Article:

Banza, Paula, MacGregor, Callum James orcid.org/0000-0001-8281-8284, Belo, Anabela DF et al. (3 more authors) (2019) Wildfire alters the structure and seasonal dynamics of nocturnal pollen-transport networks. Functional Ecology. ISSN 0269-8463

https://doi.org/10.1111/1365-2435.13388

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

Running head: Wildfire affects nocturnal pollination

Paula Banza[a,b], Callum J. Macgregor[c,d,e,f,1], Anabela D.F. Belo[g], Richard Fox[e], Michael J.O. Pocock[d] & Darren M. Evans[c]

[a]: Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Instituto de Investigação e Formação Avançada, Universidade de Évora, Núcleo da Mitra, Ap. 94, 7006-554, Évora, Portugal.

[b]: A Rocha Portugal, Mexilhoeira Grande, Portugal

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

[d]: Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB, UK.

[e]: Butterfly Conservation, Manor Yard, East Lulworth, Wareham, Dorset, BH20 5QP, UK.

[f]: Department of Biology, University of York, Wentworth Way, York, YO10 5DD, UK.

[g]: Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Núcleo da Mitra, Ap. 94, 7006-554, Évora, Portugal.

[1]: corresponding author. Current address: Department of Biology, University of York, Wentworth Way, York, YO10 5DD, UK. Email: callumjmacgregor@gmail.com. Tel: (+44) 01904 328623. No fax available.

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.

Resumo Português (Second language abstract: Portuguese)

1. Os fogos florestais induzem padrões mundiais de biodiversidade, afectando 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 nocturnos 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 nocturnos.
2. Investigámos os efeitos dos fogos florestais nas redes de transporte de pólen por polinizadores nocturnos. 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 nocturnas 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 afectada. 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.

Keywords

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

Introduction

Wildfire drives biodiversity patterns globally through heterogeneous disturbance regimes (Kelly & Brotons, 2017). It is especially important within Mediterranean ecosystems (Faivre, Roche, Boer, McCaw, & Grierson, 2011), where wildfires have become more frequent and severe since the 1970s because agricultural abandonment has caused fuel accumulation (Moreira, Rego, & Ferreira, 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, York, Christie, & McCarthy, 2017; Ponisio et al., 2016), leading to reduced abundance of pollinators and flowers (Potts, Dafni, & Ne'eman, 2001) and reductions in plant reproductive success (Ne'eman, Dafni, & Potts, 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, York, Christie, & McCarthy, 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., 2019; Macgregor, Pocock, Fox, & Evans, 2015), and may be especially important in the Mediterranean (Banza, Belo, & Evans, 2015). They are in decline (Conrad, Warren, Fox, Parsons, & Woiwod, 2006), with probable drivers of those declines including habitat fragmentation, climate change (Fox et al., 2014), and artificial light at night (Macgregor, Evans, Fox, & Pocock, 2017; 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, Limb, Harmon, & Hovick, 2017). Fire can lead to mortality of larvae through host plant destruction (Fowles, Bailey, & Hale, 2004), subterranean pupae (Schmid, Thomas, & Rogers, 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, Waser, & Price, 2004)) than at unburned sites, because generalist species play important roles in maintaining network stability (Tylianakis, Laliberté, Nielsen, & Bascompte, 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, Ballantyne, & Willmer, 2013), it is a commonly-used proxy in pollination networks (Banza, Belo, & Evans, 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, Colwell, Lin, & Gotelli, 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, Evans, & Pocock (2017), 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, Morris, & Jordano, 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, Belo, & Evans, 2015; Devoto, Bailey, & Memmott, 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, Evans, Augustyn, & Ellis (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).

Pollen-transport

Burning and season had significant, interacting effects on four pollen-transport metrics (Table S7): the proportion of moths carrying pollen ($\chi^2 = 33.21$, $P < 0.001$), the total pollen load ($\chi^2 = 8.84$, $P = 0.032$) and number of pollen types ($\chi^2 = 11.17$, $P = 0.011$) per individual pollen-carrying moth, and the number of pollen types per sample of moths ($\chi^2 = 9.65$, $P = 0.022$). The total pollen count per sample of moths was also affected by both burning ($\chi^2 = 11.82$, $P < 0.001$) and season ($\chi^2 = 44.28$, $P < 0.001$), 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). 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.

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.

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, York, Christie, & McCarthy, 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, Ferrandis, & Martínez-Sánchez, 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, Bailey, & Hale, 2004; Gerson & Kelsey, 1997; Schmid, Thomas, & Rogers, 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, Warren, Fox, Parsons, & Woiwod, 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, Ryrholm, Stefanescu, & Hill, 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., 2019; Macgregor, Pocock, Fox, & Evans, 2015); perhaps especially in Mediterranean systems (Banza, Belo, & Evans, 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, Bullock, & Watkinson, 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, Dafni, & Potts, 2000), even for plant species that respond to fire by flowering (Geerts, Malherbe, & Pauw, 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, York, Christie, & McCarthy, 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, Evans, Augustyn, & Ellis, 2017; Olesen, Bascompte, Elberling, & Jordano, 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, Laliberté, Nielsen, & Bascompte, 2010) and restoration (Raimundo, Guimarães, & Evans, 2018), allowing resilience to be built into fire-prone ecosystems (Evans, Kitson, Lunt, Straw, & Pocock, 2016).

Acknowledgements

We thank our field assistant Todd Jenkins, and several volunteers at A Rocha Portugal who provided additional assistance in the field, in the lab, and with insect identification. We are grateful to Penny Wolf for providing funding for the field study. C.J.M. was funded by the

Natural Environment Research Council and Butterfly Conservation (Industrial CASE studentship, grant ID: NE/K007394/2). We thank Jason Tylianakis and Roy Sanderson for their helpful comments on an early draft of this manuscript.

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 C.J.M., in consultation with P.B. and D.M.E.; and C.J.M. prepared the first draft of the manuscript. All authors contributed substantially to revising the manuscript.

Data accessibility

Data will be made available from the Dryad Digital Repository upon acceptance.

References

Banza, P., Belo, A. D. F., & Evans, D. M. (2015). The structure and robustness of nocturnal Lepidopteran pollen-transfer networks in a Biodiversity Hotspot. *Insect Conservation and Diversity*, *8*(6), 538–546.

Beattie, A. J. (1972). A technique for the study of insect-borne pollen. *The Pan-Pacific Entomologist*, *47*(1), 82.

Bernays, E. A., & Chapman, R. E. (1994). Patterns of Host-Plant Use. In *Host-Plant Selection by Phytophagous Insects* (pp. 4–13). Springer US.

Brown, J., York, A., Christie, F., & McCarthy, M. (2017). Effects of fire on pollinators and pollination. *Journal of Applied Ecology*, *54*(1), 313–322.

Burgos, E., Ceva, H., Perazzo, R.P.J., Devoto, M., Medan, D., Zimmermann, M., & Delbue, A.M. (2007). Why nestedness in mutualistic networks? *Journal of Theoretical Biology*, *249*, 307–313.

Capitanio, R., & Carcaillet, C. (2008). Post-fire Mediterranean vegetation dynamics and diversity: A discussion of succession models. *Forest Ecology and Management*, *255*(3–4), 431–439.

Castroviejo, S. (Ed.). (1986-2014). *Flora Iberica. Plantas vasculares de la Península Ibérica e Islas Baleares*. Real Jardín Botanico, Madrid.

Chao, A. (1987). Estimating the population size for capture-recapture data with unequal catchability. *Biometrics*, *43*(4), 783–791.

Chao, A., Colwell, R. K., Lin, C.-W., & Gotelli, N. J. (2009). Sufficient sampling for asymptotic minimum species richness estimators. *Ecology*, *90*(4), 1125–1133.

Conrad, K. F., Warren, M. S., Fox, R., Parsons, M. S., & Woiwod, I. P. (2006). Rapid declines of common, widespread British moths provide evidence of an insect biodiversity crisis. *Biological Conservation*, *132*(3), 279–291.

Del Socorro, A. P., & Gregg, P. C. (2001). Sunflower (*Helianthus annuus* L.) pollen as a marker for studies of local movement in *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *Australian Journal of Entomology*, *40*(3), 257–263.

Derocles, S. A. P., Bohan, D. A., Dumbrell, A. J., Kitson, J. J. N., Massol, F., Pauvert, C., … Evans, D. M. (2018). Chapter One - Biomonitoring for the 21st Century: Integrating Next-Generation Sequencing Into Ecological Network Analysis. In D. A. Bohan, A. J. Dumbrell, G. Woodward, & M. Jackson (Eds.), *Advances in Ecological Research* (Vol. 58, pp. 1–62). Academic Press.

Devoto, M., Bailey, S., & Memmott, J. (2011). The “night shift”: nocturnal pollen-transport networks in a boreal pine forest. *Ecological Entomology*, *36*(1), 25–35.

Evans, D. M., Kitson, J. J. N., Lunt, D. H., Straw, N. A., & Pocock, M. J. O. (2016). Merging DNA metabarcoding and ecological network analysis to understand and build resilient terrestrial ecosystems. *Functional Ecology*, *30*(12), 1904–1916.

Faivre, N., Roche, P., Boer, M. M., McCaw, L., & Grierson, P. F. (2011). Characterization of landscape pyrodiversity in Mediterranean environments: contrasts and similarities between south-western Australia and south-eastern France. *Landscape Ecology*, *26*(4), 557–571.

Flannigan, M., Cantin, A. S., de Groot, W. J., Wotton, M., Newbery, A., & Gowman, L. M. (2013). Global wildland fire season severity in the 21st century. *Forest Ecology and Management*, *294*, 54–61.

Fowles, A. P., Bailey, M. P., & Hale, A. D. (2004). Trends in the recovery of a rosy marsh moth *Coenophila subrosea* (Lepidoptera, Noctuidae) population in response to fire and conservation management on a lowland raised mire. *Journal of Insect Conservation*, *8*(2-3), 149–158.

Fox, R., Oliver, T. H., Harrower, C., Parsons, M. S., Thomas, C. D., & Roy, D. B. (2014). Long-term changes to the frequency of occurrence of British moths are consistent with opposing and synergistic effects of climate and land-use changes. *Journal of Applied Ecology*, *51*(4), 949–957.

Geerts, S., Malherbe, S. D. T., & Pauw, A. (2011). Reduced flower visitation by nectar-feeding birds in response to fire in Cape fynbos vegetation, South Africa. *Journal of Ornithology*, *153*(2), 297–301.

Gerson, E. A., & Kelsey, R. G. (1997). Attraction and direct mortality of pandora moths, *Coloradia pandora* (Lepidoptera: Saturniidae), by nocturnal fire. *Forest Ecology and Management*, *98*(1), 71–75.

Heath, J. (1965). A genuinely portable MV light trap. *Entomologist's Record and Journal of Variation*, *77*, 236–238.

Herranz, J. M., Ferrandis, P., & Martínez-Sánchez, J. J. (1998). Influence of heat on seed germination of seven Mediterranean Leguminosae species. *Plant Ecology*, *136*(1), 95–

103.

Kelly, L. T., & Brotons, L. (2017). Using fire to promote biodiversity. *Science*, *355*(6331), 1264–1265.

Kemp, J. E., Evans, D. M., Augustyn, W. J., & Ellis, A. G. (2017). Invariant antagonistic network structure despite high spatial and temporal turnover of interactions. *Ecography*, *40*(11), 1315–1324.

King, C., Ballantyne, G., & Willmer, P. G. (2013). Why flower visitation is a poor proxy for pollination: measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods in Ecology and Evolution*, *4*(9), 811–818.

Knop, E., Zoller, L., Ryser, R., Gerpe, C., Hörler, M., & Fontaine, C. (2017). Artificial light at night as a new threat to pollination. *Nature*, *548*(7666), 206–209.

Kral, K. C., Limb, R. F., Harmon, J. P., & Hovick, T. J. (2017). Arthropods and fire: Previous research shaping future conservation. *Rangeland Ecology & Management*, *70*(5), 589–598.

Macgregor, C. J., Evans, D. M., Fox, R., & Pocock, M. J. O. (2017). The dark side of street lighting: impacts on moths and evidence for the disruption of nocturnal pollen transport. *Global Change Biology*, *23*(2), 697–707.

Macgregor, C. J., Evans, D. M., & Pocock, M. J. O. (2017). Estimating sampling completeness of interactions in quantitative bipartite ecological networks: incorporating variation in species specialisation. *bioRxiv*. doi:10.1101/195917

Macgregor, C. J., Kitson, J. J. N., Fox, R., Hahn, C., Lunt, D. H., Pocock, M. J. O., & Evans, D. M. (2019). Construction, validation, and application of nocturnal pollen transport networks in an agro-ecosystem: a comparison using light microscopy and DNA metabarcoding: Constructing nocturnal pollination networks. *Ecological Entomology*, *44*(1), 17–29.

Macgregor, C. J., Pocock, M. J. O., Fox, R., & Evans, D. M. (2015). Pollination by nocturnal Lepidoptera, and the effects of light pollution: a review. *Ecological Entomology*, *40*(3), 187–198.

Manley, C. (2008). *British Moths and Butterflies: A Photographic Guide*. London, UK: A & C Black.

Memmott, J., Waser, N. M., & Price, M. V. (2004). Tolerance of pollination networks to species extinctions. *Proceedings of the Royal Society B: Biological Sciences*, *271*(1557), 2605–2611.

Moreira, F., Rego, F. C., & Ferreira, P. G. (2001). Temporal (1958–1995) pattern of change in a cultural landscape of northwestern Portugal: implications for fire occurrence. *Landscape Ecology*, *16*(6), 557–567.

Ne'eman, G., Dafni, A., & Potts, S. G. (2000). The effect of fire on flower visitation rate and fruit set in four core-species in east Mediterranean scrubland. *Plant Ecology*, *146*(1), 97–104.

Olesen, J. M., Bascompte, J., Elberling, H., & Jordano, P. (2008). Temporal dynamics in a pollination network. *Ecology*, *89*(6), 1573–1582.

Oliver, T. H., Heard, M. S., Isaac, N. J. B., Roy, D. B., Procter, D., Eigenbrod, F., … Bullock, J. M. (2015). Biodiversity and resilience of ecosystem functions. *Trends in Ecology & Evolution*, *30*(11), 673–684.

Parmesan, C., Ryrholm, N., Stefanescu, C., & Hill, J. K. (1999). Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature*, *399*(6736), 579–583.

Pausas, J. G., & Fernández-Muñoz, S. (2011). Fire regime changes in the Western Mediterranean Basin: from fuel-limited to drought-driven fire regime. *Climatic Change*, *110*(1-2), 215–226.

Ponisio, L. C., Wilkin, K., M'Gonigle, L. K., Kulhanek, K., Cook, L., Thorp, R., … Kremen, C. (2016). Pyrodiversity begets plant–pollinator community diversity. *Global Change Biology*, *22*(5), 1794–1808.

Potts, S. G., Dafni, A., & Ne'eman, G. (2001). Pollination of a core flowering shrub species in Mediterranean phrygana: variation in pollinator diversity, abundance and effectiveness in response to fire. *Oikos*, *92*(1), 71–80.

Potts, S. G., Vulliamy, B., Dafni, A., Ne'eman, G., O'Toole, C., Roberts, S., & Willmer, P. (2003). Response of plant-pollinator communities to fire: changes in diversity, abundance and floral reward structure. *Oikos*, *101*(1), 103–112.

Price, C., & Rind, D. (1994). The impact of a 2× CO2 climate on lightning-caused fires. *Journal of Climate*, *7*(10), 1484–1494.

Raimundo, R. L. G., Guimarães, P. R., Jr, & Evans, D. M. (2018). Adaptive networks for restoration ecology. *Trends in Ecology & Evolution*, *33*(9), 664–675.

R Core Team. (2018). R: A language and environment for statistical computing (Version 3.4.4). Vienna, Austria: R Foundation for Statistical Computing. http://www.r-project.org/

Schmid, J. M., Thomas, L., & Rogers, T. J. (1981). *Prescribed Burning to Increase Mortality of Pandora Moth Pupae*. U.S.D.A. Forest Service, Rocky Mountain Forest and Range Experiment Station.

Sterling, P., & Parsons, M. (2012). *Field Guide to the Micro-Moths of Great Britain and Ireland* (First Edition). Gillingham: British Wildlife Publishing Ltd.

Stokes, K. E., Bullock, J. M., & Watkinson, A. R. (2003). *Ulex gallii* Planch. and *Ulex minor* Roth. *Journal of Ecology*, *91*(6), 1106–1124.

Tylianakis, J. M., Laliberté, E., Nielsen, A., & Bascompte, J. (2010). Conservation of species interaction networks. *Biological Conservation*, *143*(10), 2270–2279.

Uchida, K., & Ushimaru, A. (2014). Biodiversity declines due to abandonment and intensification of agricultural lands: patterns and mechanisms. *Ecological Monographs*, *84*(4), 637–658.

van Langevelde, F., Braamburg-Annegarn, M., Huigens, M. E., Groendijk, R., Poitevin, O., van Deijk, J. R., … WallisDeVries, M. F. (2018). Declines in moth populations stress the need for conserving dark nights. *Global Change Biology*, *24*(3), 925–932.

Van Nuland, M. E., Haag, E. N., Bryant, J. A. M., Read, Q. D., Klein, R. N., Douglas, M. J., … Bailey, J. K. (2013). Fire promotes pollinator visitation: implications for ameliorating declines of pollination services. *PloS One*, *8*(11), e79853.

Vázquez, D. P., Morris, W. F., & Jordano, P. (2005). Interaction frequency as a surrogate for the total effect of animal mutualists on plants. *Ecology Letters*, *8*(10), 1088–1094.

Waring, P., & Townsend, M. (2009). *Field Guide to the Moths of Great Britain and Ireland* (2nd Edition). Gillingham: British Wildlife Publishing Ltd.

Welti, E. A. R., & Joern, A. (2017). Fire and grazing modulate the structure and resistance of plant–floral visitor networks in a tallgrass prairie. *Oecologia*, 1–12.

Young, H. J., & Stanton, M. L. (1990). Influences of Floral Variation on Pollen Removal and Seed Production in Wild Radish. *Ecology*, *71*(2), 536–547.

Figures

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).

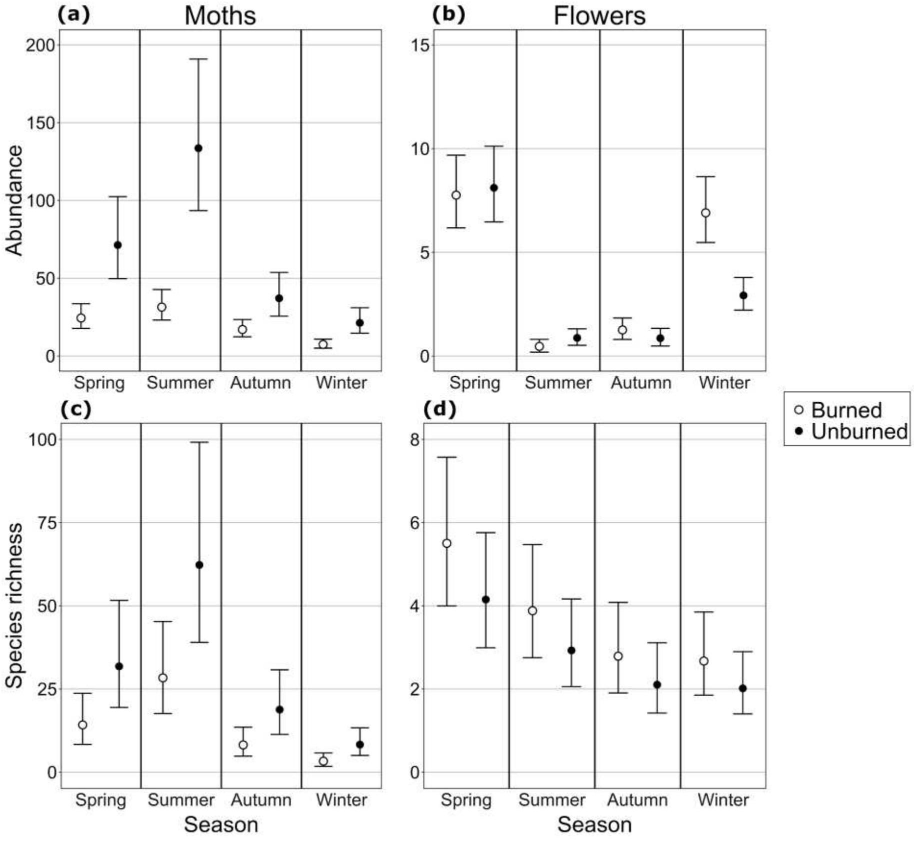

Figure 2: The effects of fire and season on the pollen loads of moths. Circles represent the model-predicted pollen load (a) and species richness (c) of pollen of individual moths, the cumulative pollen load (b) and richness (d) of all moths in a sample, and (e) the model-predicted proportion of moths 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).

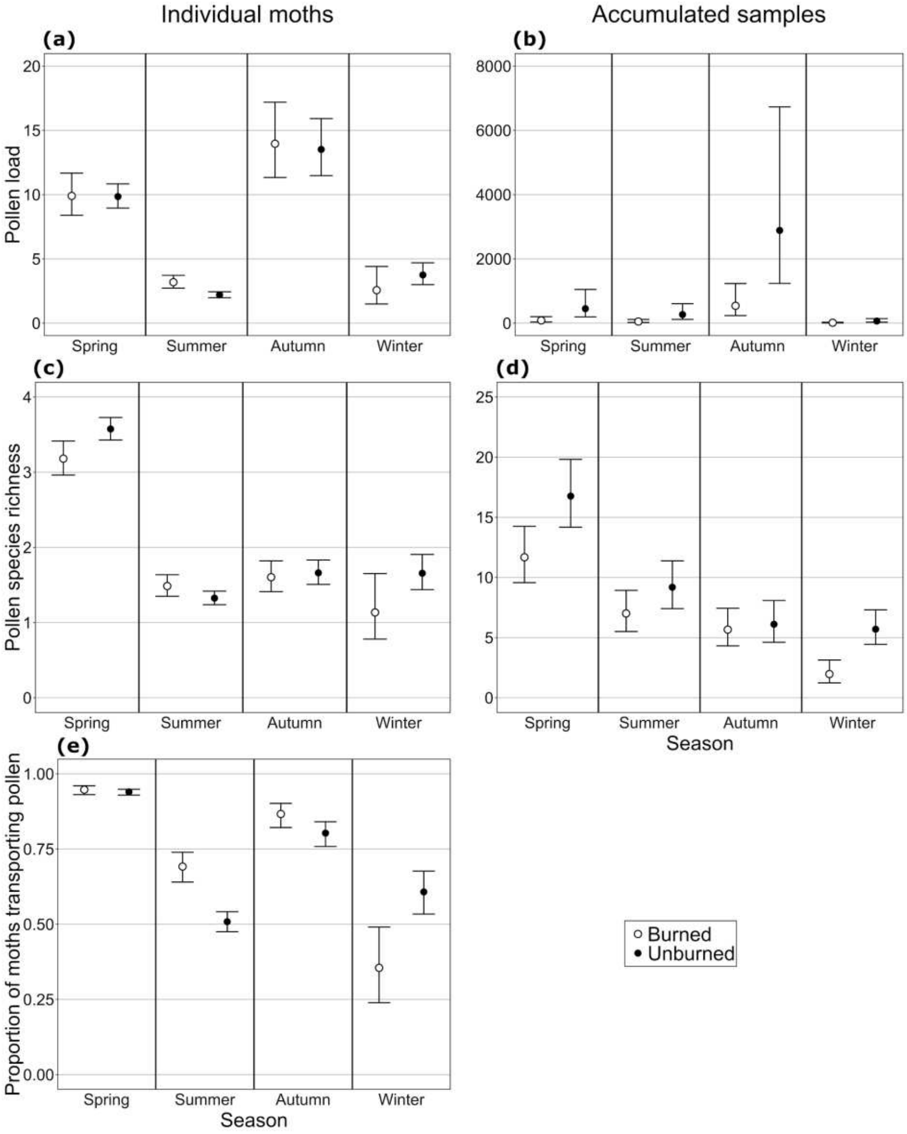

Figure 3: The effects of fire and season on a selection of network metrics (linkage density, robustness, generality of plants and generality of moths) calculated for quantitative, interaction frequency-weighted, pollen-transport networks. Points represent the model-predicted network metrics and error 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).

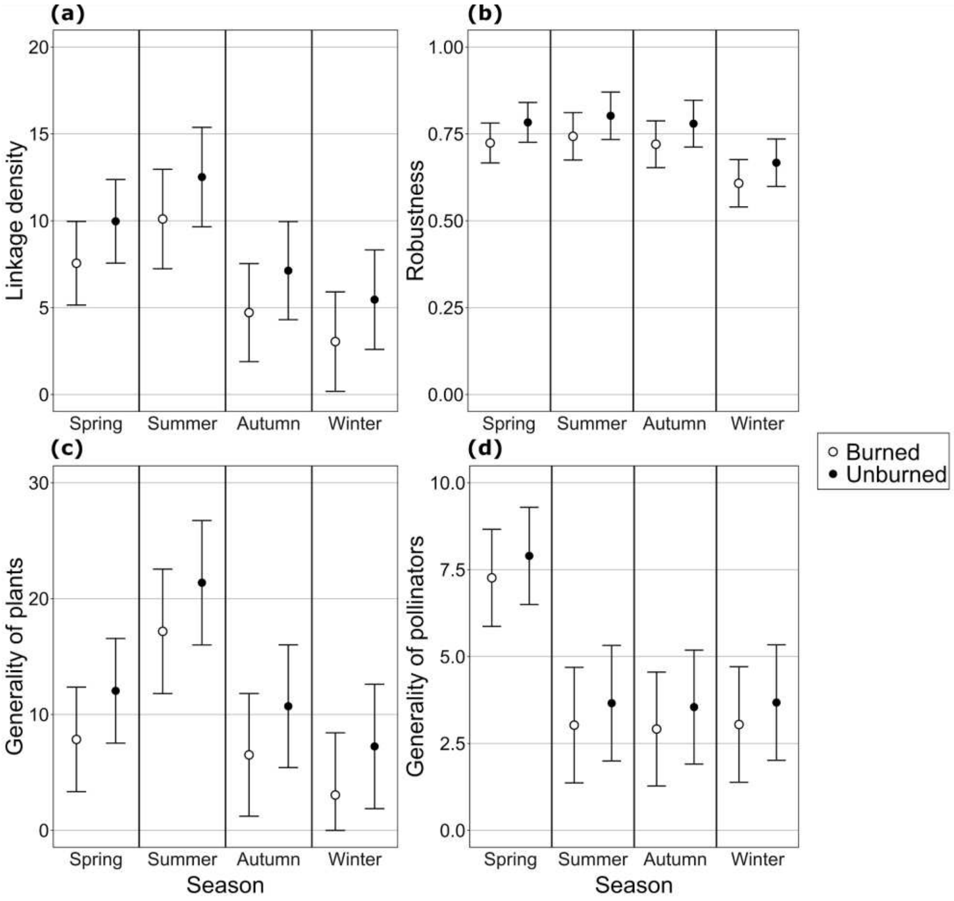

Figure 4: The quantity and causes of spatial interaction turnover between burned and unburned networks. In (a), bars show the total number of unique interactions observed in each sampling period, and coloured sections show the proportion of those interactions observed in the burned or unburned network 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).