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Short-term positive effects of wildfire on diurnal insects and pollen transport in a Mediterranean ecosystem

Paula Banza[1,2*], Darren M. Evans[3], Renata Medeiros[4], Callum J. Macgregor[3,5] & Anabela D.F. Belo[6]

[1]: MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada (IIFA), Universidade de Évora – Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

[2]: A Rocha Portugal, Ap. 41, 8501-903 Mexilhoeira Grande, Portugal.

[3]: School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK.

[4]: Cardiff School of Dentistry, Cardiff University, Heath Park, Academic Av., Cardiff, CF14 4XY, UK.

[5]: Energy and Environment Institute, University of Hull, Kingston-upon-Hull, HU6 7RX, UK.

[6]: MED – Mediterranean Institute for Agriculture, Environment and Development, Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora – Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

*: corresponding author. Email: paula.banza@arocha.org

Running title: **Wildfire and pollen transport on diurnal insects**

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 in Southern Portugal. By comparing samples collected at burned and adjacent unburned sites, we examined wildfire effects on a) abundance and species richness of insects across seasons, b) pollen being transported; c) three of the most abundant species: *Oxythyrea funesta*, *Heliothaurus ruficolis* (both Coleoptera) and *Apis mellifera* (Hymenoptera).
3. Wildfire and season had significant, interacting effects on the abundance of insects but not species richness. Abundance and species richness increased over time at both burned and unburned sites, most notably each spring.
4. Pollen loads, and species richness, found 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 sites in the spring, but in the winter was significantly higher in burned sites; there were no significant differences in summer and autumn. *H. ruficolis* abundance was higher in burned sites. *A. mellifera* abundance was unaffected.
6. Overall, across almost all the community metrics, our results suggest that wildfire affects pollen transport by diurnal insects, at least in the short term, but with time these become similar to unburned habitats.

Key words: 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). 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).

Whilst the recovery of plant communities from fire is generally understood, the importance of fire recovery on animals and plant-animal mutualisms such as pollination, only recently received more attention (Brown et al. 2017; Garcia et al. 2018; Carbone 2019; Lazarina 2019; LaManna et al. 2020; Nicholson & Egan 2020). 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 1998; Vanbergen 2013).

Carbone et al. (2019) concluded that pollinators tended to be promoted after a wildfire event, increasing after fire and during early post-fire succession stages; however, fire frequency increase has the opposite effect, decreasing pollinators, especially lepidopterans. Recent research concurs that fires induces changes at the community level and influences the dynamics of pollination service. Fire characteristics such as size, frequency, intensity ad patchiness, as well as the life history traits of organisms are crucial in determining the responses of those organisms to fire (Brown et al. 2017; Carbone et al. 2019; Pausas 2019). The magnitude of insect decline could be related to the degree of exposure to flames as well as to the mobility of insects (Swengel & Swengel 2007). Eggs and larvae are considered the most vulnerable to fire due to the lack of mobility to escape or avoid fire (Anderson et al. 1989: Huesbschman & Bragg 2000), but inactivity may be beneficial if food resources are low immediately following the fire. Different feeding guild locations affect insect responses to fire because they are exposed in different ways to flame and heat. Above ground individuals are potentially more at risk of higher mortality because there are fewer areas of refuge (Kral et al. 2017). Brown et al. (2017) refer that nest location and floral resource utilization, primarily mediate pollinator survival after fire; by nesting above ground, univoltine pollinators may be particularly vulnerable under expected fire regime changes.

Ecosystem changes affect the distribution, abundance, and effectiveness of pollinators (MA 2005; Biesmeijer et al. 2006; Vanbergen 2013; Potts et al. 2016). Successful post-fire regeneration depends upon the reinstatement of pollination services and it 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 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, 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 diurnal insects and how does this vary across seasons? 3) How does burning affect the three more abundant species? 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 area is used mainly for honey production and hunting with very few cattle and low woodland management.

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 dominant composition and physiognomy of the vegetation mentioned above was similar in burned and unburned areas before the wildfire. There were no fire events in the area between 1990-2012 (ICNF 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.

Floral resource 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). 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

One transect of 15 m was 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. For each slide we counted the number of pollen grains (“total pollen load”) up to 200 grains above which an estimate of the total was made; total number of pollen morphotypes was also registered.

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 generalized 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 calculate the estimated values.

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 in additive form 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). These three species collectively represented 43.7% of all individual insects sampled (13.8% *Apis mellifera*, 14.3% *Heliotaurus ruficollis* and 15.6% *Oxyhtyrea funesta*). 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 (LRT); 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, since LRT tests for improvement in model fit from inclusion of the term of interest, and two interacting variables are treated as a single term). 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 plants in flower were recorded in the study area (see Banza et al. 2019 for the analyses of survey results). The most representative plant families are shown in Figure 1 (and Table S5, Supporting Information). Floral resources were more abundant and species-rich in spring across all plots, and at burned sites in winter. We observed different effects of burning on spring-time floral resources provided by annual and perennial plants: annual flowers were more abundant and species-rich at burned sites than unburned sites, whereas perennial flowers were less abundant (but equally species-rich) at burned sites.

A total of 572 insects were caught and identified into 138 morphotypes (Table S4, Supporting Information). Our total sample contained at least 138 taxa of at least 44 families mainly from the Orders Coleoptera, Lepidoptera, Hymenoptera and Diptera. The assembly composition of insects is shown in Figure 2 (and Table S4, Supporting Information); all morphotypes were included in subsequent analyses, regardless of the level of identification. 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.

Almost every caught insect was found to be carrying pollen with a total of 151,422 pollen grains. Overall, insects carried pollen from 57 plant species, representing 81.5% of the 70 plant species identified on floral surveys~~.~~ 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* Welw. ex Webb; in the winter, these were *Lithodora prostrata* (Loisel.) Griseb., *Ulex argenteus*; 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).

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

Fire and sampling period had significant, interacting effects on the abundance of insects (χ2 / LRT = 23.645, d.f.= 8, p=0.0026, Figure 3, Table S4), but not on estimated species richness (LRT; χ2 = 4.663, d.f.= 8, p= 0.7929). Once the interaction was removed, estimated species richness was not significantly affected by fire alone (χ2 / LRT = 0.010, d.f. = 1, p = 0.9221) but there were significant differences among sampling periods (χ2 / LRT = 71.439, d.f. = 8, p < 0.0001 - Figure 4, Table S4). The interaction between fire and season was also not significant in explaining estimated species richness (χ2 / LRT = 0.75336, d.f.= 3, p= 0.8606).

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 (Figures 3 & 4). There was no significant effect of burning on the insects' community composition at family level *(Anosim Stat R=0.667, p=0.1,* Fig 1.), although some seasonal variation was detected, driven by phenological differences between taxa. In particular, Nymphalidae and Lycaenidae (Lepidoptera) formed a large part of the community in summer, whereas the winter community was dominated by Cetoniidae (Coleoptera).

Pollen transport

Burning and sampling period had significant interacting effects on the two pollen transport metrics that we tested (Table S7, Supporting Information): the total pollen load (Figure 5) and number of pollen morphotypes (Figure 6) per pollen-carrying individual insect. Specifically, the total pollen load and number of pollen morphotypes transported by individual insects was 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 number of pollen morphotypes tended to be greater in spring compared to the other seasons; there is a general tendency for both variables to increase across the whole sampling period (Figures 5 & 6).

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 generally a return to the state comparable to the unburned area (Figures 5 & 6).

Apis mellifera

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 ($\chi 2$ / LRT = 1.205, d.f.= 3, p=0.7517) or between burning and season ($\chi 2$ / LRT = 0.9676, d.f.= 2, p= 0.6164) in the abundance of *Apis mellifera*. There was also no individual effect of burning ($\chi 2$ / LRT = 3.4344, d.f.= 1, p=0.1161) but there was a positive marginally significant effect of sampling period (χ2 / 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

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* (χ2 / LRT = 1.2573, d.f.= 1, p= 0.2622) but there was a marginally non-significant effect of burning (χ2 / LRT = 3.5781, d.f.= 1, p= 0.0585) and a significant effect of year (χ2 / 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

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 (χ2 / 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, irrespective of fire.

Wildfires generally increase plants and pollinators abundance resulting in a homogenization of species composition of both pollinators and flowering plants (LaManna et al. 2020). However, mix-severity wildfires promote regional and local species richness as well as abundance of both pollinators and flowering plants (LaManna et al. 2020). The impact of moderate fires in communities creates openings in the vegetation creating a mosaic pattern in terms of environmental heterogeneity and resources and promotes the diversity of many pollinator guilds at local landscapes (Brown et al. 2017; Lazarina 2019). So, wildfires have mixed impacts in communities (Nicholson & Egan 2019); they can negatively affect plants and pollinators, e.g. by disturbing seed germination patterns (Pausas & Keeley 2014) and mortality rates (Ne'eman et al. 2000; Thom et al. 2015).; but they can have positive effects by creating early successional habitats with more resources such as light and soil nutrients for plants (Potts et al. 2001; Swanson et al. 2011; Van Nuland et al. 2013). In generalist plants pollinator replacement can ensure plant reproduction success, depending on the level of specialization of interactions, promoting resilience to fire disturbance (García et al. 2018). 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). Our floral resource surveys (Banza et al. 2019) showed an overall increase in winter floral abundance at burned plots, whilst in spring, annual flowers were more abundant, and perennial flowers less abundant 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. The correspondence between positive responses to burning in winter and spring for both floral resources and insects may be explained by the secondary succession flush of flowering plants after the fire, creating more opportunities for insects to feed. 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 positive 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, and 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). According to Lazarina (2019), bees and wasps are central-foragers commuting between floral resources and nest-sites to provision for their offspring; therefore, bee flight might be constrained by the species-specific foraging range (maybe related with their body size, smaller bees utilize resources at a smaller spatial scale). 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* 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). Also, beetles in general, are noncentral places foragers that appear to respond to wildfires on larger spatial scales; they tend to move more freely and depend on multiple resources (Lazarina 2019).

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). Those results are in line with the conclusions of Carbone et al. (2019) about the negative response of Lepidoptera to wildfires likely due to the higher larval susceptibility to direct fire effects. 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 a state comparable to the unburned area. 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 combined diurnal and nocturnal pollinators (e.g. Knop et al. 2017; Walton et al. 2020), whereas we analysed data on diurnal pollinators (this study) and nocturnal pollinators (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. 2017). 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 impacts of fires on insect population may require study of the immediate impacts on all stages of the insect life-cycle.

Conclusions

We found evidence that wildfire affects pollen transport by diurnal insects soon after the event, with positive effects on both pollen load and insect diversity in the short term. 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 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 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.

Data Availability Statement

Data available on request from the authors.

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Author contributions

This study was instigated by P.B., A.D.F.B. and D.M.E. Field and laboratory work was conducted by P.B. The statistical analysis was conducted by R.M. and C.J.M., in consultation with P.B. and D.M.E.; and PB prepared the first draft of the manuscript. All authors contributed substantially to revising the manuscript.

Conflict of interest Statement

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

Short Supporting Material Legends

Table S1 Locations of the six study sites.

Table S2 Summary of the plant families identified on floristic surveys.

Table S3 R packages used during analysis

Table S4 Summary of captured insects.

Table S5 – Number of pollen grains found on insects.

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.

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).

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

Figure a. Assemblage composition by species of insects caught in burned and unburned areas of Serra-do-Caldeirão, Portugal, and across seasons.

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.

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.

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.

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Figure Legends

Figure 1. 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.

Figure 2. 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.

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

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