyllaceae	52	15	10	7	20	42	10
Stachys arvensis	Lamiaceae	85	84	0	0	1	84	1
Thymus mastichina	Lamiaceae	194	174	0	3	17	92	102
Trifolium arvense	Fabaceae	5660	5374	8	213	65	5551	109
Tuberaria guttata	Cistaceae	26866	25960	301	3	602	34	26832
Ulex argenteus	Fabaceae	8846	1837	3041	0	3968	7425	1421
Ulex eriocladus	Fabaceae	9225	144	2	5351	3728	6534	2691
Ulex sp.	Fabaceae	17	0	0	0	17	17	0
Urginea maritima	Asparagaceae	176	163	3	6	4	170	6
Viburnum tinus	Adoxaceae	29	7	0	0	22	20	9
Total pollen count		**151422**						

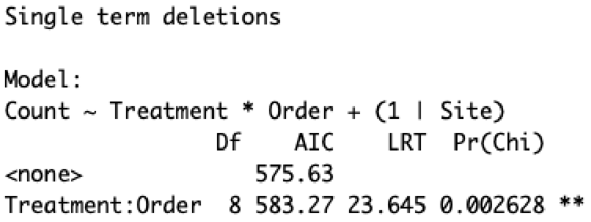
* - Species identified outside the quadrats during floristic surveys.

Table S6 R software outputs showing summary of analyses of the effects of burning and season over consecutive sampling periods on the abundance (A) and species richness (B) of insects. Intercept value represents unburned sites in autumn, sampling period 0, and is the natural logarithm of the estimate, so $e^{(intercept)}$ gives the true estimated value. For other levels of each variable, estimated value = $e^{(intercept)}$ x e^{ES}, where ES = effect size for that level from the statistical model, so e^{ES} is the multiplicative effect of the parameter in question.

A. Insect Abundance

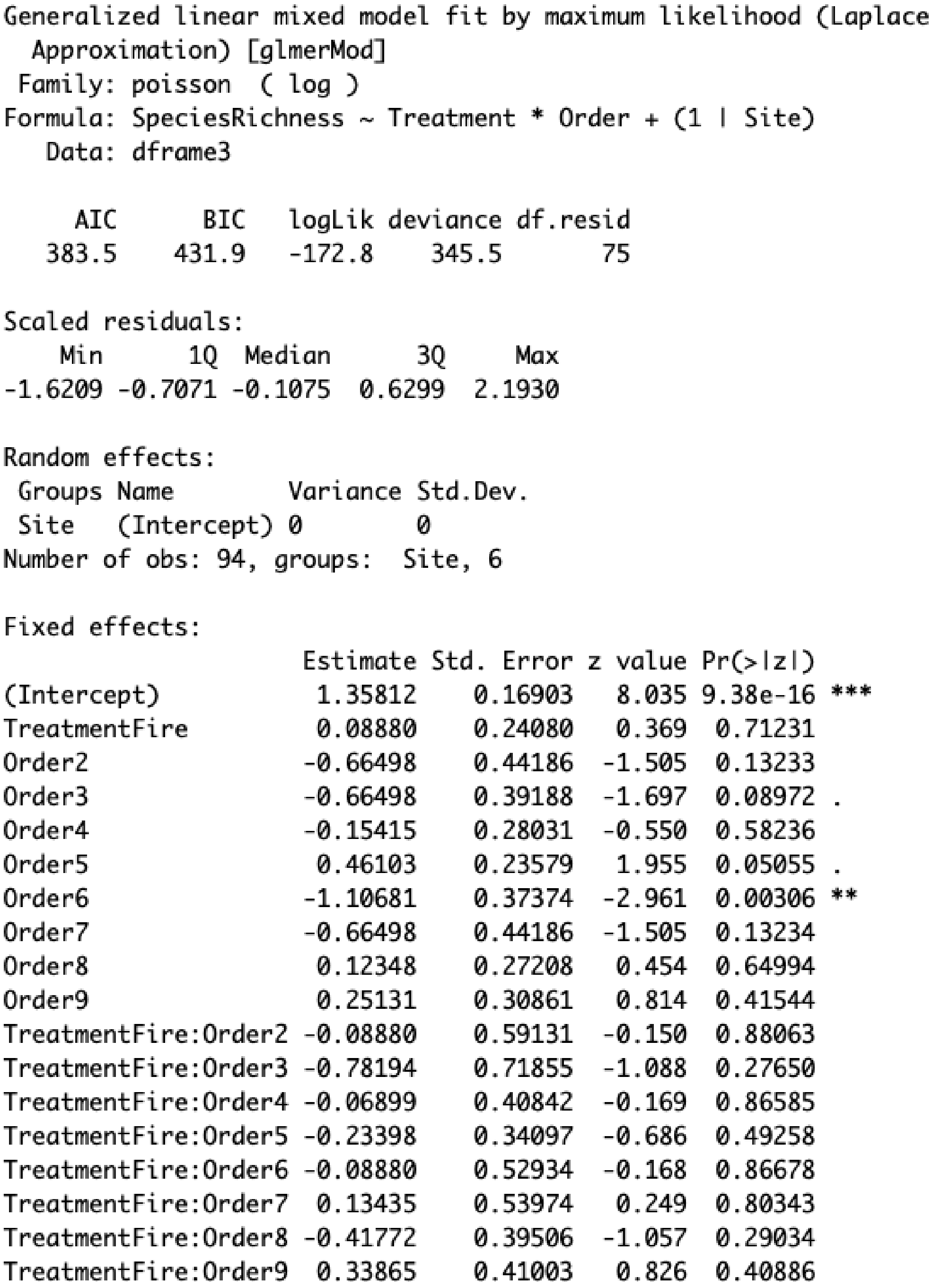


```
Generalized linear mixed model fit by maximum likelihood (Laplace
  Approximation) [glmerMod]
 Family: poisson  ( log )
Formula: Count ~ Treatment * Order + (1 | Site)
   Data: dframe3

     AIC      BIC   logLik deviance df.resid
   575.6    623.9   -268.8    537.6       75

Scaled residuals:
    Min      1Q  Median      3Q     Max
-3.2066 -0.9665 -0.2279  0.5000  4.8677

Random effects:
 Groups Name        Variance  Std.Dev.
 Site   (Intercept) 6.545e-19 8.09e-10
Number of obs: 94, groups:  Site, 6

Fixed effects:
                     Estimate Std. Error z value Pr(>|z|)
(Intercept)            1.6529     0.1459  11.332  < 2e-16 ***
TreatmentFire          0.4420     0.1915   2.309 0.020968 *
Order2                -0.9598     0.4335  -2.214 0.026836 *
Order3                -0.7366     0.3482  -2.115 0.034408 *
Order4                -0.2666     0.2509  -1.063 0.287894
Order5                 0.7894     0.1891   4.174 2.99e-05 ***
Order6                -1.2962     0.3482  -3.722 0.000197 ***
Order7                -0.9598     0.4335  -2.214 0.026836 *
Order8                 0.2640     0.2251   1.173 0.240957
Order9                 0.8594     0.2198   3.910 9.22e-05 ***
TreatmentFire:Order2  -0.4420     0.5730  -0.771 0.440458
TreatmentFire:Order3  -1.3583     0.6856  -1.981 0.047556 *
TreatmentFire:Order4   0.6731     0.3078   2.187 0.028735 *
TreatmentFire:Order5  -0.5653     0.2599  -2.175 0.029637 *
TreatmentFire:Order6  -0.3467     0.4770  -0.727 0.467351
TreatmentFire:Order7  -0.1543     0.5156  -0.299 0.764672
TreatmentFire:Order8  -0.1077     0.2892  -0.372 0.709677
TreatmentFire:Order9  -0.2028     0.2915  -0.696 0.486605
```

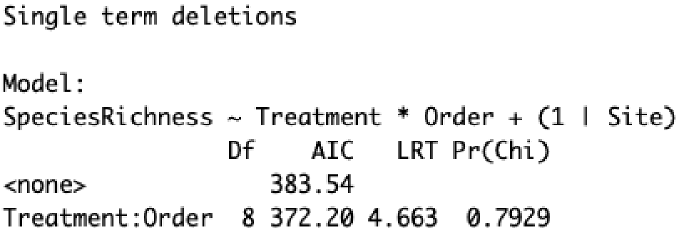


```
Single term deletions

Model:
Count ~ Treatment * Order + (1 | Site)
               Df    AIC    LRT  Pr(Chi)
<none>            575.63
Treatment:Order  8 583.27 23.645 0.002628 **
```

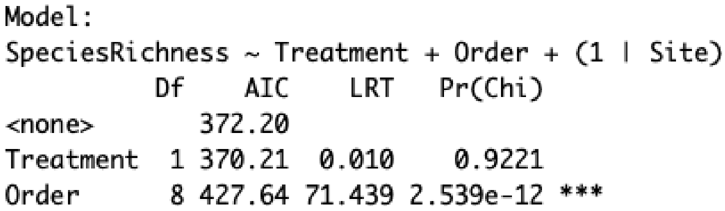
B. Insects Species Richness



```
Generalized linear mixed model fit by maximum likelihood (Laplace
  Approximation) [glmerMod]
 Family: poisson  ( log )
Formula: SpeciesRichness ~ Treatment * Order + (1 | Site)
   Data: dframe3

     AIC      BIC   logLik deviance df.resid
   383.5    431.9   -172.8    345.5       75

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.6209 -0.7071 -0.1075  0.6299  2.1930

Random effects:
 Groups Name        Variance Std.Dev.
 Site   (Intercept) 0        0
Number of obs: 94, groups:  Site, 6

Fixed effects:
                      Estimate Std. Error z value Pr(>|z|)
(Intercept)            1.35812    0.16903   8.035 9.38e-16 ***
TreatmentFire          0.08880    0.24080   0.369  0.71231
Order2                -0.66498    0.44186  -1.505  0.13233
Order3                -0.66498    0.39188  -1.697  0.08972 .
Order4                -0.15415    0.28031  -0.550  0.58236
Order5                 0.46103    0.23579   1.955  0.05055 .
Order6                -1.10681    0.37374  -2.961  0.00306 **
Order7                -0.66498    0.44186  -1.505  0.13234
Order8                 0.12348    0.27208   0.454  0.64994
Order9                 0.25131    0.30861   0.814  0.41544
TreatmentFire:Order2 -0.08880    0.59131  -0.150  0.88063
TreatmentFire:Order3 -0.78194    0.71855  -1.088  0.27650
TreatmentFire:Order4 -0.06899    0.40842  -0.169  0.86585
TreatmentFire:Order5 -0.23398    0.34097  -0.686  0.49258
TreatmentFire:Order6 -0.08880    0.52934  -0.168  0.86678
TreatmentFire:Order7  0.13435    0.53974   0.249  0.80343
TreatmentFire:Order8 -0.41772    0.39506  -1.057  0.29034
TreatmentFire:Order9  0.33865    0.41003   0.826  0.40886
```



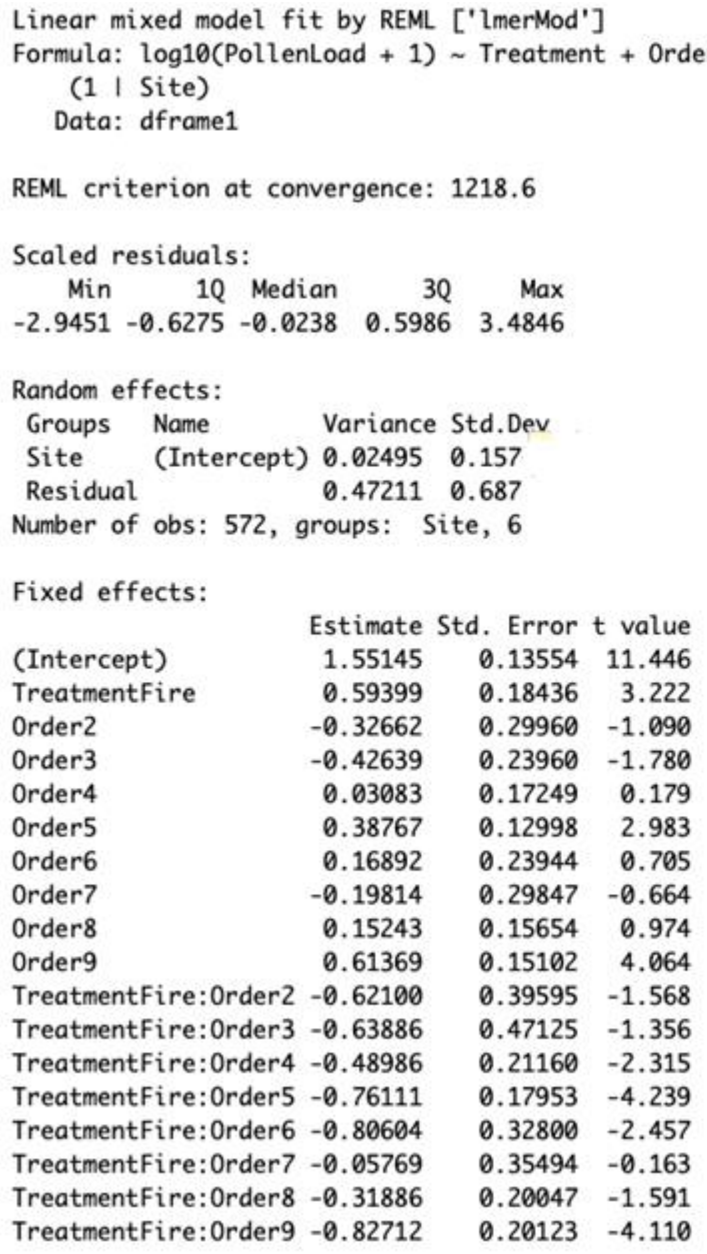
```
Single term deletions

Model:
SpeciesRichness ~ Treatment * Order + (1 | Site)
                Df    AIC   LRT Pr(Chi)
<none>             383.54
Treatment:Order  8 372.20 4.663  0.7929
```



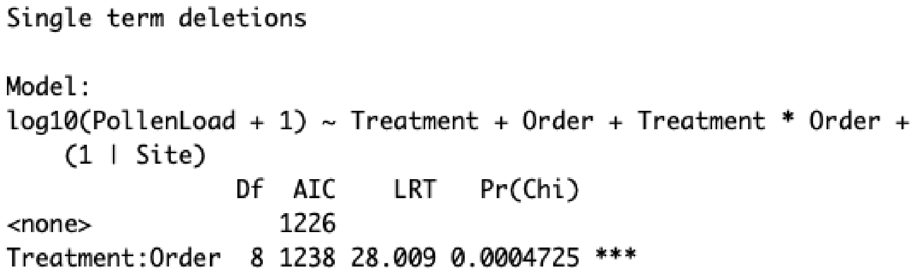
```
Model:
SpeciesRichness ~ Treatment + Order + (1 | Site)
          Df    AIC    LRT   Pr(Chi)
<none>       372.20
Treatment  1 370.21  0.010    0.9221
Order      8 427.64 71.439 2.539e-12 ***
```

Table S7 R software outputs showing summary of analyses of the effects of burning and season over consecutive sampling periods on pollen transport (A. pollen load and B. pollen type). Intercept value represents unburned sites in the first spring, and is the base 10 logarithm of the estimate, so $10^{(\text{intercept})}$ gives the true estimated value. For other levels of each variable, estimated value = $10^{(\text{intercept})}$ x 10^{ES}, where ES = effect size for that level from the statistical model, so 10^{ES} is the multiplicative effect of the parameter in question.

A. Pollen Load

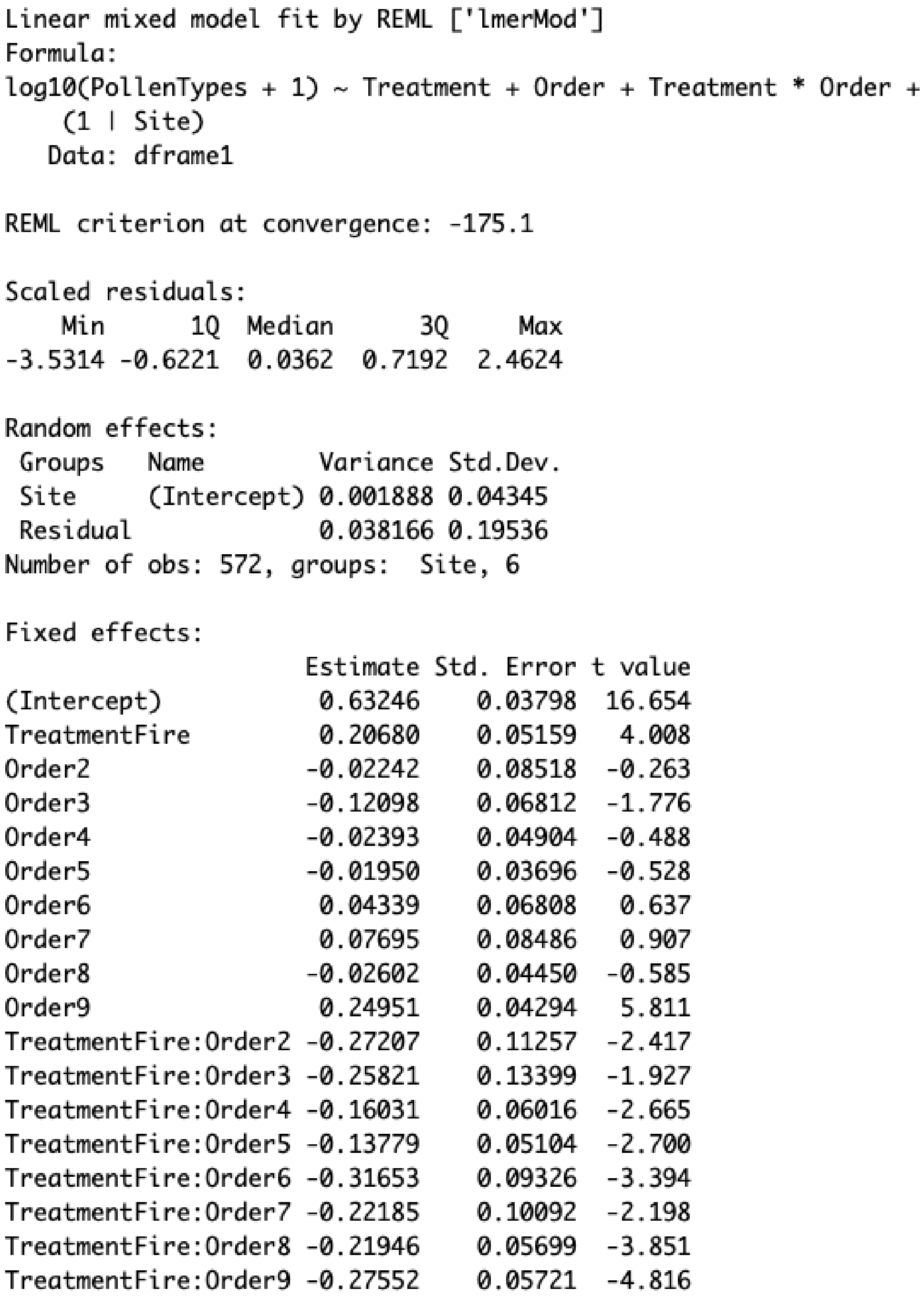


```
Linear mixed model fit by REML ['lmerMod']
Formula: log10(PollenLoad + 1) ~ Treatment + Order + Treatment * Order -
    (1 | Site)
   Data: dframe1

REML criterion at convergence: 1218.6

Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.9451 -0.6275 -0.0238  0.5986  3.4846

Random effects:
 Groups   Name        Variance Std.Dev
 Site     (Intercept) 0.02495  0.157
 Residual             0.47211  0.687
Number of obs: 572, groups:  Site, 6

Fixed effects:
                      Estimate Std. Error t value
(Intercept)            1.55145    0.13554  11.446
TreatmentFire          0.59399    0.18436   3.222
Order2                -0.32662    0.29960  -1.090
Order3                -0.42639    0.23960  -1.780
Order4                 0.03083    0.17249   0.179
Order5                 0.38767    0.12998   2.983
Order6                 0.16892    0.23944   0.705
Order7                -0.19814    0.29847  -0.664
Order8                 0.15243    0.15654   0.974
Order9                 0.61369    0.15102   4.064
TreatmentFire:Order2 -0.62100    0.39595  -1.568
TreatmentFire:Order3 -0.63886    0.47125  -1.356
TreatmentFire:Order4 -0.48986    0.21160  -2.315
TreatmentFire:Order5 -0.76111    0.17953  -4.239
TreatmentFire:Order6 -0.80604    0.32800  -2.457
TreatmentFire:Order7 -0.05769    0.35494  -0.163
TreatmentFire:Order8 -0.31886    0.20047  -1.591
TreatmentFire:Order9 -0.82712    0.20123  -4.110
```



```
Single term deletions

Model:
log10(PollenLoad + 1) ~ Treatment + Order + Treatment * Order +
    (1 | Site)
                Df  AIC    LRT   Pr(Chi)
<none>             1226
Treatment:Order  8 1238 28.009 0.0004725 ***
```

B. Pollen Type



```
Linear mixed model fit by REML ['lmerMod']
Formula:
log10(PollenTypes + 1) ~ Treatment + Order + Treatment * Order +
    (1 | Site)
   Data: dframe1

REML criterion at convergence: -175.1

Scaled residuals:
    Min      1Q  Median      3Q     Max
-3.5314 -0.6221  0.0362  0.7192  2.4624

Random effects:
 Groups   Name        Variance Std.Dev.
 Site     (Intercept) 0.001888 0.04345
 Residual             0.038166 0.19536
Number of obs: 572, groups:  Site, 6

Fixed effects:
                     Estimate Std. Error t value
(Intercept)           0.63246    0.03798  16.654
TreatmentFire         0.20680    0.05159   4.008
Order2               -0.02242    0.08518  -0.263
Order3               -0.12098    0.06812  -1.776
Order4               -0.02393    0.04904  -0.488
Order5               -0.01950    0.03696  -0.528
Order6                0.04339    0.06808   0.637
Order7                0.07695    0.08486   0.907
Order8               -0.02602    0.04450  -0.585
Order9                0.24951    0.04294   5.811
TreatmentFire:Order2 -0.27207    0.11257  -2.417
TreatmentFire:Order3 -0.25821    0.13399  -1.927
TreatmentFire:Order4 -0.16031    0.06016  -2.665
TreatmentFire:Order5 -0.13779    0.05104  -2.700
TreatmentFire:Order6 -0.31653    0.09326  -3.394
TreatmentFire:Order7 -0.22185    0.10092  -2.198
TreatmentFire:Order8 -0.21946    0.05699  -3.851
TreatmentFire:Order9 -0.27552    0.05721  -4.816
```

Table S8 – Mean temperatures taken between 12 – 16 pm on each day of fieldwork.

Year	Dates	Mean Temperatures (^0C)
2013	16 Apr	**27.8**
	14 May	**27.5**
	11 Jun	**27.8**
	09 Jul	**31.5**
	24 Sep	**32.3**
	05 Nov	**23.6**
	09 Dec	**19.3**
2014	28 Jan	**15.4**
	19 Feb	**20.3**
	25 Mar	**24.5**
	29 Apr	**33.8**
	03 Jun	**34.6**
	15 Jul	**37.4**
	12 Aug	**34.2**
	23 Sep	**24.0**
	28 Oct	**25.7**
	02 Dec	**22.5**
2015	21 Jan	**14.8**
	25 Feb	**19.3**
	25 Mar	**20.2**
	05 May	**27.5**

Additional graphs

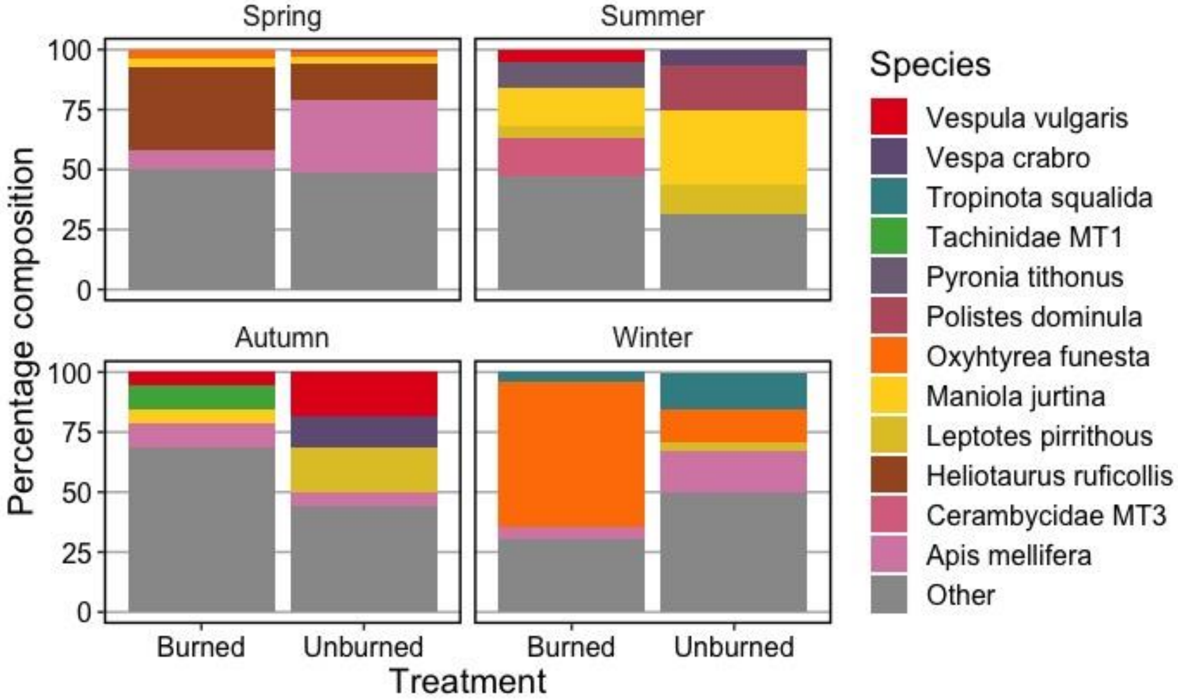


Figure a. Assemblage composition by species of insects caught in burned and unburned areas of Serra-do-Caldeirão, Portugal, and across seasons. Species never comprising >10% of individuals in any combination of season and treatment are grouped as "Others", and all other families are shown independently.

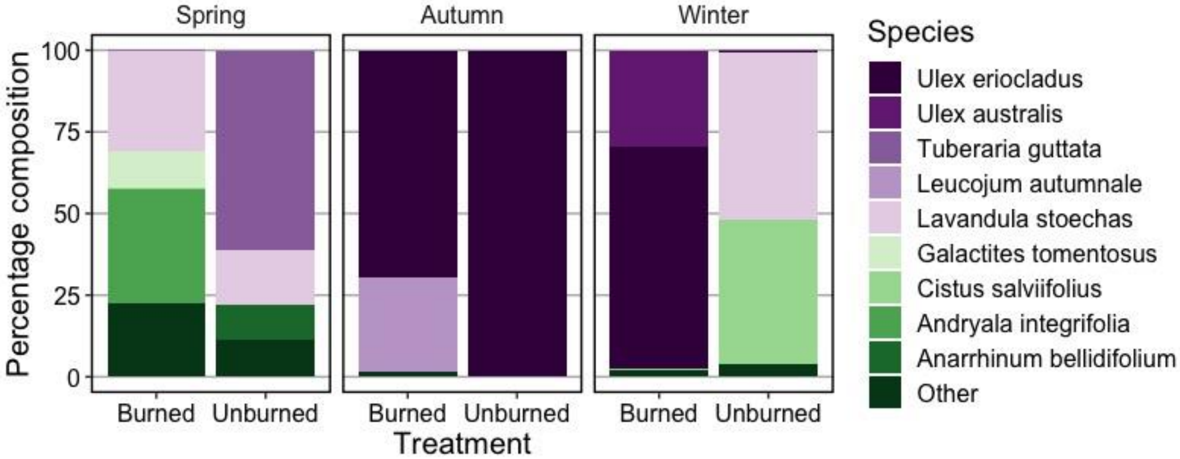


Figure b. Assemblage composition by families of pollen carried by *Apis mellifera* caught in burned and unburned plots of Serra-do-Caldeirão, Portugal, and across seasons. Families never comprising >7% of individuals in any combination of season and treatment are grouped as "Other", and all other families are shown independently.

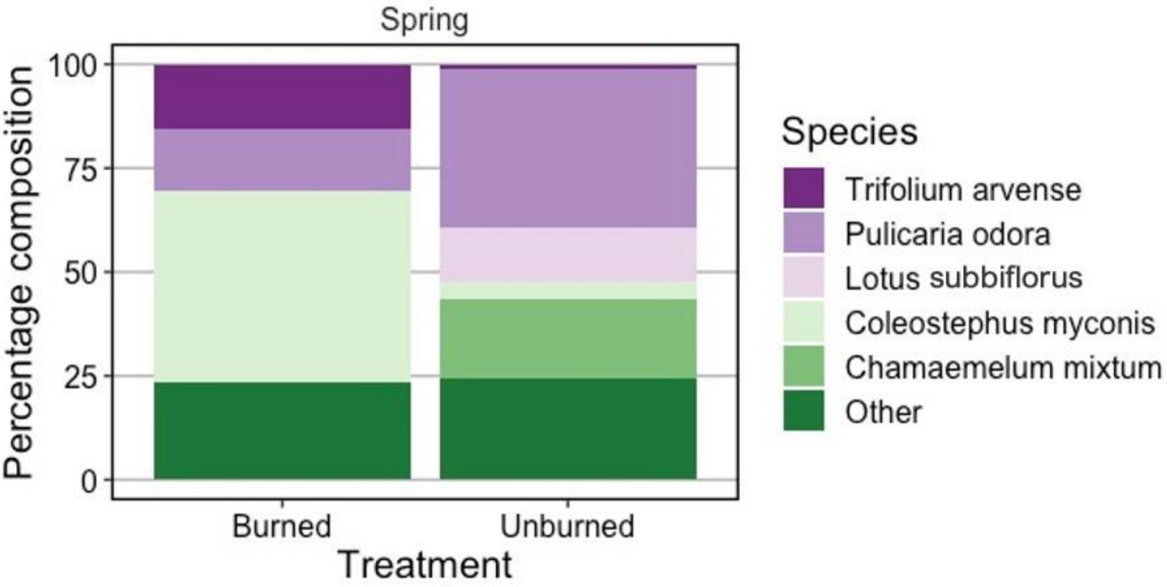


Figure c. Assemblage composition by families of pollen carried by *H. rufficolis* caught in burned and unburned plots of Serra-do-Caldeirão, Portugal, and across seasons. Families never comprising >7% of individuals in any combination of season and treatment are grouped as "Other", and all other families are shown independently.

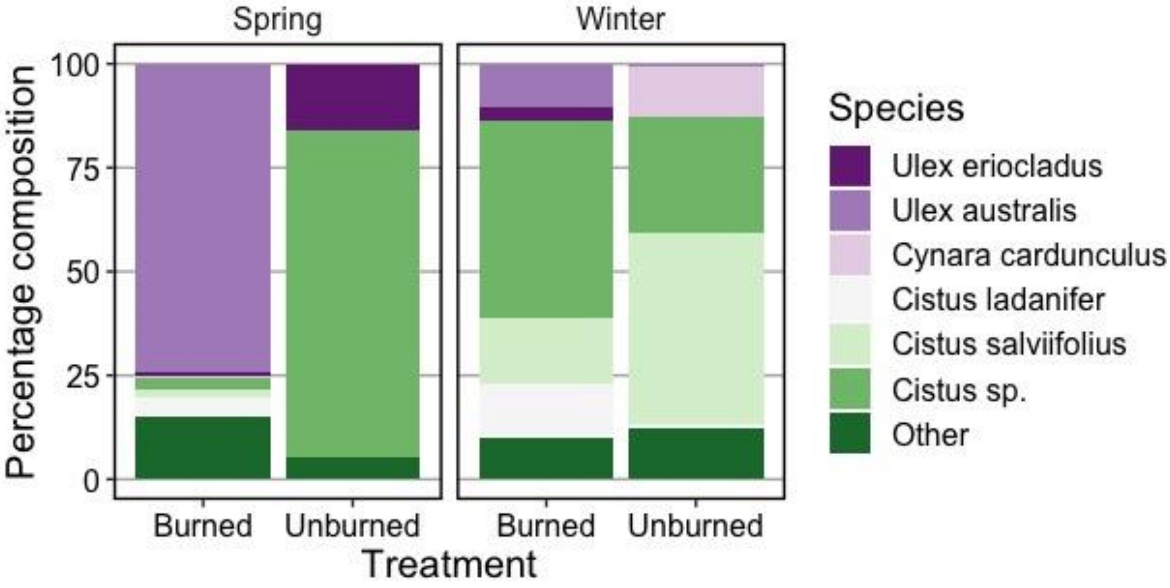


Figure d. Assemblage composition by families of pollen carried by *O. funesta* caught in burned and unburned plots of Serra-do-Caldeirão, Portugal, and across seasons. Families never comprising >7% of individuals in any combination of season and treatment are grouped as "Other", and all other families are shown independently.

Appendix 2 - Supporting Information associated with the manuscript:

Wildfire alters the structure and seasonal dynamics of
nocturnal pollen-transport networks

Table S1 Locations of the six study sites. Latitude, longitude and altitude are given for the centre of each 40 x 40 m study plot. Latitude and longitude are given in decimal degrees to 5 decimal places, and therefore are accurate to within approximately 1 m. Aspect and slope were calculated from Intermap NEXTMap 5 data, accessed through Strava.

Site	Treatment	Latitude	Longitude	Altitude (m above sea level)	Aspect	Slope
F1	Burned	37.17713	-7.86082	261	E	20%
F2	Burned	37.19641	-7.86006	372	WNW	30%
F3	Burned	37.19848	-7.85699	449	W	22.5%
NF1	Unburned	37.16919	-7.86516	340	NE	14%
NF2	Unburned	37.17334	-7.86506	317	W	12%
NF3	Unburned	37.18149	-7.86588	245	NW	10%

Table S2 R packages used during analysis. Packages were loaded into at least one script during the analytical process but may not have formed part of the final analysis.

Package	Citation
AICcmodavg	Mazerolle, M.J. (2016) AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 2.1-0. https://cran.r-project.org/package=AICcmodavg.
arm	Gelman, A. & and Su, Y.-S. (2015) arm: Data Analysis Using Regression and Multilevel/Hierarchical Models. R package version 1.8-6. https://CRAN.R-project.org/package=arm.
bipartite	Dormann, C.F., Gruber B. & Fruend, J. (2008) Introducing the bipartite Package: Analysing Ecological Networks. *R News*, **8**, 8–11.
car	Fox, J. & Weisberg, S. (2011) *An {R} Companion to Applied Regression.* Second Edition. Sage, Thousand Oaks, CA, USA.
data.table	Dowle, M., Srinivasan, A., Short, T., Lianoglou, S., Saporta, R. & Antonyan, E. (2015) data.table: Extension of Data.frame. R package version 1.9.6. https://CRAN.R-project.org/package=data.table.
effects	Fox, J. (2003) Effect Displays in R for Generalised Linear Models. *Journal of Statistical Software*, **8**, 1–27.
ggmap	Kahle, D. & Wickham, H. (2013) ggmap: Spatial Visualization with ggplot2. *The R Journal*, **5**, 144–161.
ggplot2	Wickham, H. (2009) *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag, New York, USA.
glmmADMB	Fournier, D.A., Skaug, H.J., Ancheta, J., Ianelli, J., Magnusson, A., Maunder, M., Nielsen, A. & Sibert, J. (2012) AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optimization Methods and Software*, **27**, 233–249.
gridExtra	Auguie, B. (2016) gridExtra: Miscellaneous Functions for "Grid" Graphics. R package version 2.2.1. https://CRAN.R-project.org/package=gridExtra.
lme4	Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, **67**, 1–48.
MASS	Venables, W. N. & Ripley, B. D. (2002) *Modern Applied Statistics with S*. Fourth Edition. Springer, New York, USA.
plyr	Wickham, H. (2011) The Split-Apply-Combine Strategy for Data Analysis. *Journal of Statistical Software*, **40**, 1–29.
RColorBrewer	Neuwirth, E. (2014) RColorBrewer: ColorBrewer Palettes. R package version 1.1-2. https://CRAN.R-project.org/package=RColorBrewer.

reshape2	Wickham, H. (2007) Reshaping Data with the reshape Package. *Journal of Statistical Software*, **21**, 1–20.
RVAideMemoire	Hervé, M. (2016) RVAideMemoire: Diverse Basic Statistical and Graphical Functions. R package version 0.9-56. https://CRAN.R-project.org/package=RVAideMemoire.
scales	Wickham, H. (2016) scales: Scale Functions for Visualization. R package version 0.4.1. https://CRAN.R-project.org/package=scales.
svglite	Wickham, H., Henry, L., Luciani, T.J., Decorde, M. & Lise, V. (2016) svglite: An 'SVG' Graphics Device. R package version 1.2.0. https://CRAN.R-project.org/package=svglite.
tidyr	Wickham, H. (2016) tidyr: Easily Tidy Data with \`spread()\` and \`gather()\` Functions. R package version 0.5.0. https://CRAN.R-project.org/package=tidyr.
vegan	Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H. & Wagner, H. (2016) vegan: Community Ecology Package. R package version 2.3-5. https://CRAN.R-project.org/package=vegan.

Table S3 Summary of families of captured moths, with the number of morphotypes, the number of those which were identified to species level, and the total number of individuals.

Family	No. morphotypes	No. identified species	No. individuals
Autostichidae	4	4	72
Blastobasidae	1	0	6
Choreutidae	1	1	3
Coleophoridae	2	0	35
Cosmopterigidae	2	2	2
Cossidae	1	1	1
Crambidae	27	24	233
Depressariidae	4	3	50
Drepanidae	1	1	84
Elachistidae	2	1	3
Erebidae	22	19	307
Gelechiidae	12	9	36
Geometridae	72	64	724
Gracillariidae	1	0	16
Lasiocampidae	3	3	21
Lecithoceridae	2	2	22
Limacodidae	1	1	3
Lymantriidae	2	2	4
Noctuidae	85	76	937
Nolidae	2	2	2
Notodontidae	2	2	2
Oecophoridae	4	3	19
Plutellidae	2	1	3
Psychidae	1	0	1
Pterolonchidae	1	1	2
Pterophoridae	7	3	11
Pyralidae	36	32	644
Thaumetopoeidae	1	1	5
Tineidae	5	4	13
Tortricidae	15	11	118
Yponomeutidae	2	1	7
Unidentified	4	0	20

Table S4 Summary of the plant families identified on transects, with the number of species and the total number of 1 x 1 m squares on transects in which each family was recorded. Apiaceae includes one flower type not identified beyond family level, and therefore was represented by *at least* four species.

Family	No. species	No. transects
Adoxaceae	1	4
Amaryllidaceae	2	17
Apiaceae	4*	29
Asparagaceae	2	3
Asteraceae	14	379
Boraginaceae	2	39
Brassicaceae	1	3
Campanulaceae	2	25
Caprifoliaceae	2	16
Caryophyllaceae	2	8
Cistaceae	4	167
Ericaceae	3	26
Euphorbiaceae	1	4
Fabaceae	10	285
Gentianaceae	1	36
Geraniaceae	1	2
Iridaceae	2	11
Lamiaceae	3	200
Linaceae	1	1
Orchidaceae	1	1
Papaveraceae	1	2
Plantaginaceae	1	30
Primulaceae	1	24
Ranunculaceae	2	30
Resedaceae	2	23
Rosaceae	2	12
Solanaceae	1	39
Thymelaeaceae	1	8

Table S5 Summary of analyses of the effects of burning and season over consecutive sampling periods on the abundance and species richness of moths and plants in flower. Intercept value represents unburned sites in autumn, sampling period 0, and is the natural logarithm of the estimate, so $e^{(intercept)}$ gives the true estimated value. For other levels of each variable, estimated value = $e^{(intercept)}$ x e^{ES}, where ES = effect size for that level from the statistical model, so e^{ES} is the multiplicative effect of the parameter in question. Test statistics are for Likelihood Ratio Test, and significant *P*-values (<0.05) are italicized.

Dependent variable	Model χ^2 effect (*P* value)				Parameter effect size (standard error)									
	Burning	Season	Burning x Season	Sampling period	Intercept (autumn, unburned)	Burned	Season			Sampling period	Interaction terms			
							Winter	Spring	Summer		Burned x Winter	Burned x Spring	Burned x Summer	Burned x Sampling period
Abundance of moths	-	-	36.24 (*<0.001*)	188.17 (*<0.001*)	4.13 (0.19)	-0.78 (0.25)	-0.55 (0.09)	0.65 (0.07)	1.28 (0.07)	-0.11 (0.01)	-0.28 (0.17)	-0.29 (0.12)	-0.67 (0.12)	-
Estimated species richness of moths	9.39 (*0.002*)	41.71 (*<0.001*)	N.S.	4.84 (*0.028*)	3.40 (0.31)	-0.77 (0.22)	-0.76 (0.27)	0.50 (0.29)	1.16 (0.28)	-0.09 (0.04)	-	-	-	-
Abundance of flowers	-	-	34.81 (*<0.001*)	91.42 (*<0.001*)	0.001 (0.13)	0.19 (0.16)	0.75 (0.13)	1.59 (0.13)	0.01 (0.13)	0.13 (0.01)	0.51 (0.18)	-0.23 (0.18)	-0.44 (0.19)	-
Estimated species richness of flowers	1.88 (0.170)	17.96 (*<0.001*)	N.S.	0.04 (0.841)	0.72 (0.24)	0.28 (0.19)	-0.04 (0.21)	0.68 (0.20)	0.33 (0.21)	0.01 (0.03)	-	-	-	-

† Significant interaction between treatment and sampling period: χ^2 and *P* values are given for Burning:Sampling period interaction term in Sampling period column.

Table S6 Summary of analyses testing for effects of burning and season on community composition at family level of moths and flowers, and at species level of moths, flowers and interactions.

Level	Community	Fixed effects	Model *F* (interaction effect)	*P*	Model *F* (treatment effect)	*P*	Model *F* (season effect)	*P*
Family	Moths	Treatment x Season	1.31	0.157	2.27	*<0.001*	6.71	*<0.001*
	Flowers	Treatment x Season	2.09	*0.002*	6.74	*<0.001*	12.46	*<0.001*
Species	Moths	Treatment x Season	0.72	0.164	0.86	*0.039*	0.35	0.063
	Flowers	Treatment x Season	0.21	0.664	1.34	*0.012*	0.40	0.063
	Interactions	Treatment x Season	0.78	0.121	0.84	*0.027*	2.26	0.063

Table S7 Summary of analyses of the effects of burning and season over consecutive sampling periods on pollen transport. Intercept value represents unburned sites in autumn, sampling period 0, and is the natural logarithm of the estimate, so $e^{(\text{intercept})}$ gives the true estimated value. For other levels of each variable, estimated value = $e^{(\text{intercept})} \times e^{ES}$, where ES = effect size for that level from the statistical model, so e^{ES} is the multiplicative effect of the parameter in question. Test statistics are for Likelihood Ratio Test, and significant *P*-values (<0.05) are italicized.

Threshold of 5 pollen grains	Dependent variable	Model χ^2 effect (*P* value)				Parameter effect size (standard error)									
		Burning	Season	Burning x Season	Sampling period	Intercept	Burned	Season			Sampling period	Interaction terms			
								Winter	Spring	Summer		Burned x Winter	Burned x Spring	Burned x Summer	Burned x Sampling period
Threshold not applied	Proportion of moths carrying pollen	-	-	33.21 (*<0.001*)	20.23 (*<0.001*)	1.76 (0.19)	0.47 (0.27)	-0.97 (0.20)	1.34 (0.22)	-1.37 (0.15)	-0.10 (0.02)	-1.50 (0.39)	-0.32 (0.43)	0.31 (0.29)	-
	Total pollen load per pollen-carrying moth	-	-	8.84 (*0.032*)	7.99 (*0.005*)	2.73 (0.12)	0.03 (0.16)	-1.28 (0.14)	-0.32 (0.10)	-1.82 (0.10)	-0.04 (0.01)	-0.41 (0.32)	-0.03 (0.17)	0.34 (0.17)	-
	No. pollen types per pollen-carrying moth	-	-	11.17 (*0.011*)	11.27 † (*<0.001*)	0.69 (0.07)	-0.21 (0.12)	-0.003 (0.09)	0.77 (0.06)	-0.23 (0.06)	0.05 (0.01)	-0.34 (0.23)	-0.08 (0.10)	0.15 (0.11)	0.05 (0.02)
	Total pollen count per sample	11.82 (*<0.001*)	44.28 (*<0.001*)	N.S.	8.93 (*0.003*)	9.05 (0.58)	-1.68 (0.37)	-3.79 (0.51)	-1.86 (0.55)	-2.38 (0.53)	0.23 (0.08)	-	-	-	-
	No. pollen types per sample	-	-	9.65 (*0.022*)	14.84 (*<0.001*)	2.12 (0.17)	-0.08 (0.21)	-0.07 (0.19)	1.01 (0.17)	0.41 (0.18)	-0.07 (0.02)	-0.99 (0.34)	-0.29 (0.24)	-0.19 (0.26)	-
Threshold applied	Proportion of moths carrying pollen	-	-	20.55 (*<0.001*)	6.30 † (*0.012*)	-0.49 (0.22)	-0.16 (0.36)	-1.42 (0.22)	0.63 (0.15)	-2.28 (0.17)	0.01 (0.03)	-1.53 (0.59)	-0.09 (0.28)	0.65 (0.29)	0.13 (0.05)
	Total pollen load per pollen-carrying	-	-	8.38 (*0.039*)	5.01 (*0.025*)	4.56 (0.15)	-0.62 (0.19)	-1.52 (0.21)	-1.66 (0.11)	-1.57 (0.15)	-0.04 (0.02)	-0.003 (0.57)	0.45 (0.19)	0.59 (0.24)	-

	moth														
	No. pollen types per pollen-carrying moth	2.79 (0.095)	79.04 (<*0.001*)	N.S.	4.90 (*0.027*)	0.30 (0.10)	-0.16 (0.09)	0.15 (0.17)	0.51 (0.08)	-0.09 (0.11)	-0.03 (0.01)	-	-	-	-
	Total pollen count per sample	10.49 (*0.001*)	40.79 (<*0.001*)	N.S.	7.46 (*0.006*)	9.22 (0.74)	-1.74 (0.47)	-4.59 (0.66)	-2.65 (0.70)	-3.16 (0.68)	-0.27 (0.10)	-	-	-	-
	No. pollen types per sample	7.49 (*0.006*)	28.82 (<*0.001*)	N.S.	3.52 (0.061)	1.69 (0.27)	-0.50 (0.18)	-0.93 (0.29)	0.52 (0.24)	-0.20 (0.25)	-0.07 (0.04)	-	-	-	-

† Significant interaction between treatment and sampling period: χ^2 and *P* values are given for Burning:Sampling period interaction term in Sampling period column.

Table S8 Summary of analyses of the effects of burning and season over consecutive sampling periods on quantitative, pollen load-weighted, pollen-transport networks (n = 9 pairs). Intercept value represents unburned sites in autumn, sampling period 0, and is the natural logarithm of the estimate, so $e^{(\text{intercept})}$ gives the true estimated value. For other levels of each variable, estimated value = $e^{(\text{intercept})} \times e^{ES}$, where ES = effect size for that level from the statistical model, so e^{ES} is the multiplicative effect of the parameter in question. Test statistics are for Likelihood Ratio Test, and significant *P*-values (<0.05) are italicized.

Threshold of 5 pollen grains	Dependent variable	Model χ^2 effect (*P* value)				Parameter effect size (standard error)									
		Burning	Season	Burning x Season	Sampling period	Intercept	Burned	Season			Sampling period	Interaction terms			
								Winter	Spring	Summer		Burned x Winter	Burned x Spring	Burned x Summer	Burned x Sampling period
Threshold not applied	Linkage density	2.15 (0.168)	10.20 (*0.001*)	N.S.	4.68 (0.051)	4.82 (1.23)	-1.17 (0.80)	-0.17 (1.21)	4.67 (1.09)	3.93 (1.21)	-0.35 (0.16)	-	-	-	-
	Generality of plants	1.85 (0.199)	5.60 (*0.012*)	N.S.	1.87 (0.197)	7.07 (2.46)	-2.17 (1.59)	-1.34 (2.41)	5.17 (2.18)	6.91 (2.41)	-0.44 (0.32)	-	-	-	-
	Generality of pollinators	0.09 (0.766)	12.20 (*<0.001*)	N.S.	5.13 (*0.043*)	2.58 (0.87)	-0.17 (0.56)	1.00 (0.85)	4.16 (0.77)	0.95 (0.85)	-0.25 (0.11)	-	-	-	-
	Robustness	5.03 (*0.045*)	4.68 (*0.022*)	N.S.	3.40 (0.090)	0.83 (0.04)	-0.06 (0.03)	-0.11 (0.04)	0.004 (0.04)	0.02 (0.04)	-0.01 (0.01)	-	-	-	-
	Niche overlap	0.38 (0.550)	1.82 (0.198)	N.S.	0.35 (0.564)	0.29 (0.11)	-0.04 (0.07)	-0.15 (0.11)	0.08 (0.10)	-0.01 (0.11)	0.01 (0.01)	-	-	-	-
Threshold applied	Linkage density	0.93 (0.356)	3.92 (*0.040*)	N.S.	4.76 (0.052)	4.54 (1.23)	-0.80 (0.83)	-0.33 (1.33)	3.02 (1.09)	0.66 (1.20)	-0.36 (0.16)	-	-	-	-
	Generality of plants	0.85 (0.377)	2.38 (0.125)	N.S.	1.71 (0.218)	6.38 (2.38)	-1.48 (1.60)	-1.67 (2.56)	4.07 (2.11)	1.42 (2.33)	-0.41 (0.32)	-	-	-	-
	Generality of pollinators	0.09 (0.771)	7.03 (*0.007*)	N.S.	14.47 (*0.003*)	2.70 (0.59)	-0.12 (0.40)	1.01 (0.64)	1.97 (0.53)	-0.10 (0.58)	-0.30 (0.08)	-	-	-	-
	Robustness	4.09 (0.068)	3.99 (*0.038*)	N.S.	2.79 (0.123)	0.84 (0.05)	-0.06 (0.03)	-0.13 (0.05)	-0.05 (0.04)	-0.13 (0.05)	-0.01 (0.01)	-	-	-	-
	Niche overlap	1.07 (0.323)	1.22 (0.348)	N.S.	0.16 (0.693)	0.38 (0.16)	-0.11 (0.10)	-0.23 (0.17)	0.06 (0.14)	-0.04 (0.15)	0.01 (0.02)	-	-	-	-

Table S9 Summary of analyses of the effects of burning and season over consecutive sampling periods on quantitative, interaction frequency- weighted, pollen-transport networks (n = 9 pairs). Intercept value represents unburned sites in autumn, sampling period 0, and is the natural logarithm of the estimate, so $e^{(intercept)}$ gives the true estimated value. For other levels of each variable, estimated value = $e^{(intercept)} \times e^{ES}$, where ES = effect size for that level from the statistical model, so e^{ES} is the multiplicative effect of the parameter in question. Test statistics are for Likelihood Ratio Test, and significant *P*-values (<0.05) are italicized.

Threshold of 5 pollen grains	Dependent variable	Model χ^2 effect (*P* value)				Parameter effect size (standard error)									
		Burning	Season	Burning x Season	Sampling period	Intercept	Burned	Season			Sampling period	Interaction terms			
								Winter	Spring	Summer		Burned x Winter	Burned x Spring	Burned x Summer	Burned x Sampling period
Threshold not applied	Linkage density	4.77 (*0.049*)	6.83 (*0.006*)	N.S.	7.34 (*0.019*)	10.13 (1.70)	-2.41 (1.10)	-1.67 (1.67)	2.84 (1.51)	5.39 (1.67)	-0.60 (0.22)	-	-	-	-
	Generality of plants	4.10 (0.066)	7.10 (*0.005*)	N.S.	3.60 (0.082)	14.66 (3.20)	-4.20 (2.07)	-3.47 (3.14)	1.33 (2.84)	10.66 (3.14)	-0.79 (0.42)	-	-	-	-
	Generality of pollinators	0.97 (0.344)	13.13 (*<0.001*)	N.S.	10.19 (*0.008*)	5.60 (0.99)	-0.63 (0.64)	0.13 (0.97)	4.35 (0.88)	0.11 (0.97)	-0.41 (0.13)	-	-	-	-
	Robustness	5.04 (*0.044*)	4.69 (*0.022*)	N.S.	3.40 (0.090)	0.83 (0.04)	-0.06 (0.03)	-0.11 (0.04)	0.004 (0.04)	0.02 (0.04)	-0.01 (0.01)	-	-	-	-
	Niche overlap	0.87 (0.370)	2.44 (0.115)	N.S.	0.06 (0.813)	0.40 (0.09)	-0.05 (0.06)	-0.20 (0.09)	-0.01 (0.08)	-0.06 (0.09)	0.003 (0.01)	-	-	-	-
Threshold applied	Linkage density	0.84 (0.379)	2.05 (0.165)	N.S.	3.93 (0.073)	5.74 (1.61)	-0.99 (1.08)	-0.44 (1.73)	2.81 (1.42)	0.63 (1.57)	-0.42 (0.21)	-	-	-	-
	Generality of plants	0.67 (0.431)	1.33 (0.316)	N.S.	1.84 (0.202)	8.33 (3.05)	-1.68 (2.06)	-1.79 (3.30)	3.79 (2.71)	1.63 (2.99)	-0.55 (0.41)	-	-	-	-
	Generality of pollinators	0.34 (0.572)	4.23 (*0.032*)	N.S.	8.43 (*0.014*)	3.16 (0.76)	-0.30 (0.51)	0.91 (0.82)	1.82 (0.68)	-0.37 (0.75)	-0.30 (0.10)	-	-	-	-
	Robustness	4.16 (0.066)	3.99 (*0.038*)	N.S.	2.78 (0.124)	0.84 (0.05)	-0.06 (0.03)	-0.13 (0.05)	-0.05 (0.04)	-0.13 (0.04)	-0.01 (0.01)	-	-	-	-
	Niche overlap	1.16 (0.305)	1.37 (0.302)	N.S.	0.13 (0.724)	0.38 (0.15)	-0.11 (0.10)	-0.22 (0.16)	0.08 (0.13)	-0.04 (0.15)	0.01 (0.02)	-	-	-	-

Table S10 Summary of the causes of interaction turnover between networks from burned and unburned treatments within the same sampling period (Fig. 4).

Threshold of 5 pollen grains	Sampling period	No. interactions present in both networks	No. interactions only in burned network	No. interactions only in unburned network	β (change in flowers and pollinators)	β (change in flowers)	β (change in pollinators)	β (interaction rewiring: no change in flowers and pollinators)	Jaccard β-diversity (total change in interactions)
Threshold not applied	Spring_1	94	84	780	0.121	0.073	0.503	0.198	0.902
	Summer_1	50	159	206	0.063	0.065	0.412	0.340	0.880
	Autumn_2	23	45	64	0.023	0.045	0.538	0.220	0.826
	Winter_2	2	10	58	0.471	0.157	0.214	0.129	0.971
	Spring_2	65	70	206	0.070	0.067	0.460	0.211	0.809
	Summer_2	21	52	161	0.051	0.038	0.594	0.226	0.910
	Autumn_3	19	58	42	0.025	0.025	0.613	0.176	0.840
	Winter_3	2	7	68	0.377	0.195	0.299	0.104	0.974
	Spring_3	7	17	33	0.070	0.053	0.491	0.263	0.877
Threshold applied	Spring_1	29	35	268	0.136	0.120	0.476	0.181	0.913
	Summer_1	7	26	33	0.167	0.061	0.561	0.106	0.894
	Autumn_2	6	14	21	0.024	0.024	0.610	0.195	0.854
	Winter_2	0	1	6	1.000	0	0	0	1.000
	Spring_2	28	26	87	0.156	0.163	0.355	0.128	0.801
	Summer_2	0	6	18	0.167	0.042	0.750	0.042	1.000
	Autumn_3	7	23	16	0.130	0.043	0.652	0.022	0.848
	Winter_3	1	2	26	0.552	0	0.379	0.034	0.966
	Spring_3	0	4	8	0.167	0.250	0.417	0.167	1.000

Table S11 Sampling completeness of species and interactions for each of 18 networks.

Year	Season	Treatment	Observed insect species	Insect species % sampling completeness	Observed plant species	Plant species % sampling completeness	No. pollen transporting insect species	Weighted mean % interaction completeness of species
1	Spring	Fire	35	47.3	36	68.9	33	52.4
1	Spring	No fire	118	51.5	27	81.9	116	62.5
1	Summer	Fire	87	45.9	3	78.3	77	66.1
1	Summer	No fire	94	51.5	4	82.8	82	63.6
2	Autumn	Fire	28	34.6	4	51.6	27	72.7
2	Autumn	No fire	40	59.4	1	100.0	33	82.7
2	Winter	Fire	20	36.0	15	72.5	8	64.3
2	Winter	No fire	38	51.9	7	79.7	29	75.4
2	Spring	Fire	26	13.8	28	88.1	26	75.5
2	Spring	No fire	61	41.0	16	82.2	59	74.4
2	Summer	Fire	52	58.6	5	48.4	36	71.2
2	Summer	No fire	127	59.9	4	69.2	80	65.7
3	Autumn	Fire	34	42.7	9	46.4	29	84.3
3	Autumn	No fire	33	66.7	4	82.8	30	88.0
3	Winter	Fire	7	36.8	9	81.8	7	93.0
3	Winter	No fire	25	23.1	6	93.1	21	60.7
3	Spring	Fire	6	49.0	13	90.3	6	94.7
3	Spring	No fire	8	100.0	9	51.9	8	75.2

Table S12 Summary of analyses of the effects of burning and season over consecutive sampling periods on the abundance and species richness of plants in flower, for two subsets of the data: annuals and biennials only (‘annuals’), and all other flowers (‘perennials’, also including bulbs, shrubs and trees). Intercept value represents unburned sites in autumn, sampling period 0, and is the natural logarithm of the estimate, so $e^{(\text{intercept})}$ gives the true estimated value. For other levels of each variable, estimated value = $e^{(\text{intercept})} \times e^{\text{ES}}$, where ES = effect size for that level from the statistical model, so e^{ES} is the multiplicative effect of the parameter in question. Test statistics are for Likelihood Ratio Test, and significant *P*-values (<0.05) are italicized.

Dependent variable	Model χ^2 effect (*P* value)				Parameter effect size (standard error)									
	Burning	Season	Burning x Season	Sampling period	Intercept	Burned	Season			Sampling period	Interaction terms			
							Winter	Spring	Summer		Burned x Winter	Burned x Spring	Burned x Summer	Burned x Sampling period
Abundance of annuals	-	-	47.14 (*<0.001*)	13.30 (*0.004*)	1.69 (0.18)	0.06 (0.28)	-0.81 (0.20)	-0.45 (0.18)	0.40 (0.28)	0.12 (0.02)	-0.26 (0.38)	0.47 (0.30)	-0.55 (0.52)	-
Species richness of annuals	-	31.08 (*<0.001*)	N.S.	5.99 † (*0.014*)	0.37 (0.15)	0.38 (0.12)	-0.08 (0.12)	0.28 (0.11)	-0.20 (0.15)	0.01 (0.02)	-	-	-	-0.05 (0.02)
Abundance of perennials	-	-	8.20 (*0.042*)	125.86 (*<0.001*)	1.91 (0.24)	-0.34 (0.27)	0.15 (0.23)	0.18 (0.23)	-0.21 (0.25)	0.12 (0.01)	0.28 (0.26)	0.06 (0.26)	0.56 (0.32)	-
Species richness of perennials	0.004 (0.952)	5.17 (0.160)	N.S.	6.87 (*0.009*)	0.48 (0.07)	-0.002 (0.04)	-0.06 (0.06)	-0.05 (0.06)	-0.17 (0.08)	0.01 (0.005)	-	-	-	-

† Significant interaction between treatment and sampling period: χ^2 and *P* values are given for Burning:Sampling period interaction term in Sampling period column.

Table S13 Summary of analyses of the effects of burning and season in winter on floral abundance and pollen transport in winter, with the dataset divided in two: data concerning *Ulex argenteus* only, and all other data. Intercept value represents unburned sites, sampling period 0, and is the natural logarithm of the estimate, so $e^{(\text{intercept})}$ gives the true estimated value. For other levels of each variable, estimated value = $e^{(\text{intercept})} \times e^{ES}$, where ES = effect size for that level from the statistical model, so e^{ES} is the multiplicative effect of the parameter in question. Test statistics are for Likelihood Ratio Test, and significant *P*-values (<0.05) are italicized.

Dependent variable	Model χ^2 effect (*P* value)			Parameter effect size (standard error)			
	Burning	Sampling period	Burning x Sampling period	Intercept	Burned	Sampling period	Burned x Sampling period
Floral abundance of *U. argenteus*	-	-	6.52 (*0.011*)	2.60 (0.12)	-0.32 (0.15)	0.08 (0.02)	0.05 (0.02)
Floral abundance of other flowers	4.99 (*0.026*)	4.63 (*0.031*)	N.S.	0.77 (0.27)	0.62 (0.28)	0.07 (0.03)	-
Proportion of moths carrying *U. argenteus* pollen	11.04 (*<0.001*)	14.02 (*<0.001*)	N.S.	-2.67 (0.53)	-3.31 (1.09)	0.32 (0.09)	-
Proportion of moths carrying other pollen	0.87 (0.352)	38.26 (*<0.001*)	N.S.	-2.39 (0.44)	-0.44 (0.42)	0.44 (0.08)	-
Total pollen load per moth of *U. argenteus*	5.27 (*0.022*)	13.14 (*<0.001*)	N.S.	-0.32 (0.39)	2.04 (0.90)	0.23 (0.06)	-
Total pollen load per moth of other species	-	-	4.03 (*0.045*)	-0.34 (0.32)	1.13 (0.79)	0.24 (0.05)	-0.24 (0.12)
Total pollen count per sample of *U. argenteus*	4.06 (*0.044*)	0.10 (0.750)	N.S.	1.86 (1.52)	-1.83 (0.93)	0.07 (0.23)	-
Total pollen count per sample of other species	8.33 (*0.004*)	2.52 (0.112)	N.S.	2.33 (0.93)	-1.84 (0.57)	0.22 (0.14)	-

Table S14 Summary of analyses testing for effects of burning on degree distribution of moths and plants. D value obtained from a one-tailed Kolmogorov-Smirnov test, for which the null hypothesis was that the cumulative frequency distribution of degree was not greater for unburned sites than burned sites.

Level	Season	Mean burned degree (standard error)	Mean unburned degree (standard error)	D value	*P*
Moths	Overall	3.74 (0.31)	5.63 (0.36)	0.190	*<0.001*
	Overall (threshold applied)	1.04 (0.13)	1.78 (0.16)	0.147	*0.007*
	Spring	5.52 (0.64)	6.97 (0.45)	0.130	0.250
	Summer	2.36 (0.20)	2.42 (0.21)	0.052	0.689
	Autumn	2.48 (0.28)	2.25 (0.25)	0.044	0.899
	Winter	0.75 (0.20)	2.21 (0.32)	0.429	*0.001*
Plants	Overall	10.76 (1.74)	24.58 (3.45)	0.274	*0.007*
	Overall (threshold applied)	3.00 (0.77)	7.79 (1.58)	0.273	*0.007*
	Spring	4.67 (0.99)	16.26 (2.71)	0.303	*0.002*
	Summer	4.15 (1.26)	5.98 (1.72)	0.061	0.785
	Autumn	1.95 (0.71)	1.94 (0.78)	0.030	0.941
	Winter	0.32 (0.11)	1.88 (0.49)	0.258	0.013

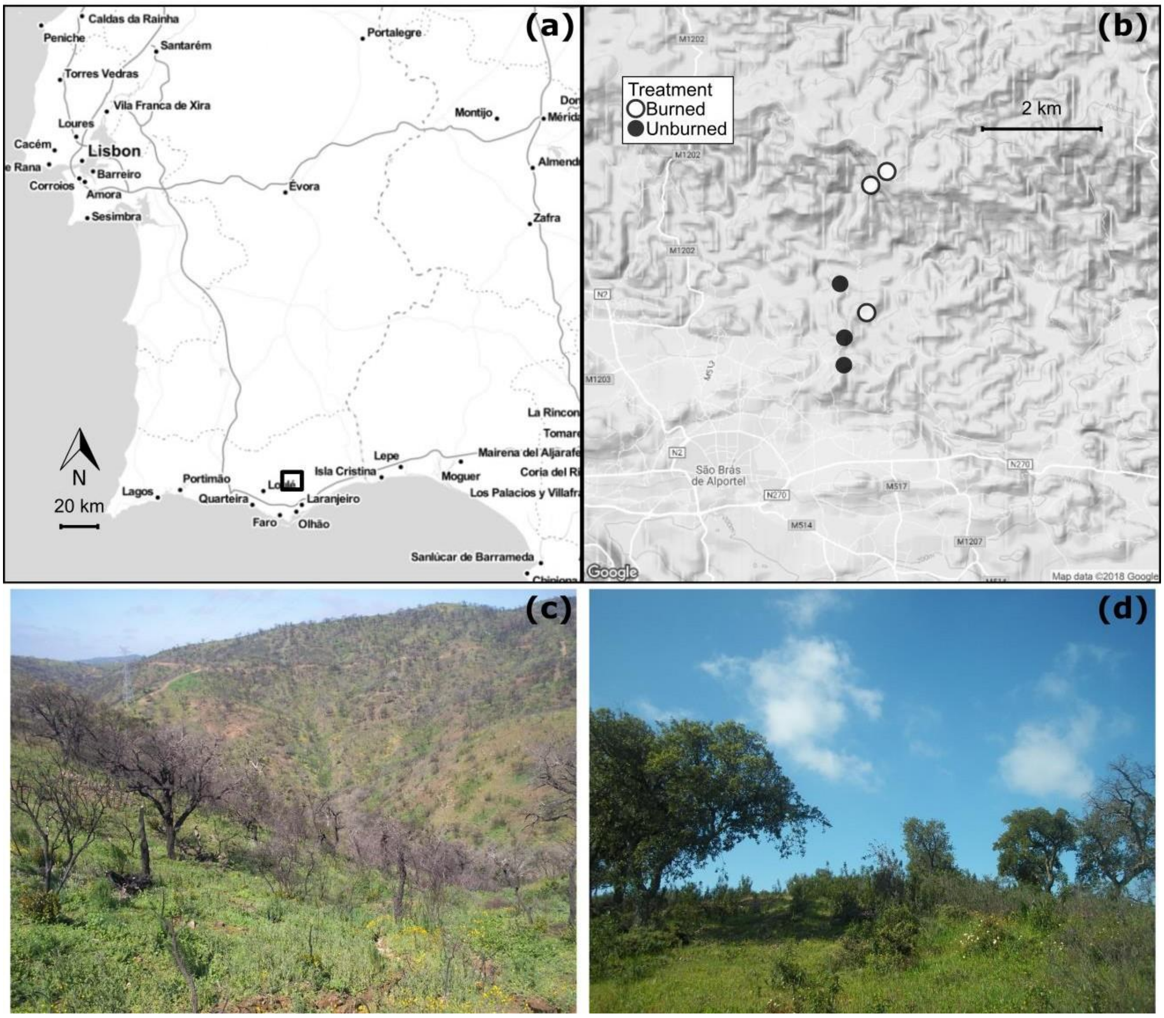


Figure S1 Location of the study sites within southern Portugal. Box on map (a) indicates the location of map (b). Points on map (b) indicate the location of the six study sites. Photographs depict typical burned (c) and unburned (d) sites and were both taken on 31st March 2013, shortly before the commencement of fieldwork. Map data © 2018 Google.

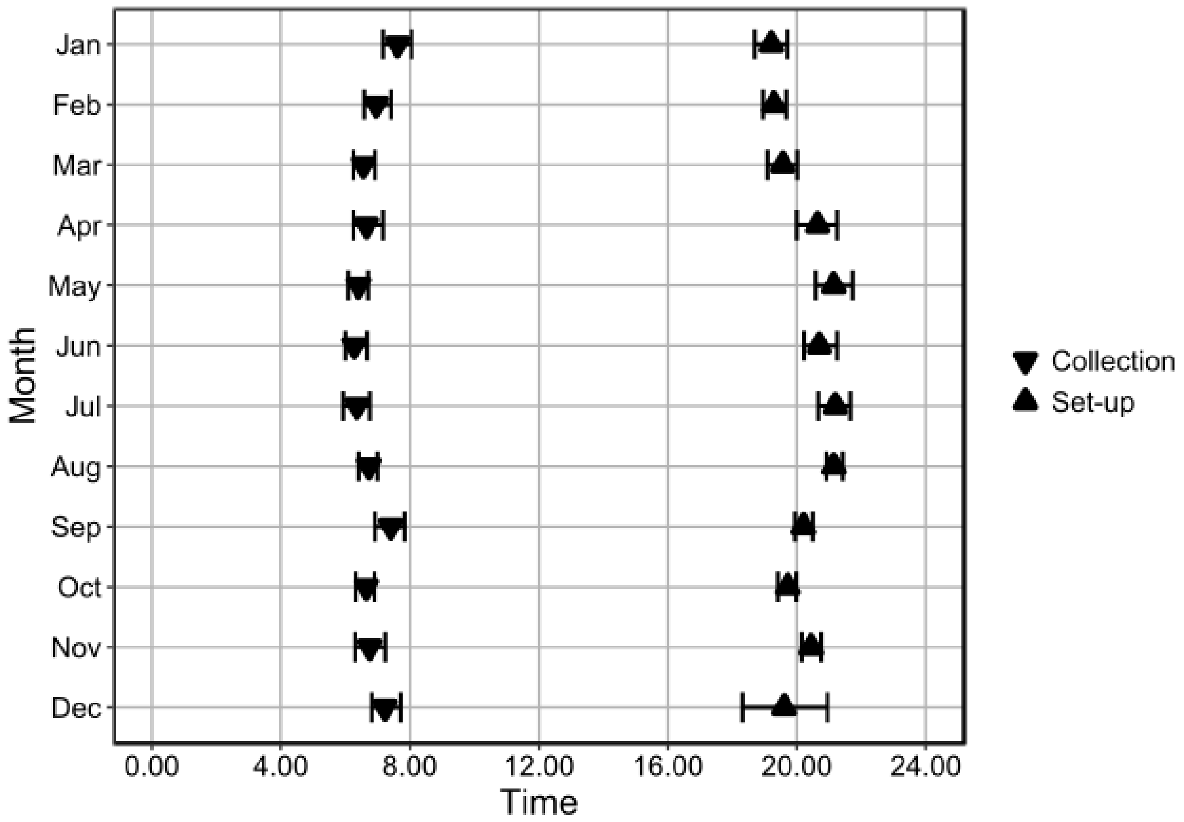


Figure S2 Monthly variation in set-up and collection time for light traps. Points show the mean monthly time for set-up and collection of light traps across all sites, treatments and years. Error bars show the range of times.

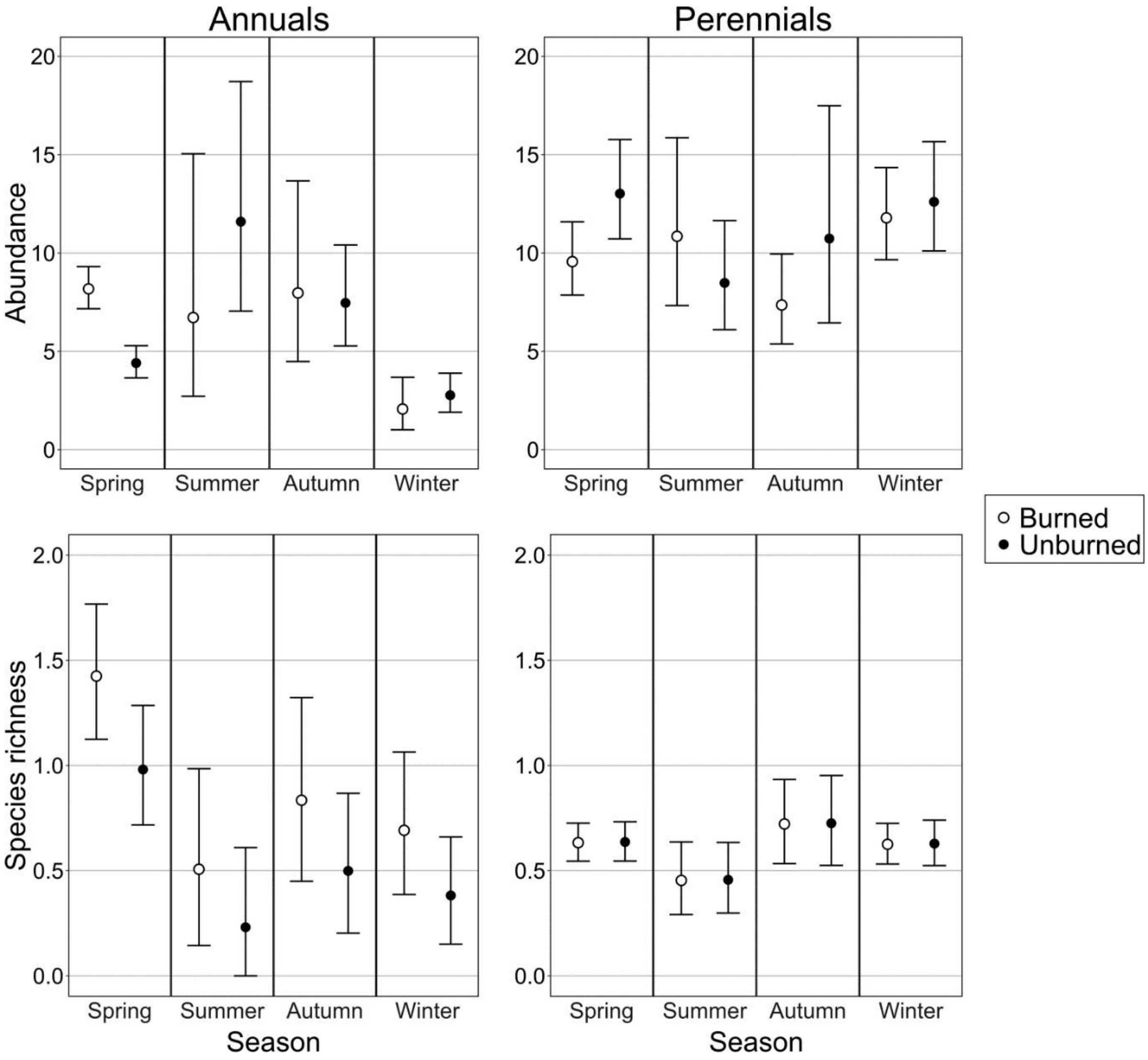


Figure S3 The effects of fire on abundance and species richness of flowers, accounting for different life-histories (Table S12). Graphs in the left-hand column show analyses for a subset of the data containing annuals and biennials only, and graphs in the right-hand column show the same analyses for a subset of the data containing all other flowers (perennials, bulbs, shrubs and trees). Stars show significance; *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

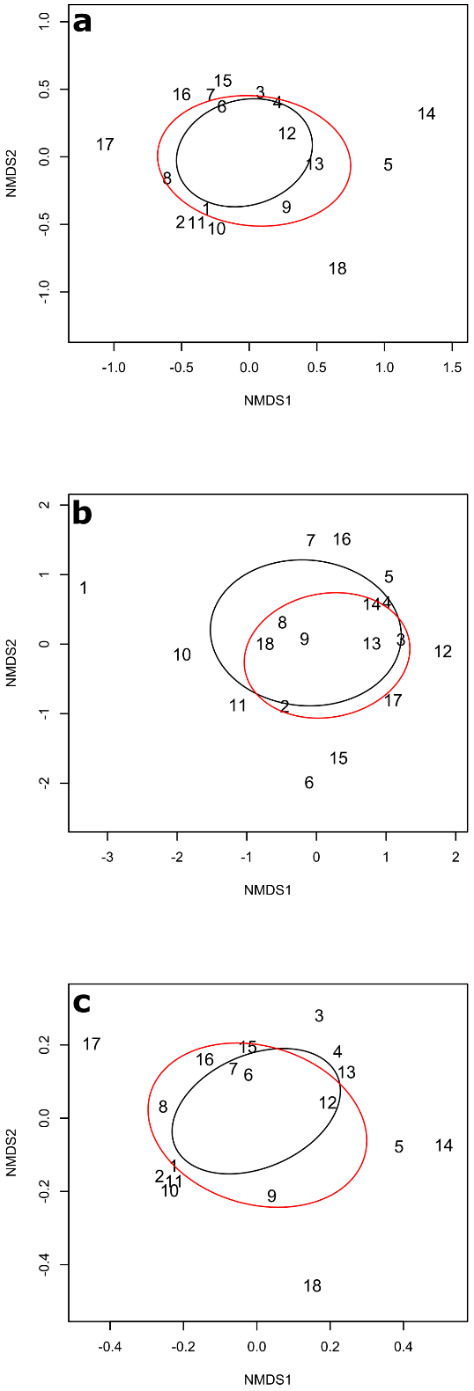


Figure S4 The effects of fire on community composition of moths (a), flowering plants (b) and interactions (c), represented by non-metric multidimensional scaling (Table S6). Each network in the study (n = 18, one burned and one unburned network sampling period) is indicated on the NMDS axes by a number; numbers 1-9 indicate unburned networks and numbers 10-18 indicate burned networks. Ellipses indicate clustering of networks within treatments: black ellipses show unburned networks and red ellipses show burned networks.

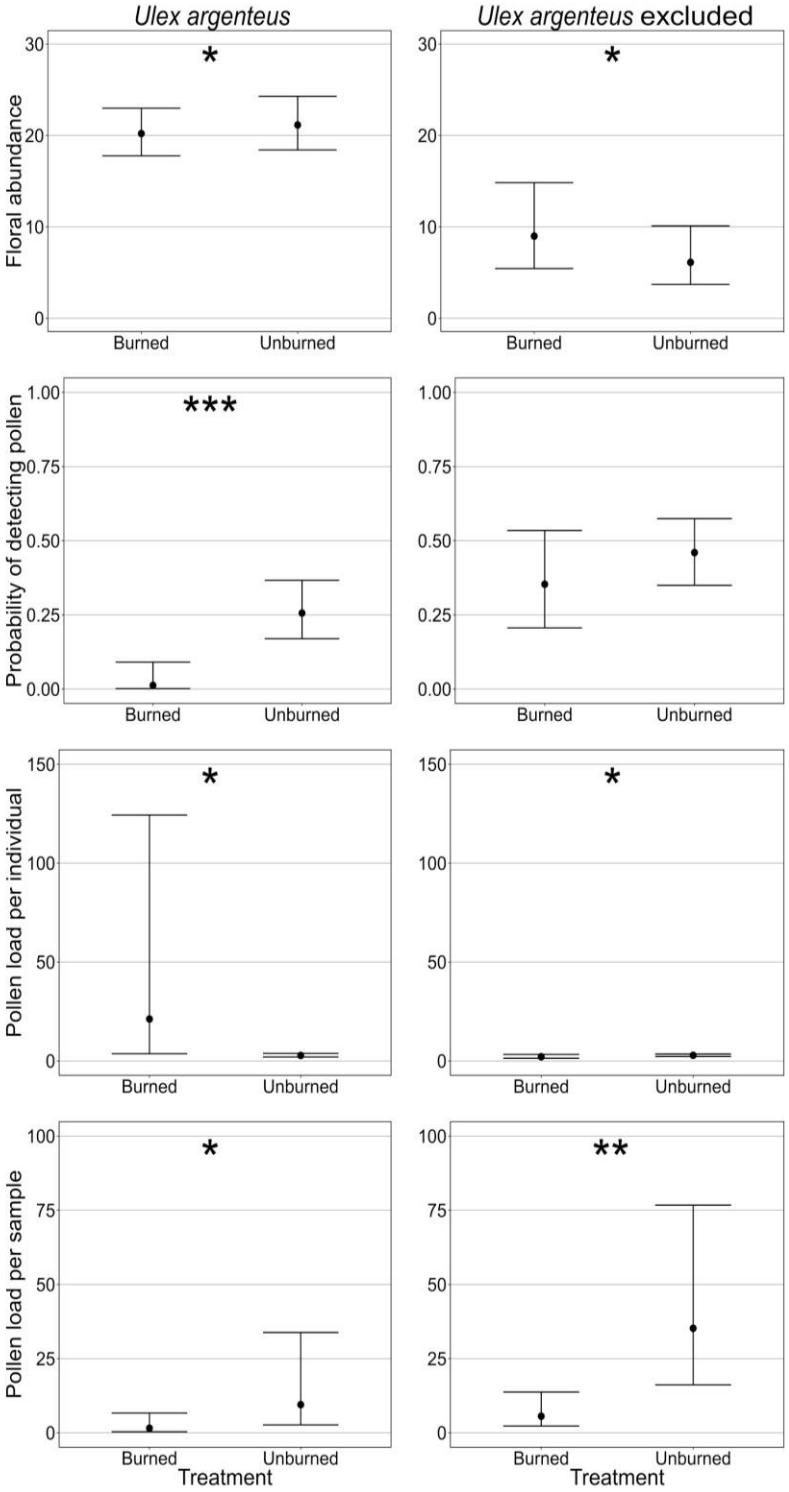


Figure S5 The effects of fire on floral abundance and pollen transport in winter, accounting for the influence of the most abundant flower, *Ulex argenteus* (Table S13). The dataset was divided in two: data concerning *U. argenteus* only, and all other data. The same analyses were conducted on each dataset. Graphs in the left-hand column show analyses for *U. argenteus* only, and graphs in the right-hand column show the same analyses for all other flowers combined. Stars show significance; *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

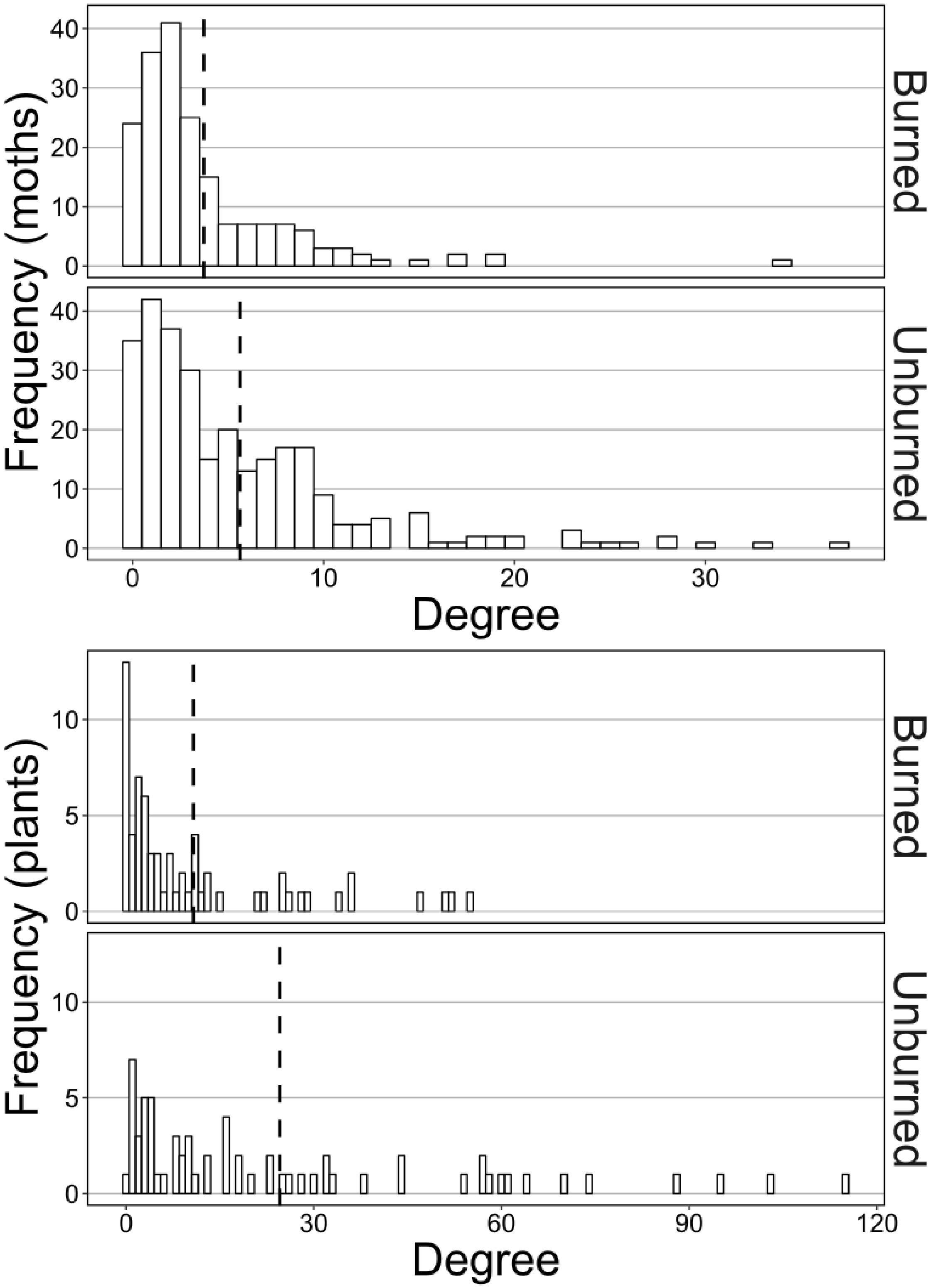


Figure S6 Degree distributions of insect and plant species in burned and unburned treatments (Table S14). Dashed lines show mean degree.

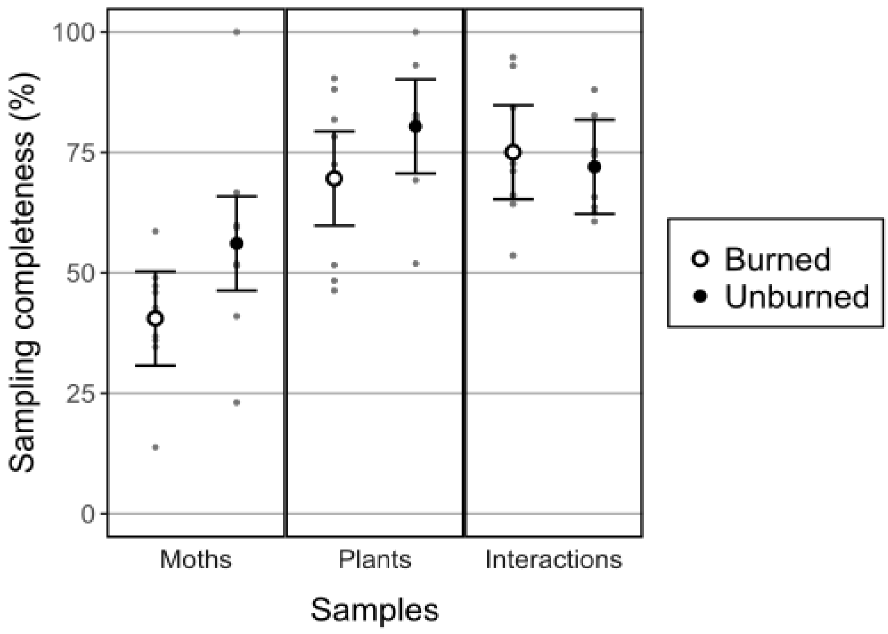


Figure S7 The level of sampling completeness for each of the 18 networks for moths, plants and interactions, separated by burning treatment (Table S11). Points in grey are the values for individual networks. Circles are the model-predicted mean values (open = burned sites, closed = unburned sites), and error bars the 95% confidence intervals.

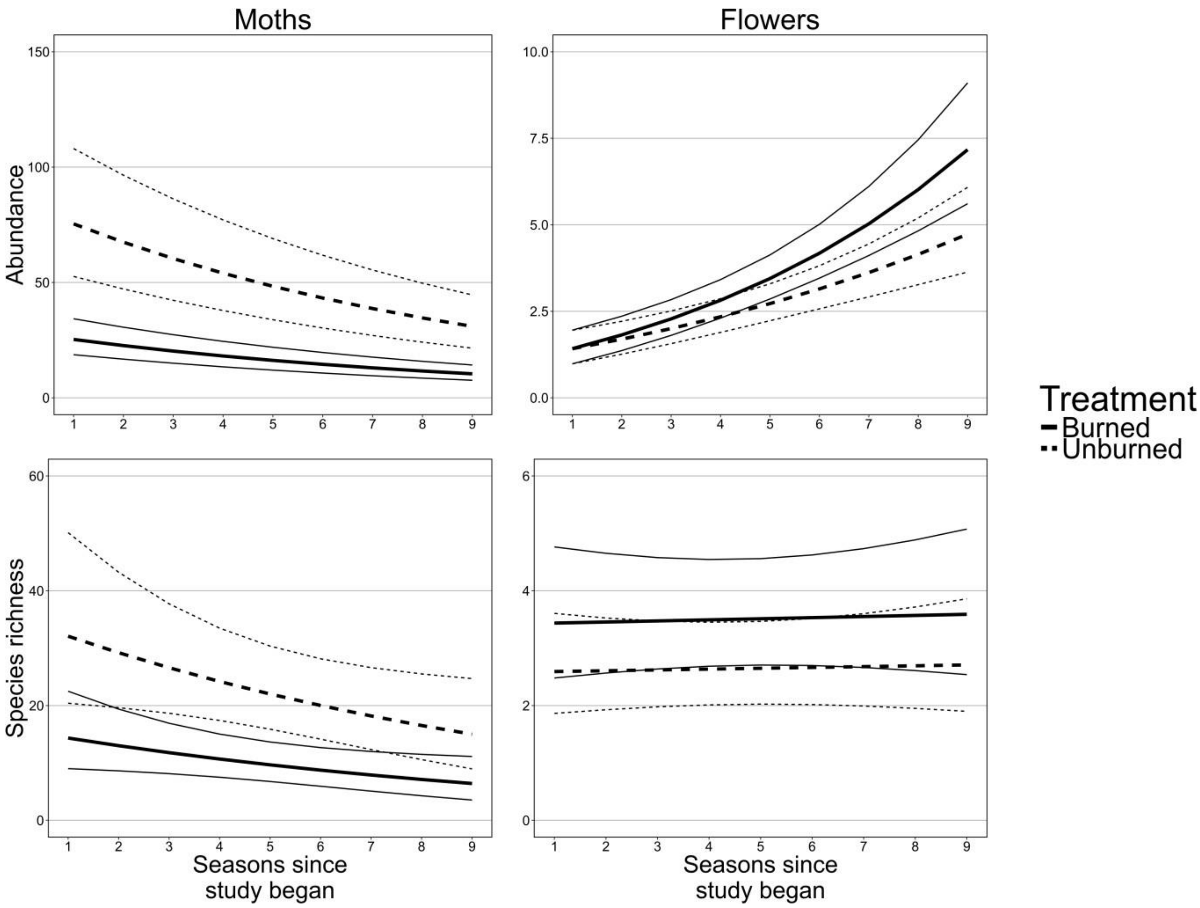


Figure S8 The effects of fire and sampling period (seasons since the study began) on the abundance and species richness of moths and flowers. For moths, lines represent the model- predicted abundance and species richness per trap; for flowers, lines represent the model- predicted percentage cover and species richness per transect. Quantile lines show 95% confidence intervals. For clarity, the effect of season was omitted when making predictions, so lines effectively show the trend if all seasons were autumn; however, no significant interactions were found between season and sampling period in any analysis, so trends are expected to be the same in all seasons. Analyses of moth abundance and species richness were based on moth-trap samples (n = 73); analyses of floral abundance and species richness were based on 1 x 1 m quadrats (n = 1260).

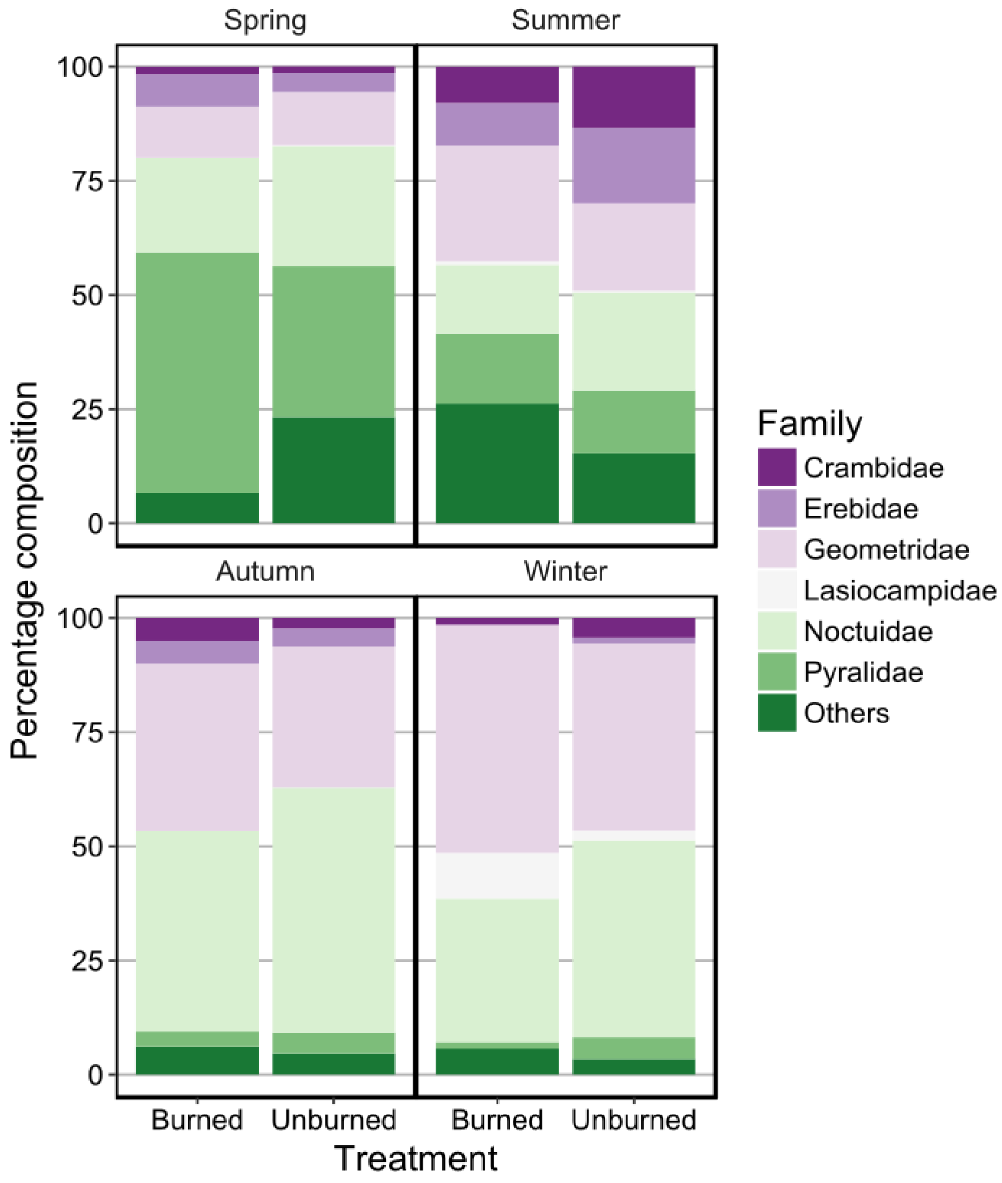


Figure S9 Assemblage composition by family of moths for each treatment and season. Families never comprising >10% of individuals in any combination of season and treatment are grouped as “Others”, and all other families are shown independently.

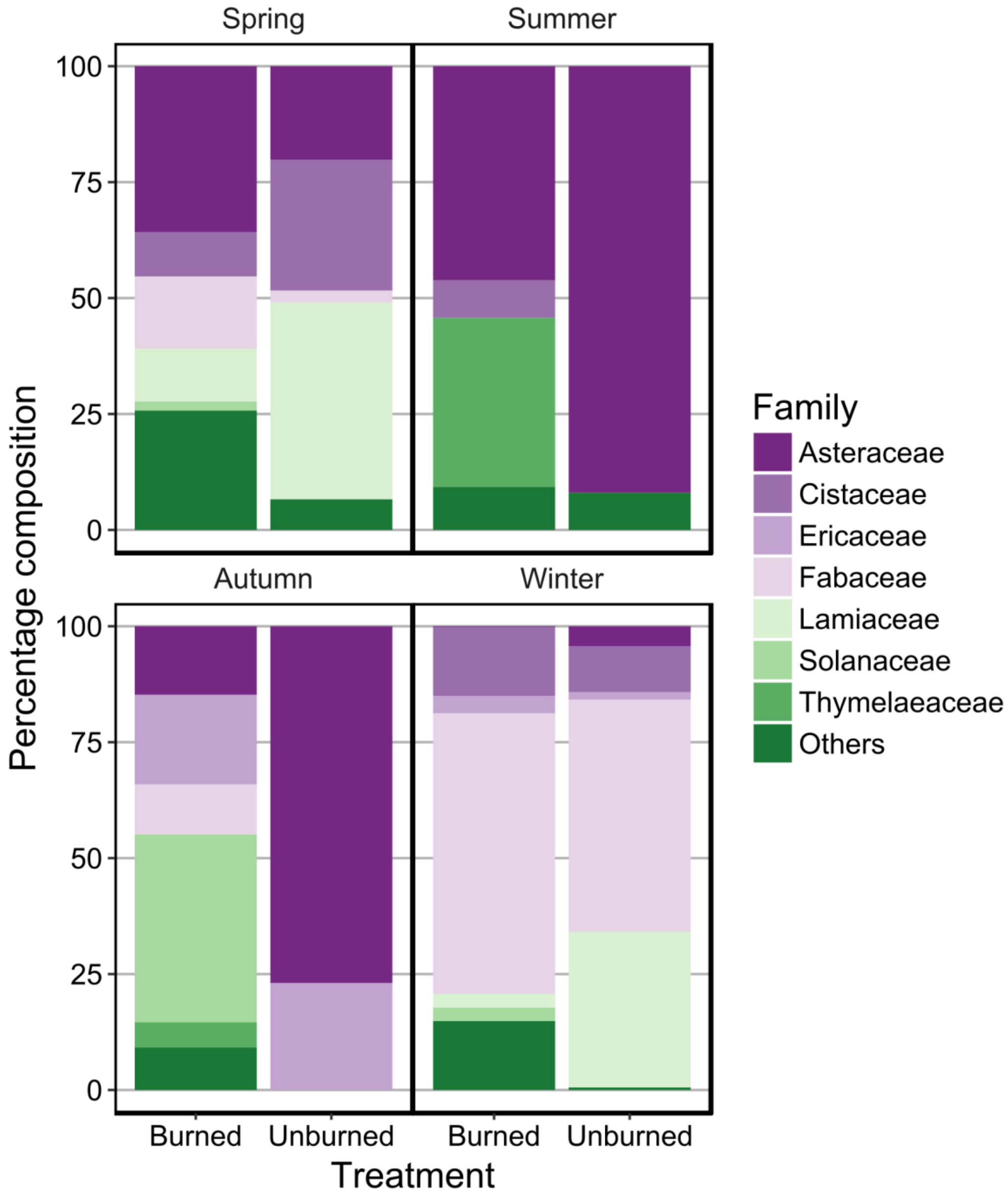


Figure S10 Assemblage composition by family of flowering plants for each treatment and season. Families never comprising >10% of individuals in any combination of season and treatment are grouped as "Others", and all other families are shown independently.

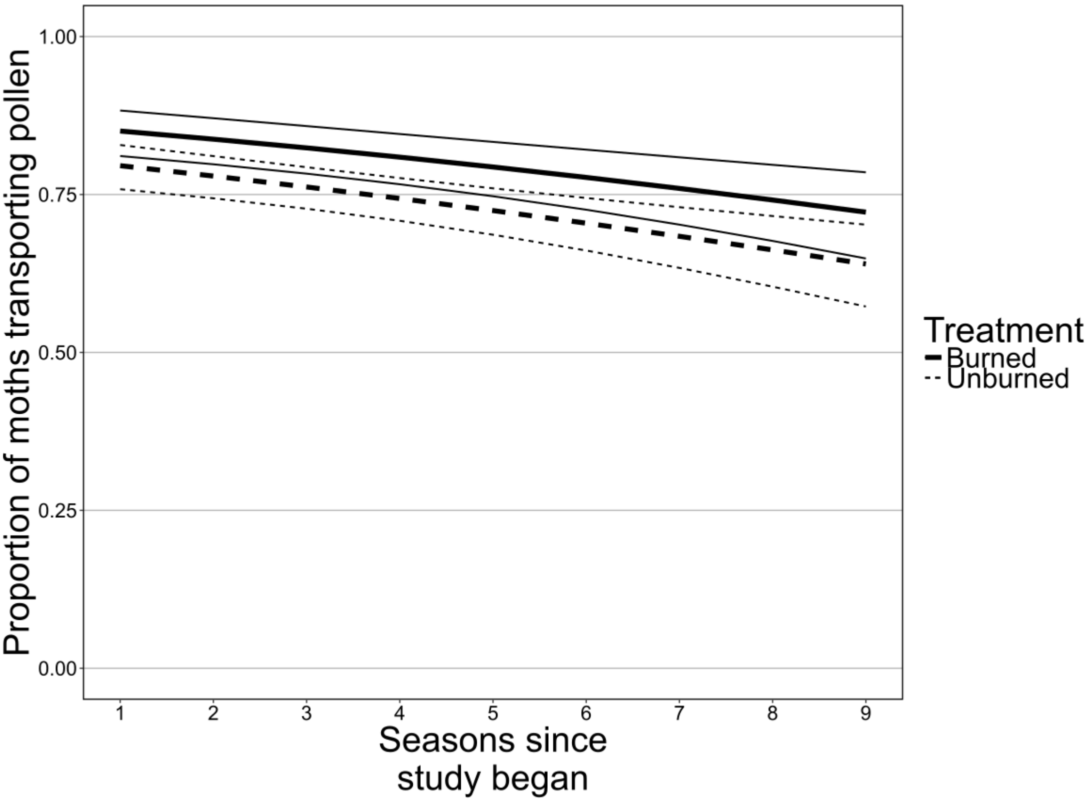


Figure S11 The effects of fire and sampling period (seasons since the study began) on the likelihood of detecting pollen on a moth. Lines represent the model-predicted proportion of moths found to be carrying pollen. Quantile lines show 95% confidence intervals. For clarity, the effect of season was omitted when making predictions, so lines effectively show trends if all seasons were autumn; however, no significant interaction was found between season and sampling period, so trends are expected to be the same in all seasons. Analyses were based on individual moths (n = 3406).

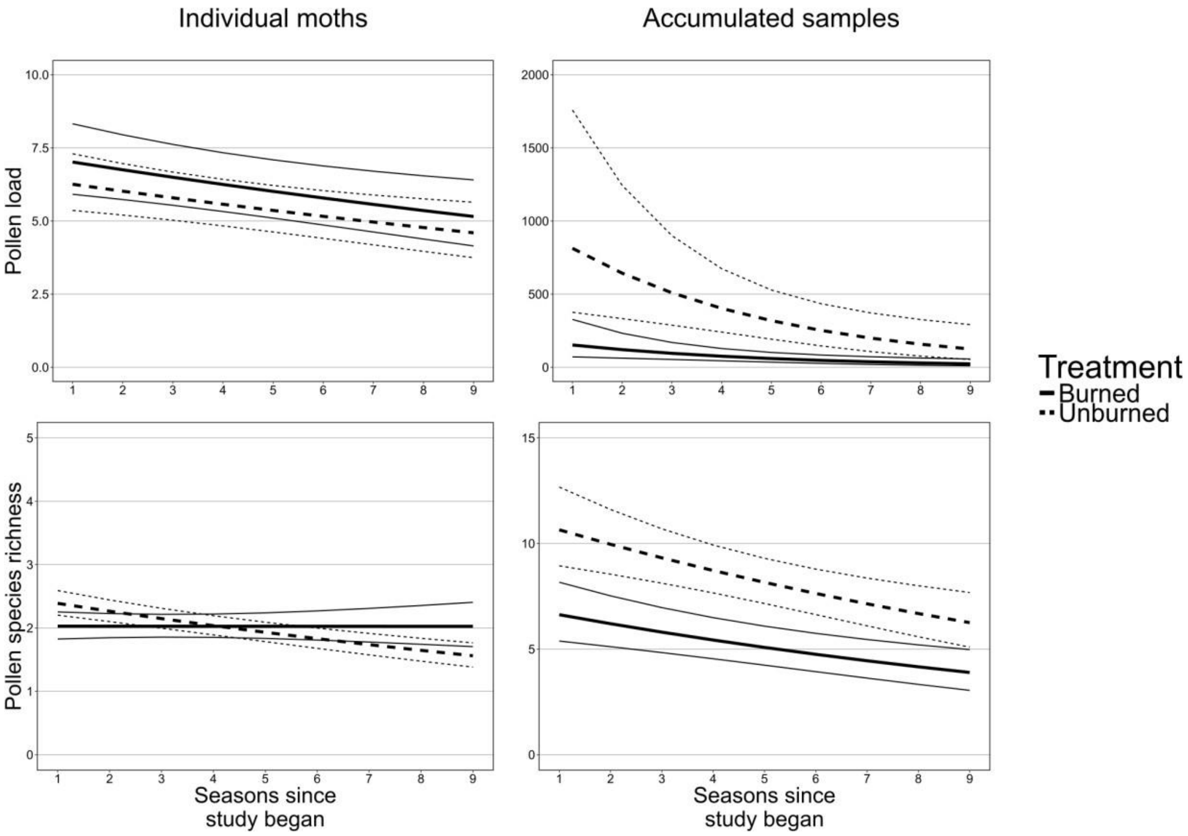


Figure S12 The effects of fire and sampling period (seasons since the study began) on the pollen loads of moths. Lines represent the model-predicted pollen load and species richness of pollen of individual moths and the cumulative pollen load and richness of all moths in a sample. Quantile lines show 95% confidence intervals. For clarity, the effect of season was omitted when making predictions, so lines effectively show trends if all seasons were autumn; however, no significant interactions were found between season and sampling period in any analysis, so trends are expected to be the same in all seasons. Analyses of the pollen loads of individual moths were based on pollen-carrying moths (n = 2394); analyses of accumulated samples of pollen were based on moth-trap samples (n = 73).

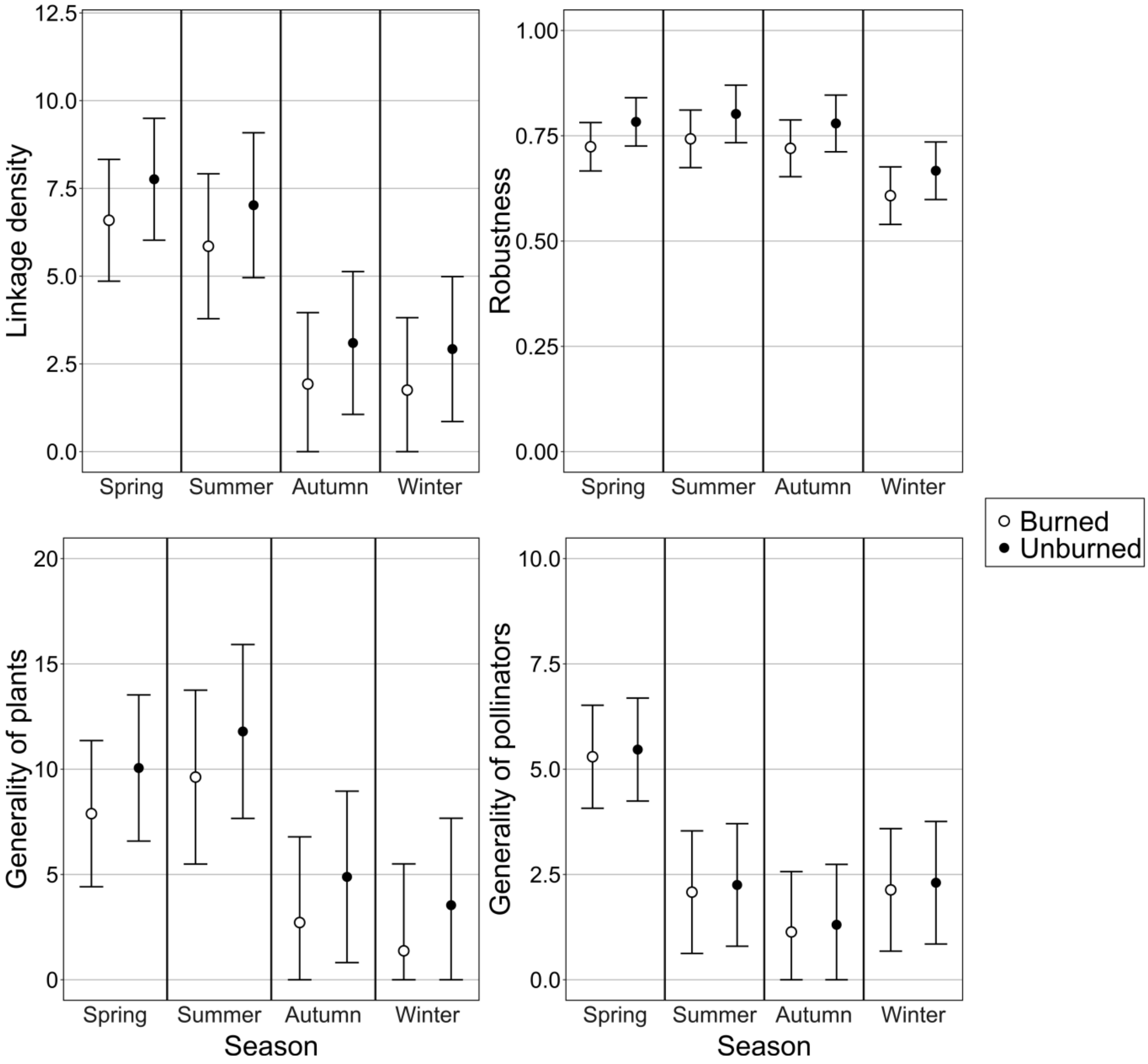


Figure S13 The effects of fire and season on a selection of network metrics (linkage density, robustness, generality of plants and of pollinators) calculated for quantitative, pollen load- weighted, pollen-transport networks. Circles represent the model-predicted network metrics (open = burned networks, closed = unburned networks) and error bars show 95% confidence intervals. Analyses were based on one burned network and one unburned network for each sampling period in the study (n = 18).

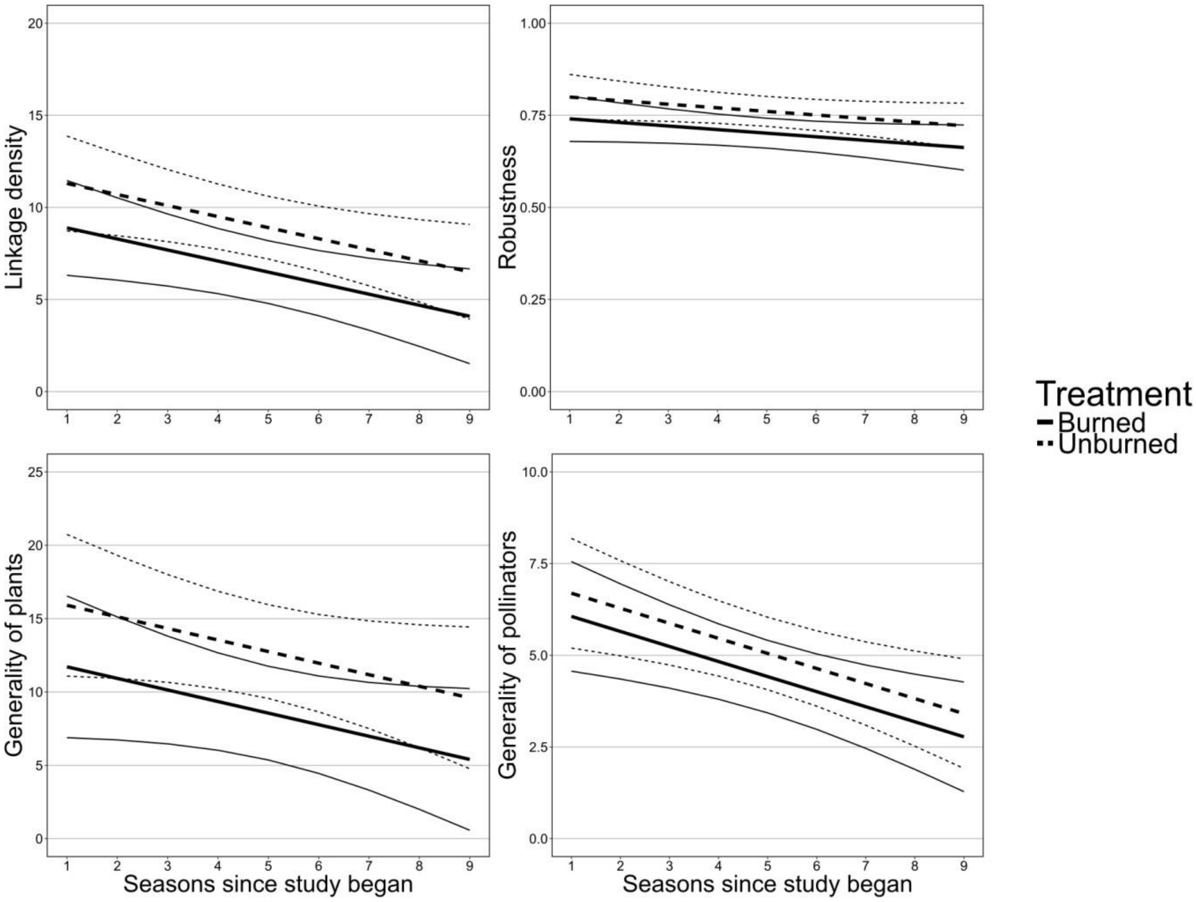


Figure S14 The effects of fire and sampling period (seasons since the study began) on quantitative, interaction frequency-weighted, pollen-transport network metrics. Lines represent the model-predicted network metrics. Quantile lines show 95% confidence intervals. For clarity, the effect of season was omitted when making predictions, so lines effectively show trends if all seasons were autumn; however, no significant interactions were found between season and sampling period in any analysis, so trends are expected to be the same in all seasons. Analyses were based on one burned network and one unburned network for each sampling period in the study (n = 18).

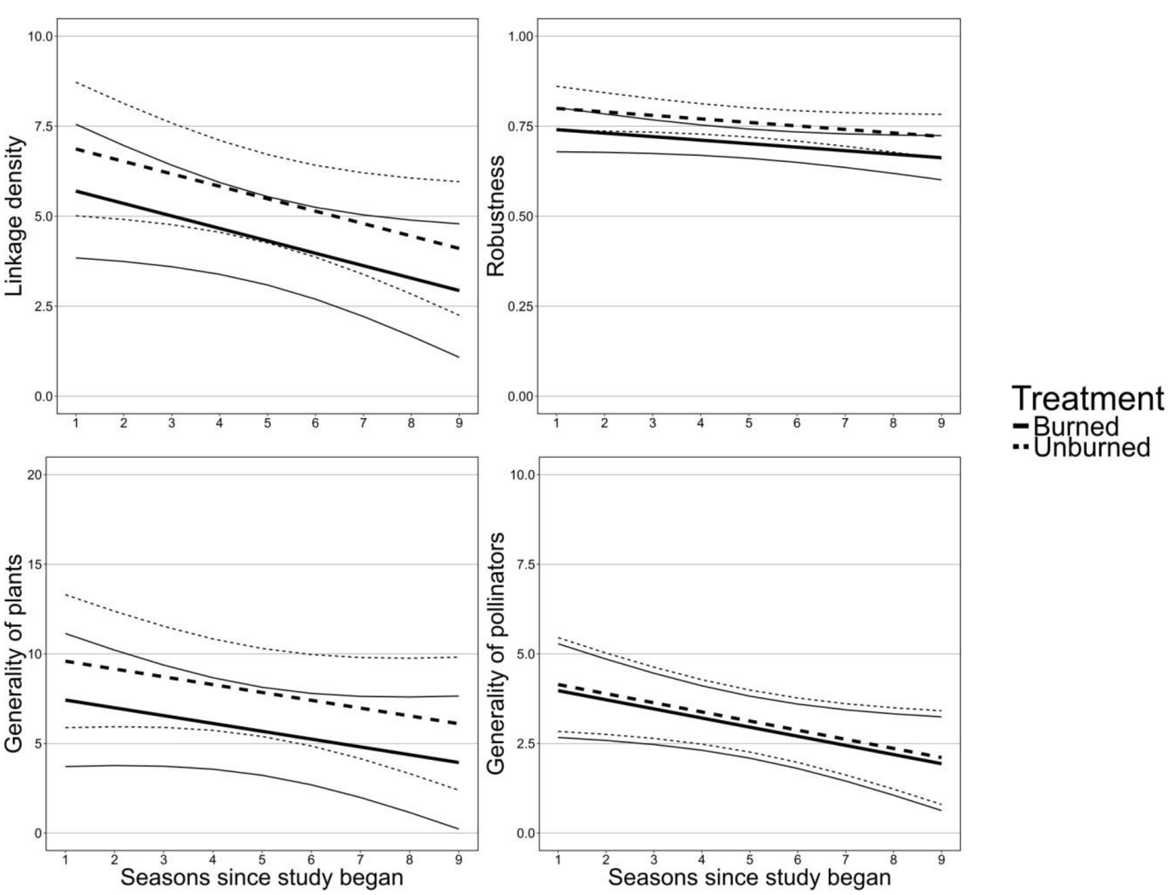


Figure S15 The effects of fire and sampling period (seasons since the study began) on quantitative, pollen load-weighted, pollen-transport network metrics. Lines represent the model-predicted network metrics. Quantile lines show 95% confidence intervals. For clarity, the effect of season was omitted when making predictions, so lines effectively show trends if all seasons were autumn; however, no significant interactions were found between season and sampling period in any analysis, so trends are expected to be the same in all seasons. Analyses were based on one burned network and one unburned network for each sampling period in the study (n = 18).