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Plant-animal interactions in fire-prone ecosystems

TESIS DOCTORAL

Yedra García García

PROGRAMA DE DOCTORADO EN BIODIVERSIDAD Y BIOLOGÍA EVOLUTIVA

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CERTIFICAN:

Que los trabajos de investigación desarrollados en la memoria de tesis doctoral: "*Plant-animal interactions in fire-prone ecosystems*", han sido realizados bajo su supervisión y son aptos para ser presentados por Dña. Yedra García García ante el tribunal que en su día se consigne, para aspirar al Grado de Doctora en el Programa de Doctorado de Biodiversidad y Biología Evolutiva de la Universidad de Valencia.

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List of publications and manuscripts derived from the thesis

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- García Y, Castellanos MC, Pausas JG. Do fire-induced changes in plant volatile organic compounds promote pollinator switches? Under review.

- García Y, Castellanos MC, Dantas VL, Wolowski M, Sazima, M, Pausas JG. Plant-animal interactions contribute to the assembly of forest-savanna communities. Article in preparation.

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SUMMARY

There is a general concern about the increasing biodiversity loss in different ecosystems. In many cases, different disturbances are behind the extinction of species and ecological interactions. Understanding how plant-animal interactions respond to disturbance is thus one of the priorities faced by scientists. However, most of the studies on this topic have focused on anthropogenic disturbances, while the impacts of natural disturbances (e.g. wildfires) have received less attention. This thesis is about the responses of plant-animal interactions from environments with a long fire history (i.e. fire-prone ecosystems) to wildfires and the ultimate effects on plant reproductive performance. In addition, it explores how these interactions may contribute to the assembly of fire-prone communities from Neotropical forest-savanna mosaics, where fire is a strong abiotic filter. In ***Chapter I***, we assessed whether wildfires, through the postfire age and distance to the unburnt (i.e. fire extent), disrupted antagonistic plant-animal interactions (i.e. seed predation and herbivory). We also evaluated whether the effects of fire differed with the degree of specialization of the interactions involved (generalized vs specialized). To do that, we studied herbivory and seed predation interactions by insects in two Mediterranean plants, *Ulex parviflorus* and *Asphodelus ramosus*, with fast postfire responses. Our results showed that wildfires disrupted plant antagonistic interactions with specialist seed predators leading to temporary beneficial effects on plant reproductive success. We did not reported significant differences on generalist herbivores that showed a fast recovery at recently burnt areas. In addition, fire created spatial patterns in the strength of seed predation interactions by specialist insects inside the burnt. In ***Chapter II***, we studied the effects of wildfires, through the postfire age and distance to the unburnt, in the specialized pollination system of the Mediterranean dwarf palm, *Chamaerops humilis*. We expected that wildfires disrupted the interaction between the palm and its nursery weevil pollinator, with negative consequences on plant reproduction. Our

analyses, however, detected a complete recovery in the palm's fruit set at most of the study areas only three years after the fires. The replacement at recently burnt sites by an additional beetle pollinator, previously unnoticed, may have provided plant reproductive resilience. Our study suggests that even in highly specialized pollination systems from fire-prone ecosystems, a differential postfire response by a few pollinator species might ensure plant reproduction. In ***Chapter III***, we explored whether wildfires can change plant scents mediating pollination interactions and, in turn, their attractiveness to pollinators. We studied the pollination system of *C. humilis* by two beetle pollinators. The palm's leaf scent mediates the interaction. Our analysis showed only slightly changes on scent composition after the fires. In addition, we did not detected pollinator preferences on the palm's scent from burnt or unburnt sites. Our study indicates that wildfires can modify plant chemical signals mediating plant-pollination interactions, which may have further effects on pollinator attraction. However, in our study system, the limited scent changes reported together with the high specificity between the plant signal and the pollinators may have contributed to the fast postfire recovery of *C. humilis* pollination. Finally, in ***Chapter IV*** we evaluated the potential contribution of plant-animal mutualisms (through plant reproductive traits shaped by these interactions) into the assembly of forest-savanna mosaics from fire-prone ecosystems. We estimated the functional (based on floral and fruit traits) and phylogenetic structure in both habitat types (i.e. forest and savannas). Savannas exhibited overdispersed functional patterns contrasting with forests where patterns of functional similarity emerged. This reinforced previous evidences on the functional dichotomy of these tropical formations. In addition, our results suggested that multiple ecological processes including fire and plant-animal mutualisms (i.e. pollination, seed dispersal) may drive species coexistence in forest-savanna mosaics by acting on different plant traits (i.e. fire-related and reproductive traits).

RESUMEN

Síntesis

Estudiar cómo responden las interacciones ecológicas a las perturbaciones es clave para abordar la creciente pérdida de biodiversidad en diferentes ecosistemas. En la Tierra existen especies que han evolucionado ante la presencia recurrente de perturbaciones naturales, como ocurre en ecosistemas con incendios frecuentes. En ellos el fuego se originó poco después de la aparición de las primeras plantas terrestres y también algunos de los patrones de incendios característicos que todavía permanecen. Sin embargo, las actividades humanas están alterando los patrones naturales de incendios, lo que puede suponer una amenaza incluso para las especies que presentan una rápida recuperación posfuego. Numerosos estudios han abordado las respuestas de plantas y animales a los incendios, aunque las de sus interacciones han recibido un menor interés. Explorar cómo hacen frente las interacciones planta-animal al fuego en ecosistemas con una larga historia de incendios, puede enseñarnos acerca de los mecanismos que mantienen la biodiversidad. Esta tesis se centra en el estudio de los efectos del fuego en diferentes interacciones planta-animal y sus consecuencias en la reproducción de las plantas implicadas. Además, explora la posible contribución de los mutualismos planta-animal, como la polinización y dispersión de semillas, en los procesos de ensamblaje de comunidades con incendios recurrentes en las que el fuego actúa como un fuerte filtro ambiental.

Nuestros resultados muestran diferentes mecanismos por los que la reproducción de plantas de ambientes con incendios recurrentes (con rápida respuesta posfuego), podría verse recuperada en poco tiempo e incluso beneficiada tras el fuego. En concreto, el fuego es capaz de modificar las interacciones antagonistas entre plantas y animales (p. ej. depredación de semillas y herbivoría en *Ulex parviflorus* y *Asphodelus ramosus*) con efectos temporales positivos para la

reproducción de las plantas (*Capítulo I*). En el caso de interacciones de polinización, la distinta capacidad de respuesta al fuego de los polinizadores puede promover la resiliencia ante los incendios incluso en sistemas de polinización especializados, como el del palmito *Chamaerops humilis* (*Capítulo II*). Por otro lado, los incendios pueden alterar las fragancias emitidas por las plantas que favorecen la atracción de sus polinizadores (*Capítulo III*). En el sistema de polinización por escarabajos de *C. humilis*, los cambios moderados en composición de la esencia emitida por la planta y la fuerte especificidad entre ésta y los polinizadores, incluso después del fuego, permitieron que la atracción no se viese afectada. Por último, esta tesis sugiere que, junto al filtro abiótico ejercido por el fuego en comunidades vegetales de ecosistemas con incendios recurrentes, como los mosaicos bosque-sabana del Cerrado brasileño, los mutualismos planta-animal también pueden contribuir en el ensamblaje de las mismas al actuar sobre los rasgos reproductivos de las plantas. Esto da lugar a la presencia de patrones de diversidad funcional no aleatorios y contrastados en los dos tipos de hábitats (i.e. bosque y sabana). En concreto, detectamos patrones sobredispersados en las comunidades de sabana (i.e. más diversas) y agregados en las comunidades de bosque (i.e. menos diversas, *Capítulo IV*).

Antecedentes

Existe una creciente preocupación por las altas tasas de pérdida de biodiversidad en numerosos ecosistemas (Steffan-Dewenter et al. 2005; Dirzo et al. 2014; Valiente-Banuet et al. 2015). A la pérdida de especies hay que añadir la de las interacciones ecológicas, que puede preceder a la extinción de las primeras (Tylianakis et al. 2008; Aizen et al. 2012; Valiente-Banuet et al. 2015). Diferentes estudios han aportado evidencias de perturbaciones ligadas a actividades humanas como causantes de la acelerada pérdida de biodiversidad actual (Memmott et al. 2007; Traveset and Richardson 2006; Tylianakis et al. 2008; González-Varo et al. 2013; Dirzo et al. 2014; Goulson et al. 2015). Por otro lado, hay organismos que han lidiado con perturbaciones naturales desde hace milenios. Este es el caso de las especies de ecosistemas con incendios recurrentes, en los que el origen de los incendios y de sus regímenes naturales (caracterizados por tamaño, estacionalidad, intensidad y frecuencia concretos) está unido a la aparición de las primeras plantas terrestres (Glasspool et al. 2004; Pausas and Keeley 2009). Estudiar cómo las interacciones entre estas especies hacen frente a los incendios nos puede enseñar acerca de los mecanismos que mantienen la biodiversidad. En esta tesis se exploran las respuestas al fuego de diferentes interacciones entre plantas y animales procedentes de ecosistemas con incendios recurrentes y cómo ello repercute en el éxito reproductivo de las plantas.

Numerosos trabajos han analizado los efectos del fuego en plantas y animales (Swengel et al. 2001; Bond and Keeley 2005; Keeley et al. 2011; 2012; New 2014; Pausas y Parr 2018). En ecosistemas con incendios recurrentes, muchos organismos presentan rasgos que les confieren ventajas adaptativas en presencia de regímenes de fuego naturales (Keeley et al. 2011; Keeley et al. 2012; Koltz et al. 2018; Pausas y Parr 2018). Aun así, los humanos estamos alterando estos patrones, por ejemplo, incrementando la frecuencia y la extensión de incendios en algunos ecosistemas, lo que puede suponer una amenaza incluso para las especies nativas de ambientes con una larga historia de incendios (Lavorel et al. 2007; Keeley et al. 2011; Pausas y Paula 2012). Los efectos del fuego en las interacciones planta-animal han recibido, sin embargo, un menor interés, aunque el número de trabajos ha aumentado en los últimos años (Dafni et al. 2012; Ponisio et al. 2016, Brown et al. 2017, Kelly et al. 2018). A pesar de la variación en los resultados encontrados, hay al menos tres características de los regímenes de incendios que parecen influir en la capacidad de respuesta de las interacciones planta-animal y que son: la frecuencia, la extensión y la edad posfuego (tiempo transcurrido desde el último incendio). El grado de especialización trófica de los organismos implicados (generalistas *vs* especialistas) también puede influir en su respuesta, aunque esto se ha explorado poco en el contexto de sus interacciones (e.g. Mihuc et al. 2015; Lybbert et al. 2018; *Capítulos I-III*). Además, en el caso de la polinización los trabajos previos se han centrado en rasgos y recursos florales como la cantidad de polen y néctar o densidad de flores (e.g. Potts et al. 2003; van Nuland et al. 2013; LoPresti et al. 2018). Sin embargo, los posibles efectos del fuego en las fragancias emitidas por las plantas que son claves para atraer a sus polinizadores en muchos sistemas de polinización (Schiestl 2015), no han sido explorados todavía (*Capítulo II*).

El fuego es también un proceso ecológico clave en el ensamblaje de comunidades en ecosistemas con incendios recurrentes, donde actúa como un potente filtro ambiental (Verdú y Pausas 2007; Pausas y Verdú 2008; Ojeda et al. 2010; Cianciaruso et al. 2012; Hoffmann et al. 2012; Dantas et al. 2013). Como resultado, el fuego puede favorecer comunidades en las que predominan especies con rasgos comunes en relación a su respuesta a los incendios (Pausas y Verdú 2008; Cianciaruso et al. 2012; Dantas et al. 2013). Si además estos rasgos están conservados filogenéticamente, es decir, a lo largo de la evolución de las especies que los presentan, se puede dar una sobrerrepresentación de especies estrechamente emparentadas y fenotípicamente similares (p. ej. Verdú y Pausas 2007; Dantas et al. 2013). Por otro lado, existe un número creciente de trabajos que demuestran el papel de las interacciones planta-animal como motores adicionales del ensamblaje de las comunidades (Sargent y Ackerly 2008; Pellissier et al. 2010, 2012; Pringle et al. 2016; Woloswki et al. 2017; Kemp et al. 2018; Bartomeus y Godoy 2018). Distintos estudios han mostrado que la variación espacial en interacciones planta-animal de comunidades con condiciones ambientales contrastadas, puede dar lugar a patrones no aleatorios en los rasgos moldeados por estas interacciones (p. ej. rasgos florales sobre los que actúan polinizadores; Pellissier et al. 2010; Koski y Ashman 2015, Pringle et al. 2016; Wolowski et al. 2017). En este contexto los mosaicos bosque-sabana que caracterizan el Cerrado brasileño son sistemas ideales para investigar posibles patrones de ensamblaje en rasgos moldeados por interacciones mutualistas en ambos tipos de hábitat (i.e. bosques y sabanas), ya que estos difieren en su historia de incendios y en sus condiciones ambientales, y además poseen una alta diversidad de especies (Gottsberger y Silberbauer-Gottsberger 2006). Pese a ello, hasta ahora los estudios que conocemos sobre el ensamblaje en estas comunidades se han centrado principalmente en el papel del filtrado abiótico (Hoffmann et al. 2012; Dantas et al. 2013; Laureto y Cianciaruso 2015; Maracahipes et al. 2018; pero ver Maruyama et al. 2014; *Capítulo IV*).

Objetivos

El objetivo general de esta tesis es estudiar el efecto del fuego en interacciones planta-animal de ecosistemas con incendios recurrentes y las consecuencias para la reproducción de las plantas implicadas. Además, esta tesis explora el posible papel de los mutualismos planta-animal en el ensamblaje de mosaicos bosque-sabana en ecosistemas tropicales con incendios recurrentes. Los objetivos específicos que se abordan a largo de los cuatro capítulos son:

1) Estudiar si los incendios forestales, a través del tiempo transcurrido y la distancia al interior del incendio, afectan a las interacciones antagonistas planta-animal (i.e. herbivoría y depredación de semillas) y esto beneficia al éxito reproductivo de las plantas implicadas. Además, se analiza si el efecto difiere según el grado de especialización trófica de los animales antagonistas (***Capítulo I***).

2) Estudiar si los incendios forestales, a través del tiempo transcurrido y la distancia al interior del incendio, afectan a las interacciones de polinización especializadas con efectos negativos en la reproducción de las plantas implicadas (***Capítulo II***).

3) Analizar si el fuego modifica las señales químicas emitidas por las plantas que median en sus interacciones de polinización. Evaluar si dichos efectos tienen consecuencias en la atracción de polinizadores (***Capítulo III***).

4) Estudiar la posible contribución de los mutualismos planta-animal (i.e. polinización y dispersión de semillas), a través de rasgos reproductivos moldeados por estas interacciones, en el ensamblaje de comunidades de mosaicos bosque-sabana en ecosistemas con incendios recurrentes (***Capítulo IV***).

Metodología general

Áreas de estudio y diseño experimental

El estudio de los efectos del fuego en interacciones planta-animal que incluyen los tres primeros capítulos, se llevó a cabo en siete localidades del este de España afectadas por incendios forestales. Para el *Capítulo IV*, se recopiló información de una zona en un mosaico bosque-sabana del Cerrado brasileño. Las áreas de estudio en España son matorrales de clima mediterráneo caracterizados por inviernos suaves y húmedos que contrastan con veranos calurosos y secos. El área de estudio de Brasil consistió en un mosaico de parcelas de bosque y sabana con diferente cobertura vegetal e historia de incendios (Dantas et al. 2013). En este caso el clima es tropical-húmedo con una marcada estacionalidad, caracterizada por una estación húmeda y calurosa de octubre a abril y una estación seca de mayo a septiembre. Las áreas estudiadas tanto en España como en Brasil se corresponden con ecosistemas con incendios recurrentes, en los que los organismos presentan frecuentemente rasgos que les confieren ventajas reproductivas y de supervivencia ante regímenes de incendios concretos (Keeley et al. 2011; Koltz et al. 2018).

En los tres primeros capítulos se analizaron los efectos del fuego en cada área de estudio en zonas pareadas, quemadas y sin quemar. En el *Capítulo I* se estudiaron los efectos en interacciones antagonistas (p. ej. depredación de semillas y herbivoría) y en los *Capítulos II* y *III* en interacciones de polinización especializadas. Las parcelas sin quemar se seleccionaron cuidadosamente para que fuesen representativas de las condiciones anteriores al paso del fuego, en términos de composición de especies y tipo de suelo. Para analizar los efectos de la distancia hasta la vegetación no quemada (subrogado de la extensión del incendio) y el tiempo desde el último incendio, las interacciones se muestrearon en diferentes categorías de distancias (*Capítulo I*) o a lo largo de transectos (*Capítulo II*) desde el perímetro del incendio al interior y en zonas con diferentes edades posincendio (*Capítulos I* y *II*). Las distancias desde cada parcela o individuo fueron calculadas con el programa Quantum GIS (QGIS Team 2013). Para el estudio de los efectos del fuego, las interacciones fueron muestreadas mediante censos en campo de diferentes especies de insectos sobre las plantas con las que interactúan. La respuesta en la reproducción de las plantas se evaluó mediante estimas de depredación de semillas o de producción de frutos en el laboratorio.

En el *Capítulo III* se analizaron los posibles cambios producidos por los incendios en las señales químicas emitidas por las plantas en forma de esencias, que median en su polinización. Estas señales contienen compuestos orgánicos volátiles (COVs). El sistema de polinización del palmito, *Chamaerops humilis* L., fue el sistema de estudio utilizado para analizar estos posibles efectos. La fragancia emitida por la planta se muestreó en dos áreas de estudio tras incendios recientes en España. En cada área se muestreó una zona quemada y una adyacente sin quemar. Durante la floración de la planta, coincidiendo con el pico de emisión de olor, se recolectó la esencia emitida por las hojas de *C. humilis*. La recolección se llevó a cabo embolsando una hoja de la planta (N= 60 individuos) conectada a una bomba de vacío portátil y a una trampa de volátiles con compuestos adsorbentes, en la que quedaban retenidos los COVs del palmito. Los COVs de la esencia se analizaron mediante cromatografía de gases acoplada a espectrometría de masas. Además, se llevaron a cabo bioensayos de olfactometría en el laboratorio con los polinizadores del palmito y esencias del mismo, procedentes de las zonas de estudio (quemadas y no quemadas), para analizar si los cambios en la señal interferían en su capacidad de atracción de polinizadores (ver Apéndice C para detalles del muestreo de la fragancia y los bioensayos).

Por último, en el *Capítulo IV* se exploró la posible contribución de interacciones mutualistas entre plantas y animales al ensamblaje de comunidades en mosaicos bosque-sabana con historias de fuego contrastadas. Para ello se realizó una base de datos de 12 rasgos reproductivos (de flores y frutos) de las plantas presentes en un mosaico de parcelas de bosques y sabana en un área de Cerrado en Brasil. La información sobre la composición de las parcelas y la estructura de la cobertura vegetal fue obtenida de un trabajo anterior de Dantas et al. (2013). Los datos de los rasgos se recopilaron mediante una búsqueda bibliográfica y medidas directas en pliegos digitalizados del Herbario del Jardín Botánico de Río de Janeiro (Apéndice D para más detalles sobre la base de datos).

La matriz de ocurrencia de las especies de plantas (N=75 especies) en las parcelas de bosque y sabana (N=98 parcelas), junto con los datos reproductivos, se utilizaron para evaluar si la estructura funcional en bosques y sabanas difería de patrones esperados por azar obtenidos mediante modelos nulos. Además, se estimó la estructura filogenética en ambos tipos de hábitats. La métrica de estructura funcional utilizada fue la distancia media de rasgos entre los pares de especies (aquí llamada distancia media funcional, MFD). Para la estructura filogenética, el índice utilizado fue la distancia filogenética media entre los pares de especies (MPD). La presencia de patrones de ensamblaje no aleatorios en ambas comunidades se evaluó comparando ambos índices con la distribución obtenida mediante modelos nulos generados al azar (*Capítulo IV* y Apéndice D para más detalles).

Interacciones planta-animal estudiadas

- *Ulex parviflorus-Exapion fasciolatum* (*Capítulo I*)

Ulex parviflorus Pourr. (Fabaceae), conocida comúnmente por aliaga, es una especie de arbusto espinoso nativo de la cuenca mediterránea. Esta leguminosa es considerada una especie *semilladora*, que recluta desde un banco de semillas en el suelo después del fuego, con semillas que rompen su dormancia e inician la germinación bajo las altas temperaturas alcanzadas en los incendios (Paula et al. 2009; Moreira et al. 2010). La planta es atacada por un gorgojo especialista, el curculiónido *Exapion fascilatum* Wagner (Brentidae, Apioninae), que se alimenta de sus semillas. Las hembras del gorgojo ponen sus huevos en el interior de los frutos inmaduros de *U. parviflorus* y los adultos emergen durante la dehiscencia de los mismos. Además, en los frutos es frecuente observar la presencia de avispas parásitas (Chalcidoidea) de las larvas y pupas del gorgojo. Los efectos del fuego sobre la depredación de semillas en este sistema fueron estudiados en el *Capítulo I*.

- *Asphodelus ramosus* y depredadores antagonistas (*Capítulo I*)

El gamón, *Asphodelus ramosus* L. (Liliaceae), es un geófito mediterráneo con una rápida respuesta al fuego gracias a su rizoma (Pantis y Margaris 1988; Paula et al. 2009). Esta planta sufre herbivoría por parte de insectos generalistas como los escarabajos *Tropinota squalida* Scop y *Oxythyrea funesta* Poda (Cetoniinae), o el pentatómido *Carpocoris fuscipinus* Boh. (Pentatomidae). Además, es atacado por la chinche especialista *Horistus orientalis* Gmelin (Miridae) que presenta un ciclo de vida estrechamente ligado a la planta, en el que tanto las ninfas como los adultos se alimentan especialmente de las flores y frutos. La abundancia de estos depredadores antagonistas sobre la planta tras el fuego, así como sus efectos en la producción de frutos fueron estudiados en el *Capítulo I*.

- *Chamaerops-humilis* y polinizadores especialistas (*Capítulos II* y *III*)

El palmito, *Chamaerops humilis* L. (Arecaceae) es una palmera dioica originaria del oeste de la cuenca del Mediterráneo. Presenta una rápida respuesta al fuego gracias al rebrote desde yemas apicales y a su capacidad de floración la primavera siguiente a un incendio (Paula et al. 2009; Tavşanoğlu y Pausas 2018). Esta palmera presenta un sistema de polinización de tipo criadero ("*nursery pollination*") con el curculiónido *Derelomus chamaeropis* Fabricius (Curculionidae), que se desarrolla dentro de las inflorescencias viejas, preferentemente de individuos masculinos (Anstett 1999; Dufaÿ y Anstett 2004; Jácome-Flores et al. 2018). En la primavera, coincidiendo con la floración, las hojas en lugar de las flores (que prácticamente no emiten fragancia, Dufaÿ et al. 2003), emiten una fuerte señal química que atrae a los adultos de *D. chamaeropis* que se han desarrollado dentro de las inflorescencias. La emergencia de los adultos del escarabajo coincide con el final de floración de los individuos masculinos y el inicio de los femeninos favoreciendo la polinización (Dufaÿ 2010). Las plantas femeninas y masculinas emiten esencias con composición similar, lo que facilita la visitas a los individuos femeninos a pesar de que ofrecen menos recompensa al polinizador (i.e. ausencia de polen e inflorescencias menos atractivas para su desarrollo, Dufaÿ et al. 2003; Dufaÿ et al 2004; Jácome Flores et al. 2018). Además, la planta es visitada por el escarabajo nitidúlido *Meligethinus pallidulus* Erichson (Nitidulidae), que tal y como se muestra en el *Capítulo II* puede ser especialmente relevante en condiciones posfuego. La abundancia de ambos escarabajos en las inflorescencias del palmito tras incendios, así como la producción de frutos de la planta fueron estudiadas en el *Capítulo II.*

Resultados

En esta tesis mostramos que los incendios alteran las interacciones entre plantas y animales a través de características espaciales y temporales de los mismos. Esto da lugar a la formación de patrones de variación espacio-temporal en las interacciones dentro del área quemada. Las consecuencias en la reproducción de las plantas dependerán de la capacidad de respuesta de dichas interacciones (*Capítulos I* y *II*). Además, el grado de especialización trófica de los organismos implicados en las interacciones, también puede influir en la capacidad de recuperación de éstas tras el fuego (*Capítulo I*). Sin embargo, incluso en interacciones, por ejemplo, de polinización con un alto grado de especialización, una rápida respuesta por parte de alguna de las pocas especies involucradas puede asegurar el éxito reproductivo de las plantas (*Capítulo II*). Estas relaciones de polinización podrían también verse alteradas después del fuego por la modificación de las señales químicas con las que las plantas atraen a sus polinizadores (*Capítulo III*). En el sistema de polinización estudiado, los tenues cambios observados en la composición de la fragancia y la ausencia de efectos significativos en su capacidad de atracción, parecen favorecer también la rápida respuesta tras el fuego (*Capítulo III*). Es importante remarcar que estos estudios fueron realizados en interacciones entre pares de especies concretos involucrando un pequeño número de especies y que por tanto nuevos trabajos, por ejemplo, a nivel de comunidad, son necesarios para evaluar si en otros sistemas o a escalas más amplias emergen resultados similares. Finalmente, esta tesis sugiere que el ensamblaje de comunidades vegetales de mosaicos bosque-sabana en ambientes con incendios recurrentes, es el resultado de múltiples procesos entre los que además del fuego, que es un fuerte filtro abiótico, se encuentran los mutualismos planta-animal. Estos últimos actúan sobre los rasgos reproductivos de las plantas generando patrones de diversidad funcional contrastados en comunidades de bosque y sabana (*Capítulo IV*).

En el *Capítulo I* (García et al. 2016), detectamos que el fuego puede tener efectos temporales beneficiosos en la reproducción de las plantas a través de su impacto sobre las interacciones con animales antagonistas especialistas. Estos resultados coinciden con evidencias anteriores de los efectos negativos del fuego en poblaciones de herbívoros que pueden suponer un alivio temporal para las plantas que muestran una rápida respuesta al fuego (Knight y Holt 2005). La menor presión por parte de los antagonistas, unida a un aumento de nutrientes en el suelo o a los claros abiertos por el fuego que reducen la competencia por luz y recursos, podrían tener efectos positivos en la dinámica de las poblaciones vegetales. Además de los efectos temporales, nuestro estudio detectó la presencia de patrones de variación espacial en las interacciones dentro de las zonas quemadas (García et al. 2016). Este trabajo se centró en interacciones de depredación pre-dispersiva; sin embargo, el fuego puede modificar las interacciones post-dispersivas afectando también a la reproducción de las plantas (Ordóñez and Retana 2004; Broncano et al. 2008; Puerta-Piñero et a.l 2010). Por tanto, estudios integrativos que evalúen cómo el fuego modifica el balance entre distintas interacciones en zonas quemadas son necesarios para entender los efectos últimos sobre el éxito reproductivo de las plantas.

En el *Capítulo II* (García et al. 2018), nuestros resultados muestran cómo la diferente capacidad de respuesta al fuego de las especies de polinizadores, pueden favorecer una rápida recuperación incluso en sistemas de polinización especializados. Esto es consistente con trabajos previos centrados en interacciones generalistas, que demuestran que la diversidad de respuestas de los polinizadores puede tamponar los efectos negativos del fuego en las especies de polinizadores más sensibles (Potts et al. 2001; Campbell et al. 2007; van Nuland et al. 2013, Lybbert et al. 2018). Como consecuencia, la producción de frutos se vió recuperada en la mayor parte de las zonas de estudio tan solo tres años después de los incendios (García et al. 2018). Futuros trabajos podrían evaluar si esta resiliencia al fuego en sistemas de polinización especializados es común en otros ecosistemas con incendios recurrentes. Este trabajo señala también la importancia de los co-polinizadores en los sistemas de polinización de tipo criadero "*nursery*" (Kephart et al. 2006; Hossaert-McKey et al. 2010), especialmente en ambientes con perturbaciones recurrentes (García et al. 2018; *Capítulo III*). Futuros trabajos que incluyan otros sistemas de polinización o escalas más amplias, como es el caso de estudios de redes de polinización, podrían aportar información relevante sobre la resiliencia de las comunidades de plantas y polinizadores a la perturbación.

En el *Capítulo III*, detectamos que los incendios pueden alterar las señales químicas emitidas por las plantas que atraen a sus polinizadores, lo que podría interferir en sus interacciones de polinización. Trabajos anteriores han aportado evidencias de que perturbaciones ligadas a los humanos como el incremento de temperatura, ozono o sequía, pueden modificar estas señales químicas (Farré-Armengol et al., 2013, 2014, 2016; Burkle y Runyon 2016, 2017; Jürgens y Bischoff 2017). En nuestro sistema de estudio, los cambios en la fragancia fueron moderados y esta mostró una alta especificidad por los polinizadores incluso después del fuego, lo que también facilitó la resiliencia del sistema de polinización del palmito ante los incendios (*Capítulo III*). Las particularidades de la emisión de la fragancia en el palmito (emitida por las hojas) y la especialización de los polinizadores implicados (Dufaÿ et al. 2003; García et al. 2018), sugieren tomar con precaución los resultados obtenidos y señalan la necesidad de estudiar otras interacciones de polinización mediadas por esencias florales. Sin embargo, el hecho de que el fuego es capaz de modificar diversos factores que han demostrado alterar las fragancias florales por sí mismos, como cambios en las bacterias asociadas a las plantas, en las presiones de herbivoría, en el contenido hídrico del suelo o en temperatura (Kessler et al. 2011; Farré-Armengol et al. 2014; Burkle and Runyon 2016; Helletsgruber et al. 2017), apuntan la posibilidad de que el fuego también modifique las fragancias emitidas por flores.

Por último, el *Capítulo IV* sugiere que las interacciones mutualistas tales como la polinización y dispersión de semillas, pueden ser procesos adicionales en el ensamblaje de las comunidades vegetales de mosaicos bosque-sabana con distintas historias de incendios. Esto resulta en patrones no aleatorios en los rasgos reproductivos de las comunidades vegetales. En concreto, las comunidades de bosque mostraron patrones agregados en estos rasgos, indicando similaridad funcional que contrasta con la dispersión funcional detectada en sabanas. Estos resultados refuerzan la dicotomía funcional entre las dos formaciones ya señalada por trabajos previos (Hoffmann et al. 2012; Dantas et al. 2013, Sfair et al. 2016; Maracahipes et al. 2018), y muestran que también se ve reflejada en rasgos relacionados con procesos de polinización y dispersión en estos ecosistemas tropicales. Este estudio remarca la importancia de incluir rasgos reproductivos junto a los típicamente analizados (i.e. ecofisiológicos, vegetativos, Keddy 1992; Kraft et al. 2008) en trabajos de ensamblaje de comunidades vegetales. Por último, la variación en los patrones observados refuerza la necesidad de combinar enfoques basados en rasgos y en la filogenia de las especies para entender las distintas fuerzas que subyacen a los patrones de ensamblaje de las comunidades (Cavender-Bares et al. 2009; Pausas y Verdú 2010; de Bello 2017).

Conclusiones

1. El fuego modifica las interacciones antagonistas entre plantas y animales y esto puede beneficiar a la reproducción de las plantas hasta que los antagonistas se restablecen e interactúan de nuevo con ellas. En concreto, este efecto se observa en interacciones con antagonistas especialistas (p. ej. depredadores de semillas), que a diferencia de los generalistas requieren más tiempo para recuperar sus interacciones.

2. Los incendios modifican las interacciones entre las plantas y sus polinizadores. Una capacidad de respuesta rápida por parte de alguna de las especies de polinizadores, puede proveer de resiliencia incluso a sistemas de polinización especializados, lo que resulta en una rápida recuperación de la reproducción de la planta.

3. Los incendios pueden generar patrones de variación espacial y temporal en las interacciones entre plantas y animales.

4. El fuego puede alterar las señales químicas que median en las interacciones de polinización lo que su vez podría tener efectos en la capacidad de atracción de polinizadores. En el caso de sistemas de polinización especializados, la alta especificidad entre la señal y los polinizadores incluso

tras un incendio, podría favorecer que la polinización no se vea afectada negativamente.

5. En ecosistemas con incendios recurrentes, además del filtro abiótico impuesto por el fuego, las interacciones mutualistas entre plantas y animales pueden contribuir al ensamblaje de sus comunidades. En mosaicos bosque-sabana, observamos que múltiples procesos ecológicos (i.e. fuego e interacciones mutualistas) pueden actuar sobre distintos rasgos de las plantas (i. e. vegetativos y reproductivos) dando lugar a patrones de ensamblaje diferentes a los esperados por azar.

6. La distinción funcional entre formaciones de bosque y sabana tropicales con historias de incendios contrastadas, se observa también en rasgos reproductivos, con una mayor diversidad funcional en las comunidades de sabana (patrones dispersados) que en las de bosque (patrones agregados).

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GENERAL INTRODUCTION AND AIMS

There is a general concern about the increasing loss of species and ecological interactions in different ecosystems (Steffan-Dewenter et al. 2005; Dirzo et al. 2014; Valiente-Banuet et al. 2015). Anthropogenic disturbances are in many cases behind this decline in biodiversity (Kearns et al. 1998; Traveset and Richardson 2006; Memmott et al. 2007; Tylianakis et al. 2008; Winfree et al. 2009; González-Varo et al. 2013; Dirzo et al. 2014; Goulson et al. 2015). Understanding how plant-animal interactions respond to disturbance is thus one of the priorities in the current ecological agenda. However, in some environments species and their interactions may have faced natural disturbances for millennia. This is the case of fire-prone ecosystems where fire appeared soon after the first land plants (Glasspool et al. 2004; Pausas and Keeley 2009; Bond and Scott 2010). Surprisingly, the effects of wildfires on plant-animal interactions in these ecosystems have received much less attention than other disturbances (Dafni et al. 2012; Ponisio et al. 2016). Furthermore, humans are currently changing natural fire patterns in many regions (Lavorel et al. 2007; Keeley et al. 2011; Pausas and Paula 2012). Studying how plant-animal interactions cope with wildfires provides insights on the mechanisms maintaining biodiversity. This thesis is about the responses of plant-animal interactions from fire-prone ecosystems to wildfires and the ultimate effects on the plant reproductive performance. In addition, it explores how these interactions may contribute to the assembly of fire-prone communities.

Fire as a natural disturbance

The first records of fossil charcoal place the origin of wildfires on Earth in the Paleozoic during the Silurian period (440 mya) linked to the appearance of land plants (Glasspool et al. 2004; Pausas and Keeley 2009; Bond and Scott 2010). These early plants might have coped with some of the current fire regimes (i.e. particular associations of fire spread pattern, intensity, frequency and seasonality in a given area) since crown and understory surface fire regimes were already present at the beginning of the history of fire on Earth (Falcon-Lang 2000; Cressler 2001; Pausas and Keeley 2009). Fire imposes a plethora of changes on ecosystems that can affect species persistence such as alterations in soil properties, moist content, temperature, habitat structure, and in the availability and quality of resources (DeBano 1976; Wan et al. 2001; Bond and Keeley 2005; Certini 2005; Keeley et al. 2012). Today, with over a half of the Earth's land considered as fire-prone (Keeley et al. 2012), the role of fire as a global natural disturbance modifying abiotic and biotic components of ecosystems is widely accepted within the scientific community.

Many studies assessing the effects of fire on different aspects of biodiversity have been conducted in fire-prone ecosystems such as Mediterranean shrublands and tropical savannas (Hoffman et al. 2003, 2012; Bond and Keeley 2005; Pausas et al. 2009; Keeley et al. 2012). These flammable environments show particular fire regimes under which native plants and animals may have evolved (Keeley et al. 2011, 2012; Koltz et al. 2018; Pausas and Parr 2018). For instance, Mediterranean shrublands experience high-intensity crown fires with fire-free intervals of several decades (Keeley et al. 2012). In tropical savannas and especially in those from humid regions as the Brazilian Cerrado, low-intensity fires of high frequency (i.e. several fires within a decade) conform the dominant regime (Bond and Keeley 2005; Hoffman et al. 2003, 2012). Nevertheless, humans are currently changing natural fire regimes leading to increased fire sizes and reduced fire-free intervals in some regions that may also threat species from these fire-prone ecosystems (Bond and van Wilgen 1996; Lavorel et al. 2007; Keeley et al. 2011; Pausas and Paula 2012).

Fire responses in plants and animals

Fires can alter the reproductive performance of plants and animals and induce demographic and genetic changes on population structure (Keeley et al. 2012; New 2014; Smith et al. 2014a; Paniw et al. 2018; Pausas and Parr 2018). In fire-prone ecosystems, fire is an important force shaping plant communities (Verdú and Pausas 2007; Ojeda et al. 2010; Dantas et al. 2013), where it can also structure arthropod communities (Parr et al. 2004; Moretti et al. 2009; Arnan et al. 2013; Lazarina et al. 2017).

Plants exhibit a variety of traits that provide fitness advantages under fire pressures (reviewed by Keeley et al. 2011). This has allowed ecologists to establish a functional classification of plant traits involved in postfire response (Pausas et al. 2004; Keeley et al. 2012). For instance, in Mediterranean ecosystems under intense crown fire regimes, plants show contrasting traits that provide persistence at individual level, in the case of postfire resprouting, and at population level in the case of postfire seeding from a seed-bank (Pausas et al. 2004; Keeley et al. 2012; Pausas and Keeley 2014). Obligate resprouters (sensu Pausas and Keeley 2014) regenerate from belowground buds or from basal epicormic buds (i.e. stem) through a variety of structures that protect them from fire (e.g. rizhomes, lignotubers, bulbs, thick barks, Moreira et al. 2012; Pausas and Keeley 2017; Pausas et al. 2018). In contrast, obligate seeder species (sensu Pausas and Keeley 2014) recruit after fires from seeds stored in aerial or soil seed-banks (Keeley et al. 2011; Moreira and Pausas 2012; Smith et al. 2014b;). Smoke and high temperatures generated during fires are two important factors triggering germination in seeder species (Keeley 1991; Moreira and Pausas 2012). Facultative seeders combine both resprouting and recruiting traits to cope with fires (Pausas and Keeley 2014).

Studies on fire responses in animals have mainly focused on the effects of fire events on individuals or populations rather on traits that provide postfire persistence (Kelly et al. 2018). This may be explained, at least in part, by the fact that behavioral traits are an important component of fire-response strategies in animals and they can be difficult to study especially at field (Pausas and Parr 2018). Animals show disparate responses to cope with fires that can provide a quick recovery or even beneficial effects in postfire conditions (Swengel 2001; New 2014; van Mantgem et al. 2015; Koltz et al. 2018; Pausas and Parr 2018). These responses involve behavioural (e.g. fleeing, burrowing into the soil, scape to unburnt refuge), morphological (e.g. fire and infrared sensors), and life-history traits (e.g. endogenous survival in diapause, soil-nesting species, high dispersal, feed-generalists and habitat-generalists; Schütz et al 1999; New 201; Lazarina et al. 2016, 2017; van Mantgem et al. 2015; Koltz et al 2018). However, the mechanisms behind these responses are still poorly understood, making generalizations difficult, although recent studies have proposed some functional classifications (van Mantgem et al 2015; Pausas 2018).

From an evolutionary perpective, some of the adaptive fire traits in plants may have their origin on fire pressures (i.e. fire adaptations) while others may be exaptations originated by different selective forces that have acquired an adaptive value in fire-prone environments (Keeley et al. 2011; Bowman et al. 2012; He et al. 2012; Pausas and Schwilk 2012; Moreira et al. 2014; Castellanos et al. 2015). This may also be the case of fire-related traits in animals, although the evolutionary role of fire in shaping traits on this taxa has only recently gained attention (Koltz et al. 2018; Pausas and Parr 2018). Some insights of adaptive morphological traits in animal species under fire pressures come from studies on insects, such as the presence of smoke detectors in some cerambycid beetles antennae and infrared sensors in some buprestid beetle species (Schütz et al. 1999; Schmitz et al. 2016; Koltz et al. 2018).

Recent studies have proposed new frameworks and general classifications that aim to provide a more integrative view of plant and animal responses to fires (Kelly et al. 2018; Pausas 2018). These new approaches may contribute to a better understanding of the effects of fire on biodiversity. Furthermore, under the assumption that the variability of fire regimes may promote a high biotic diversity (i.e. "pyrodiversity begets biodiversity", Martins and Sapsis 1992; Parr and Andersen 2006), patch mosaic burning has been applied in different ecosystems with management purposes. However, empirical evidence supporting this hypothesis is still scarce and limited to certain groups of animals and ecological interactions (Taylor et al. 2012; Farnsworth et al. 204; Griffiths et al. 2015; Bowman and Legge 2016; Ponisio et al. 2016), which also calls for further integrative studies providing a more comprehensive view of the impacts of fire on biodiversity.

Fire and plant-animal interactions

Despite the considerable amount of research on postfire responses in plants and animals, the effects of wildfires on their interactions have received less attention, although interest in this topic is increasing (Dafni et al. 2012; Brown et al. 2017; Kelly et al. 2018). Assessing the effect of disturbances on plant-animal interactions is particularly important, as these interactions may become extinct even before the complete loss of species (Tylianakis et al. 2008; Aizen et al. 2012; Valiente-Banuet et al. 2015). Many of the studies on the effects of fire on plant-animal interactions have been conducted in experimental, prescribed fires that are smaller and less heterogeneous and intense than wildfires making generalizations difficult (e.g. Vickery 2002; Campbell et al. 2007; van Nuland et al. 2013). As mentioned before, fires can differentially affect plant and animal species. Such mismatches across trophic levels may lead to the disruption of their interactions (Knight and Holt 2005). From the plant's perspective, the disruptions may have contrasting effects on its reproductive success depending on the sign of the interaction (i.e.

mutualistic and antagonistic interactions *Chapters I* and *II*). Therefore, while plants may benefit by disruptions of herbivory or seed predation interactions (e.g. Knight and Holt 2005, *Chapter I*), they may experience detrimental effects by the disruption of their mutualistic interactions (e.g. LoPresti et al. 2018, *Chapter II*).

Studies on the impacts of fire on herbivory have mainly focused on large mammalian grazers, especially in grasslands and tallgrass prairies where herbivores interact with the predominant surface fires (Parr et al. 2014; Forrestel et al. 2015; Burkepile et al. 2016). In fact, the interaction between grazers and fire is used with management purposes in these ecosystems (*pyric herbivory*; Fuhlendorf et al. 2009; Limb et al. 2011; McGranahan et al. 2012), and the rewilding of large grazers has been proposed in order to restore natural fire regimes (Johnson et al. 2018). Research on fire effects on herbivory by insects shows disparate results, that is, while in some cases herbivores are rapidly attracted to the young, green leaves into the burnt increasing plant damage (Radho-Toly et al. 2001; Lopes and Vasconcelos 2011), other studies have reported a slow recovery of herbivore populations that can last for several years resulting in reduced herbivory pressures inside the burnt areas (Whelan and Main 1979; Knight and Holt 2005).

There are also examples of fire effects on seed predation and biotic-dispersal interactions (Whelan 1986; Andersen 1988; Ordoñez and Retana 2004; Parr et al. 2007; Broncano et al. 2008; Zwolak et al. 2010; Tasker et al. 2011; Beaumont et al. 2013; St Clair et al. 2016; Paolucci et al. 2016; Setterfield and Andersen 2018). Once again, the contrasting results make difficult to stablish generalized response patterns. Thus, while in many studies seed predators and dispersers, especially feed-generalists and fire-opportunistic species, can strongly increase in burnt areas linked to the availability in food resources and changes in habitat structure (e.g landscape simplification, Parr and Andersen 2007) with marked effects on seed predation/dispersal rates (Saracino et al. 2004; Ordoñez and Retana 2004; Parr and Andersen 2007; Broncano et al. 2008; Lucas-Borja et al. 2010); others have shown postfire negative impacts on seed predator and disperser populations resulting in different outcomes on plant reproductive performance (Whelan 1986; Vickery 2002). Past work on the effects of fire on seed dispersal by animals has focused on dispersal by ants (i.e. myrmecochory) because of its predominance in some fire-prone ecosystems as tropical savannas (Parr and Andersen 2007; Paolucci et al. 2016), while our knowledge on other groups of dispersers (e.g. birds) is still scarce. Under a management perspective, the interactive effects of fire, land-use changes and logging on herbivory, seed predation and dispersal interactions have also received attention (Puerta-Piñero et al. 2010, 2012; Castro et al. 2011; Hahn and Orrock 2015).

Particularly in the last decade, there has been increasing interest on the effects of fire on plant pollination (Ne'eman et al. 2000; Potts et al. 2001, 2003, 2006; Campbell et al. 2007; Pauw 2007; Dafni et al. 2012; van Nuland et al. 2013; Brown et al. 2016; Ponisio et al. 2016; Brown and York 2017; Carbone and Aguilar 2017; Peralta et al. 2017; Lybbert et al. 2018; LoPresti et al. 2018). Different studies show that despite fires disrupt plant-pollinator interactions, plant communities can be quite resilient with short recovery times of pollinator populations and plant reproductive performance (Potts et al. 2001; Campbell et al. 2007; van Nuland et al. 2013, Peralta et al. 2017; Lybbert et al. 2018). This may be explained by the generalized nature of most of the pollination systems assessed (but see Pauw 2007; Brown and York 2016; Brown et al. 2017), where the lack of some pollinators can be buffered by other species with high dispersal or soil-nesting preferences (Moretti et al. 2009; Lazarina et al. 2016; Peralta et al. 2017). Most studies on fire and pollination have mainly focused on bee pollinators and on typically explored floral traits and rewards (e.g. changes on floral patch density, pollen and nectar rewards, Ne'eman et al. 2000; Potts et al. 2003; van Nuland et al. 2013; Peralta et al. 2017; LoPresti et al. 2018). However, the effects of wildfires in other groups of pollinators and on less studied plant traits such as scents mediating plant-pollinator interactions is still lacking (*Chapter IV*). These chemical signals emitted by plants allow them to attract pollinators even from long distances and are crucial in many pollination systems (Schiestl 2015). Recent studies assessing the effects of anthropogenic-driven disturbances on scents involved in pollination have reported significant changes on these chemical signals that ultimately may alter pollinator attraction (Farré-Armengol et al. 2014, 2016; Burkle and Runyon, 2016, 2017; Jürgens and Bischoff 2017).

Despite the varying results, three characteristics of fire regimes emerged from previous literature as important factors in understanding the response of plant-animal interactions to wildfires. These factors can interact with each other, with other characteristics of fires regimes (e.g. intensity, seasonality) and with certain traits of the species involved (e.g. body size, trophic and nesting preferences). First, the **fire frequency** of a particular fire regime allows plants and animals to recover before the next fire event occurs (Keeley et al. 2011, 2012; New 2014). However, increased frequencies reduce the fire-free interval and may not allow plants and animals to recover, which may negatively impact on their interactions (Moretti et al. 2006; Keeley et al. 2011; Dafni et al. 2012; Griffiths et al. 2015; Carbone and Aguilar 2017; Koltz et al. 2018). In addition, high fire frequencies promote soil erosion and change soil composition (i.e. soil nutrients and water content) that can modify plant and soil arthropod postfire responses (Huebner et al. 2012; Carbone and Aguilar 2017). Research assessing the effects of fire frequency on plant-insect interactions is still scarce, although regarding pollination, a few studies on pollinator communities suggest that they can be less resilient under higher fire frequencies, and that generalist species (e.g polylectic bees) may be favored in frequently burnt areas (Moretti et al. 2006; Brown et al. 2016; Lazarina et al. 2017; Carbone and Aguilar 2017).

The **postfire age** (i.e time since last fire) is a key factor in determining the interaction response as it indicates the successional stage and in turn, the availability and quality of nesting sites, hosts and food resources for animals

(Moretti et al. 2009; Lazarina et al. 2016; Peralta et al. 2017). From the plant's perspective, if the mortality of herbivores or seed predators is high, the postfire age may also indicate the time lapse between a release from antagonistic pressures and the complete interaction recovery (*Chapter I*). In addition, time since the last fire can be also relevant in shaping the balance between seed dispersal and seed predation interactions at burnt sites (Dafni et al. 2012). For antagonistic species with broad trophic niches or those with high dispersal abilities, faster postfire recolonization rates than for specialists or less mobile species are expected (Mihuc et al. 1995; Arnold et al. 2017; Pausas and Parr 2018; Koltz et al. 2018), although this has been rarely studied in the context of ecological interactions (Mihuc et al. 1995, *Chapter I*). Regarding plant pollination, the time since fire also shapes the availability of pollinators (Potts et al. 2003, 2005; Pauw 2007; Moretti et al. 2009; Lazarina et al. 2016; Peralta et al. 2017). For instance, in fire-prone ecosystems the abundance of floral resources provided by seeders and species with fire-induced flowering (e.g. resprouting geophytes) together with the increased availability of bare ground, attracts a great amount of pollinators to recently burnt sites and plant reproduction can experience a fast postfire response (Potts et al. 2001; 2003, 2005; Pauw 2007; Campbell et al. 2007; Peralta et al. 2017). However, in specialized pollination systems this recovery may be constrained by the mutual dependence among the interacting species (*Chapter II*).

The **fire extent** can also influence the effects of fires on plant-animal interactions (Vickery 2002; Knight and Holt 2005; Parr et al. 2007, Arnold et al. 2017). Fires create patchy structures with burnt areas surrounded by adjacent unburnt sites than can alter biotic interactions at local and landscape scales (Vickery 2002; Knight and Holt 2005; Ponisio et al. 2016). In addition, fires directly kill many animals or they die after at burnt sites (i.e. starvation or increased predation). Their recovery will depend on dispersal from surrounding unburnt areas or fire refugia (Swengel 2001; Brennan et al. 2011; New 2014; Koltz et al. 2018; Pausas and Parr 2018). Therefore, while recolonization proceeds, fire can also lead to the appearance of spatial gradients on plant-animal interactions inside the burnt (Vickery 2002; Knight and Holt 2005; Parr et al. 2007; Arnold et al. 2017). For instance, Knight and Holt (2005) found that the abundance of orthopteran herbivores in a sandhill ecocosystem decreased with the distance from the fire perimeter (a surrogate of fire extent) towards the interior, creating spatial gradients in plant herbivory pressures within the burnt area. Hence, similarly to postfire age, this spatial variation might be exacerbated in plant-animal interactions depending on animal species with low dispersal abilities or particular feeding requirements (i.e. specialists) that may need more time to reach the fire interior (*Chapters I and II*).

The role of plant-animal interactions in the assembly of fire-prone communities

As previously mentioned, fire acts as a strong abiotic filter driving species coexistence in fire-prone communities where it promotes plant assemblages with similar fire-related traits (i.e. phenotypic clustering, Verdú and Pausas 2007; Pausas and Verdú 2008; Ojeda et al. 2010; Cianciaruso et al. 2012; Hoffman et al. 2012; Dantas et al. 2013). There is also increasing evidence on the role of plant-animal interactions as additional forces contributing into the assembly of plant communities (Sargent and Ackerly 2008; Pellissier et al. 2010, 2012; Muchhala et al. 2014; Briscoe-Runquist et al. 2016; Larios et al. 2017; Bartomeus and Godoy 2018). Particularly, mutualistic interactions (e.g. pollinationa and seed dispersal) may drive species coexistence, for example, through biotic filtering or their interplay with plant-plant facilitation and competition, leading to non-random patterns of plant reproductive traits (e.g. floral, fruit, seed traits, Sargent and Ackerly 2008; Pellissier et al. 2012). Changes in the spatial distribution of these mutualistic interactions may lead to the emergence of particular assembly patterns in different habitat conditions (Pellissier et al. 2010; Koski and Ashman 2015; Wolowski et al. 2017; Bergamo et al. 2018; Kemp et al. 2018). For instance, contrasting sets of dominant pollinators can lead to community assemblages sharing similar floral colors linked to local pollinator preferences (Kemp et al. 2018).

Forest-savanna mosaics from Neotropical fire-prone ecosystems are ideal environments to test the potential contribution of plant-animal mutualisms in community assembly, because of their great biodiversity together with the contrasting environmental conditions (i.e. forest vs savanna habitats) under different fire histories. However, community ecology studies on these ecosystems have mostly focused on habitat filtering as the main assembly process (Hoffmann et al. 2003; 2012; Dantas et al. 2013, 2016; Laureto and Cianciaruso 2015; Maracahipes et al. 2018), while information on plant-animal interactions at the community level is scarce (Maruyama et al. 2014; *Chapter IV*).

AIMS

The main goal of this thesis is to get a better understanding of the effects of fire on plant-animal interactions from fire-prone ecosystems and the ultimate consequences on plant reproduction. In addition, it also explores whether these interactions contribute to the assembly of fire-prone communities where the fire filter is known as a strong assembly force. The specific aims addressed by each chapter are:

1) To assess whether wildfires, through the postfire age and distance to the unburnt (i.e. fire extent), disrupt antagonistic plant-animal interactions (i.e. seed predation and herbivory) leading to beneficial effects on plant reproduction (***Chapter I***). This chapter also evaluates whether the fire effects differ with the degree of specialization of the interactions involved (generalized vs specialized).

2) To study whether wildfires, through the postfire age and distance to the unburnt (i.e. fire extent), disrupt specialized pollination interactions leading to negative effects on plant reproductive performance (***Chapter II***).

3) To assess whether wildfires can modify the plant chemical signals mediating in plant-pollinator interactions and the consequences on pollinator attraction (***Chapter III***).

4) To evaluate the potential contribution of plant-animal mutualisms (through reproductive traits shaped by theses interactions) in the community assembly of forest-savanna mosaics from fire prone ecosystems (***Chapter IV***).

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GENERAL METHODOLOGY

Study sites and experimental design

The first three chapters of this thesis were conducted in seven locations after wildfires in eastern Spain. In *Chapter IV*, information on an eighth study area from a forest-savanna mosaic in the Brazilian Cerrado was used to test for community assembly patterns. The study areas in Spain are shrublands with Mediterranean climate characterized by contrasting mild wet winters and hot dry summers with frequent fires (Pausas 2004, see Figs. M1 and M2, and Table M1). The study area in Brazil consisted in a mosaic of forest and savanna plots with varying canopy structure and fire history from the Cerrado bioregion (Fig. M3). In this case, climate is tropical-humid with a marked seasonality defined by a warm rainy season from October to April and a dry season from May to September (Dantas et al. 2013). Both areas correspond to fire-prone ecosystems (i.e. Mediterranean shrublands and Neotropical savannas) in which organisms may have evolved different strategies under certain fire regimes (Keeley et al. 2011).

To assess the effects of wildfires on antagonistic (*Chapter I*) and mutualistic (*Chapters II* and *III*) plant-animal interactions; and on plant scents mediating pollination interactions (*Chapter III*), field sampling was performed in burnt and close unburnt sites at each study area (Fig. M1 and Table M1). Postfire changes in specialized and generalized antagonistic interactions (i.e. seed predation and herbivory) were studied in *Chapter I*, while *Chapters II* and *III* were focused on the effects in specialized pollination interactions.

Figure M1. Map of the seven locations studied after wildfires in eastern Spain. Locations 2-4 were sampled in *Chapter I*, 1 and 5-7 in *Chapter III*, and 6 and 7 in *Chapter III*.

Table M1. Information on the study locations in eastern Spain. Year: year of the fire, Samp. years: year/s of sampling. Numbers in brackets correspond to Fig. M1.

Locality	Year	Longitude	Latitude	Samp. years	Chapter
Cortes (4)	2012	39.289298	-0.799552	2014, 2015	I
Andilla (2)	2012	39.800408	-0.708005	2014, 2015	I
Segorbe (3)	2014	39.825932	-0.427716	2014, 2015	I
Tivissa (1)	2014	40.979691	0.693141	2016, 2017	I, II
Dénia (6)	2014	38.808054	0.160267	2016, 2017	II
Carcaixent (5)	2016	39.105267	-0.400584	2017	II, III
Xàbia (7)	2016	38.731141	0.169339	2017	II, III

A careful selection of the unburnt (control) sites was performed in order to choose sites that were representative of the prefire conditions in terms of species composition and soil type. To test the effects of the distance to the unburnt (a surrogate of fire extent) and time since fire in plant-animal interactions and the consequences on plant reproductive success, the study interactions were sampled at different distance categories (*Chapter I*) or along transects (*Chapter II*) from the fire's perimeter in areas with different postfire ages and involving various sampling years (*Chapters I* and *II*). Distance from each study plot (*Chapter I*) or plant individual (*Chapter II*) to the burnt perimeter was calculated by using geographical information systems with the software Quantum GIS (QGIS Team 2013). In the case of large wildfires that show unburnt patches, distance to these patches was also included (i.e. minimum distance to unburnt vegetation). The effects of fire on plant-animal interactions were investigated by field censuses of different insect species on their interacting plants. The consequences for plant reproductive performance were assessed by fruit collection at field and after estimating seed predation or plant fruit set (i.e. proportion of flowers setting fruits).

Figure M2. Mediterranean shrubland with postfire resprouter and seeder species after a wildfire in Carcaixent (Valencia, Spain). Photo by Yedra García.

Chapter *III* studied the potential effects of wildfires on plant chemical signals (i.e. scent) that mediate in pollination interactions. These signals are conformed by volatile organic compounds (VOCs). The pollination system of the Mediterranean dwarf palm, *Chamaerops humilis*, was used to test this idea. The palm scent was collected in burnt and adjacent unburnt sites from two locations after wildfires in Spain. In addition, to assess the postfire changes on plant signal attractiveness, olfactory bioassays with the palm's pollinators and the scent sampled in burnt and unburnt sites were performed at the laboratory.

Finally, *Chapter IV* explored the contribution of mutualistic plant-animal interactions to the assembly of forest-savanna mosaics. To achieve this goal, a database of 12 reproductive traits (i.e. floral and fruit traits) from plants co-occurring across a forest-savanna mosaic was built. Information on plot composition and canopy structure from a previous study by Dantas et al. (2013) was also used. Trait information was obatined from an extensive literature search and direct measures in digital herbarium records (see Appendix D for details on data compilation). The trait data base together with the species co-occurrence matrix were used to assess whether the phenotypic structure of forests and savannas departed from random community patterns derived by null models.

Figure M3. Brazilian Cerrado vegetation in Parque Nacional Serra do Cipó (Belo Horizonte, Brasil). Photo by Yedra García.

Study interactions

The effects of the distance to the unburnt and postfire age were assessed in the following antagonistic and mutualistic plant-animal interactions involving Mediterranean plant and insect species:

- *Ulex parviflorus-Exapion fasciolatum* (*Chapter I*)

  Ulex parviflorus Pourr. (Fabaceae) is a spiny shrub from the western Mediterranean Basin. This gorse is considered an obligated seeder species with seeds breaking their dormancy under high temperatures and postfire-induced germination (Paula et al. 2009, Moreira et al. 2010). The plant shows a specialist seed predator, the weevil *Exapion fascilatum* Wagner (Apioninae) that feeds on the gorse seeds. *Exapion* weevils are specialized on plants of tribu Genisteae (Fabaceae) and previous studies have shown the strong dependence among different *Exapion* weevils and *Ulex* species (e.g. *Exapion ulicis-Ulex europaeus*, Barat et al. 2007; Tarayre et al. 2007). Female individuals leave their eggs inside the gorse's green pods where the weevil develops. Adults emerge from ripe fruits during the fruit dehiscence. In addition, parasitoid wasps (Chalcidoidea) that feed on the weevil larvae and pupa are often seen inside the gorse pods. Seed predation in this specialized system was assessed in *Chapter I.*

- *Asphodelus ramosus* and antagonistic predators (*Chapter I*, Fig. M3)

  The branched asphodel *Asphodelus ramosus* L. (Liliaceae) is a Mediterranean geophyte that shows a quick postfire recovery thanks to is resprouting rhizome (Pantis and Margaris 1988; Paula et al. 2009, Fig. M4). The plant is attacked by different generalist herbivores such as the Cetoniinae beetles *Tropinota squalida* Scop and *Oxythyrea funesta* Poda and the bug *Carpocoris fuscipinus* Boh. (Pentatomidae). In addition, the specialist bug *Horistus orientalis* Gmelin (Miridae) shows a life cycle tightly dependent on the plant, where nymphs and adults feed on different organs especially on flower and fruits. The abundance of generalist and specialist predators on the plant as well as the resulting fruit set were studied in *Chapter I*.

Figure M4. *Asphodelus ramosus* in bloom after a wildfire in Segorbe (Spain). Photo by Cala Castellanos.

- *Chamaerops-humilis* and specialist beetle pollinators (*Chapters II*, *III*)

The Mediterranean dwarf palm *Chamaerops humilis* L. (Arecaceae) is a dioecious palm from the western Mediterranean Basin. It shows a rapid postfire resprouting from apical buds and has the ability to flower the spring after a fire (Paula et al. 2009; Tavşanoğlu and Pausas 2018, Fig. M5). The palm is engaged in a specialized nursery pollination system with the weevil *Derelomus chamaeropis* who develops preferentially inside the old male inflorescences (Anstett 1999; Dufaÿ and Anstett 2004; Jácome-Flores et al. 2018). In spring, during the palm flowering, the palm leaves instead of flowers (almost odorless) emit a strong scent that attracts newly emerging weevil individuals, with *C. humilis* pollen attached, to flowering palms (Dufaÿ et al. 2003). Both plant sexes show similar scent composition allowing female plants to be pollinated (Dufaÿ et al. 2003). In addition, the palm has an additional non-nursery pollinator, the sap beetle *Meligethinus pallidulus* (Nitidulidae) who as shown in *Chapter II* can be especially relevant in postfire conditions (García et al. 2018). During the palm flowering, the abundance of the two beetle species on the palm new inflorescences as well as the resulting palm fruit set were sampled (*Chapter II*).

Figure M5. Male individual of *Chamaerops humilis* flowering after a wildfire in Segorbe (Spain). Photo by Juli Pausas.

VOCs analysis and olfactory bioassays

To explore for potential effects of wildfires on *Chamaerops humilis* scent *(Chapter* 3), the palm's volatiles were sampled in 60 individuals from two areas after recent wildfires (< 1 year postfire age). VOCs were collected in a burnt and an adjacent unburnt site (control) at each study area. During the palm flowering, coinciding with the peak of scent emission, the palm's scent was sampled from healthy leaves by dynamic headspace adsorption (Fig. M6). One leaf per individual was enclosed in a polyethylene terephthalate bag connected to a portable membrane pump. The emitted volatiles were then retained inside scent traps containing a mixture of two adsorbents (see Appendix C for further details). Air blanks were also sampled to correct for VOCs contaminants in the study areas. Additional scent samples from burnt and unburnt sites were collected for the olfactory bioassays.

The palm's VOCs were analyzed by Gas Chromatography-Mass Spectrometry (GCMS) at the Mass Spectrometry Section of the Experimental Research Support Service from the University of Valencia (SCSIE). The identity and the relative amount of each volatile compound in the scent was assessed by comparison of the MS compound peak areas with mass spectral databases from NIST 11 and Willey 9 libraries (SCSIE, see Appendix C for details).

Olfactory bioassays were performed at the laboratory to test whether the postfire-induced changes on plant VOCs altered the scent attractiveness to pollinators. Bioassays were conducted in a Y-tube olfactometer with the two specialist beetle pollinators collected in the study areas. In each trial, a leaf scent sample diluted in acetone from the burnt or the unburnt sites was tested against a control sample (i.e. only acetone) in the two arms of the olfactometer (see *Chapter III* for details). Control trials with acetone in both arms were alternated with VOC trials.

Figure M6. Field sampling of *Chamaerops humilis* scent after a wildfire in Carcaixent (Valencia, Spain). Photo by Yedra García.

Functional and phylogenetic structure measures

To investigate the potential contribution of mutualistic plant-animal interactions (i.e. pollination and seed dispersal) in the assembly of forest-savanna mosaics, the functional patterns of both habitat types, based on reproductive plant traits linked to these interactions were studied. The phylogenetic structure in both habitats was also explored in order to depict the prevailing assembly forces in these complex communities. Functional and phylogenetic patterns were estimated for 75 plant species occurring in forest and savanna plots (N= 98) sampled by Dantas et al. (2013, *Chapter IV*). The standardized effect size of the Mean Pairwise Distance of reproductive traits (hereafter Mean Functional Distance, MFD) and of the Mean Phylogenetic Distance (MPD) were used as phenotypic and phylogenetic structure measures in both habitats. To calculate the phylogenetic structure, a phylogenetic tree for the species pool was obtained by using as backbone an updated version of

Zanne's phylogeny for vascular plants (Zanne et al. 2014; Qian and Jin 2016). To test for non-random functional and phylogenetic patterns, the departure of sesMFD and sesMPD values from random expectations was estimated by using null models. Differences in sesMFD and sesMPD among forests and savannas were also assessed (see *Chapter IV* for further details).

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CHAPTER I: Fires can benefit plants by disrupting antagonistic interactions

Abstract

Fire has a key role in the ecology and evolution of many ecosystems, yet its effects on plant-insect interactions are poorly understood. Because interacting species are likely to respond to fire differently, disruptions of the interactions are expected. We hypothesized that plants that regenerate after fire can benefit through the disruption of their antagonistic interactions. We expected stronger effects on interactions with specialist predators than with generalists. We studied two interactions between two Mediterranean plants (*Ulex parviflorus, Asphodelus ramosus*) and their specialist seed predators after large wildfires. In *A. ramosus* we also studied the generalist herbivores. We sampled the interactions in burned and adjacent unburned areas during two years by estimating seed predation, number of herbivores and fruit set. To assess the effect of the distance to unburned vegetation we sampled plots at two distance classes from the fire perimeter. Even three years after the fires, *Ulex* plants experienced lower seed damage by specialists in burned sites. The presence of herbivores on *Asphodelus* decreased in burned locations, and the variability in their presence was significantly related to fruit set. Generalist herbivores were unaffected. We show that plants can benefit from fire through the disruption of their antagonistic interactions with specialist seed predators for at least a few years. In environments with a long fire history, this effect might be one additional mechanism underlying the success of fire-adapted plants.

INTRODUCTION

Fire is one of the most common disturbances worldwide and can play an important role in the ecology and evolution of many ecosystems (Pausas and Keeley 2009). In environments with a long fire history, such as tropical savannas and Mediterranean ecosystems, fire structures communities and landscapes (Verdú and Pausas 2007; Keeley et al. 2011; Dantas et al. 2013). The effects of fire on plants are relatively well-known (Bond and Van Wilgen 1996; Keeley et al. 2012) and information on the effects of fire on animal populations is also increasing steadily (Swengel 2001; Izhaki 2012, New 2014). Studies on the role of fire in plant-animal interactions have been largely focused on mammal herbivory (e.g., Fuhlendorf et al. 2009; Wan et al. 2014) and, to a lesser extent, on seed predation (e.g., Bond 1984; Andersen 1988; Broncano et al. 2008). However, less is known about how fires disrupt plant-insect interactions and the implications for the plants (Vickery 2002; Knight and Holt 2005; Dafni et al. 2012).

Both antagonistic (e.g. herbivory) and mutualistic (e.g. pollination) interactions between plants and insects are crucial components of natural ecosystems and can determine ecological and evolutionary processes (Herrera and Pellmyr 2002). In ecosystems where wildfires are historically recurrent, many plant species are capable of quickly recovering via resprouting or recruitment from a fire-resistant seedbank (Pausas et al. 2004) and reproduce shortly after the fire. In contrast, fires can directly cause drastic declines in many insect populations, whose recovery then depends on the fire regime and intrinsic characteristics like movement capacity (Swengel 2001; Moretti et al. 2006). Because the different interacting species are likely to respond to fire in varying ways, disruptions of the plant-insect interactions are expected. The dynamics of these disruptions and the postfire recovery of the interactions could therefore have strong consequences for plant populations and constitute important selective pressures for species living in fire-prone environments.

The effects for plants may be different depending on whether fire disrupts mutualistic interactions, potentially decreasing reproductive success, or antagonistic interactions such as herbivory and seed predation. Fires can for example increase seed predation and herbivory when generalist insects are involved (Andersen 1988; Radho-Toly et al. 2001; Lopes and Vasconcelos 2011). However, there is also evidence of a postfire decrease in insect herbivory in different ecosystems (Whelan and Main 1979; Auld and O' Connell 1989; Vickery 2002; Knight and Holt 2005). In the latter cases, plants could benefit through a release from negative interactions that limit their performance (Hendrix 1988). The reduction of negative effects can be stronger if the interactions involve seed predators, and this can be particularly beneficial for plants in which the success of their first life stages depends on fire.

The consequences of a fire-driven disruption of antagonistic interactions on plant populations will depend, among other factors, on two important interrelated aspects. First, postfire changes in the existing habitat can have a greater impact on species that are tightly dependent on specific habitat characteristics like specialists, compared to generalists (Ewers and Didham 2006). For instance, herbivory and seed predation are often exerted by highly specialized phytophagous insects which interact only with one or a few host plants (Ehrlich and Murphy 1988; Jaenike 1990). Consequently, the alteration of their host plants may lead to changes in their abundance and distribution (Larsson et al. 2000). The recolonization of burnt areas by generalist animals might therefore be faster than by specialists, because the specialist's mobility into the interior of the burnt may be restricted to the presence and the regeneration of their only host. Second, the distance to unburned vegetation might also have an impact on the speed of recolonization and therefore on the duration of the disruption, which can result in spatial variation in the interaction from the edge towards the interior of the burned area. The contrast between specialists and generalists may be even stronger in large fires where species have to migrate long distances to reach the center of the burned area. A stronger disruption of specialized interactions compared to generalist ones would thus be expected, and this effect could be exacerbated as one moves from the edge to the center of the fire. While some previous studies show that fire may modify the plant-insect interaction, less is known about the implications for the plant's reproductive performance (e.g., Whelan and Main 1979; Auld and O'Connell 1989; Vickery 2002), and particularly on how these implications differ depending on the level of specialization of the insect.

Our hypothesis is that plants that quickly regenerate after fire may additionally benefit from it because fire disrupts antagonistic interactions, and that this effect will be exacerbated with the distance to the unburned vegetation. To test it, we studied two interactions between plants and their specialist seed predators after recent wildfires in Mediterranean shrublands of eastern Spain: (1) the Mediterranean gorse *Ulex parviflorus* and its seed predator, the weevil *Exapion fasciolatum*; and (2) the branched asphodel *Asphodelus ramosus* and the specialist mirid bug *Horistus orientalis,* together with other generalist insects. In the two systems the life cycles of the specialist insects depend entirely on their host plants. We expected that fires would have strong negative effects on the local populations of both specialist predators, and would in turn benefit the host plants by reducing seed predation.

MATERIAL AND METHODS

Plant-insect interaction I: *Ulex parviflorus-Exapion fasciolatum*

The Mediterranean gorse, *Ulex parviflorus* Pourr. (Fabaceae) is a spiny perennial shrub from the western Mediterranean Basin. It can live up to 25 years (Baeza and Vallejo 2006) and, as observed in this study, individuals can reach their mature stage as soon as two years after fire. One or two (occasionally more) seeds develop inside small pods and are dispersed explosively at the beginning of the summer. A preliminary analysis suggested that the variance in the number of seeds per pod is not related to contrasted fire regimes (mean number of seeds/pod = 1.29 and 1.34 in populations growing under high or low fire frequency respectively, N= 3206 pods examined). *Ulex parviflorus* is common in fire-prone Mediterranean shrublands where it recruits massively after fire, when high soil temperatures break seed dormancy and induce germination (postfire obligate seeder; Paula et al. 2009; Moreira et al. 2010; Moreira and Pausas 2012).

Ulex parviflorus seeds are attacked by the weevil *Exapion fasciolatum* Wagner (Brentidae: Apioninae). Information on this species is scarce, but *Exapion* species are specialist predators of the Genisteae tribe (Fabaceae; Alonso-Zarazaga 1990), including *Ulex* (Barat et al. 2007; Tarayre et al. 2007). In the closely related *Exapion ulicis-Ulex europaeus* interaction, weevil predation can damage up to 90% of the gorse pods and may explain phenological shifts in the plant to reduce predation impacts (Barat et al. 2007; Tarayre et al. 2007). The weevil's life cycle depends entirely on the host plant. In early spring, females lay their eggs inside the gorse ovaries or small green pods, where larvae and pupa develop while feeding on the seeds. Adults emerge with ripe pod dehiscence. *Ulex parviflorus* pods can also contain a parasitoid wasp (*Eurytoma* sp.) feeding on the larvae and pupae of *Exapion fasciolatum*.

Plant-insect interaction II: ***Asphodelus ramosus-Horistus orientalis***

Asphodelus ramosus L. (= *A. aestivus* Brot., Liliaceae) is a Mediterranean geophyte widely distributed along the Mediterranean basin (Lifante 1996). It has a short rhizome surrounded by tubers and a basal rosette of leaves that produces a branched flowering scape. Thanks to the resprouting capacity from the rhizome, this species is favored by heavy grazing and recurrent fires (Pantis and Margaris 1988); in fact, it flowers massively in burned areas.

Horistus orientalis Gmelin (= *Capsodes lineolatus* Br., Hemiptera: Miridae) is a phytophagous bug that feeds on *Asphodelus ramosus*. Members of the mirid family exhibit a high degree of host-plant specificity (Cassis and Schuh 2012) and in our study region this species has never been confirmed feeding on other plants (Luis Vivas pers. comm). Mirids often develop synchronously with the plant, from the deposition of the eggs within the scape tissues, until adult emergence after the nymph stage (Wheeler 2001; Cassis and Schuh 2012). Published information about *H. orientalis* is very limited, but our observations suggest that its entire life cycle occurs on the plant, as in the closely related bug *Capsodes infuscatus*. The eggs are deposited inside the inflorescence stalk in the spring and adults disperse the next spring (Ayal and Izhaki 1993; Izhaki et al. 1996). Both nymphs and adults feed on leaves and especially on flowers and fruits. In the case of *C. infuscatus*, the damage produced on *A. ramosus* can reach 100% of fruit loss (Ayal and Izhaki 1993). *Asphodelus ramosus* is also attacked by generalist herbivores; we mainly observed two phytophagous beetles from the subfamily Cetoniinae, *Tropinota squalida* Scop. and *Oxythyrea funesta* Poda, and the bug *Carpocoris fuscipinus* Boh. (Pentatomidae), among others.

Study sites and sampling

Each interaction was studied during two consecutive years in two burned locations from different wildfires in eastern Spain (Valencia; see Table 1.1). The region shows a typical Mediterranean climate with frequent fires (Pausas 2004; Pausas and Paula 2012). To study the effect of fire on the interactions we sampled plots inside each burned location plus unburned adjacent (control) plots where no fires have been registered for at least 20 years. Unburned plots were carefully chosen to be representative of the pre-fire conditions (e.g. same plant species composition, dominant species and soil characteristics), and when possible, close the to fire perimeter. The same control and burned plots were sampled during the two years of the study when possible. To assess the effect of the distance to unburned vegetation on the interactions, plots were assigned to three different categories according to their distance to the fire's perimeter: (a) control plots in the adjacent unburned areas ("Unburned"), (b) plots located inside the burned area and up to 500 meters (mean= 268) from the fire perimeter ("Edge") and (c) plots at more than 500 meters (mean= 1199, maximum= 2400) from the fire perimeter

("Center"). Plots at burned areas were carefully selected to avoid the proximity of unburned patches. Distances were estimated using geographic information tools and digital maps provided by the regional government of Valencia.

Table 1.1. Fire location name, year of fire occurrence and sampling years for each study system: a) *Ulex parviflorus* and the specialist weevil *Exapion fasciolatum* and b) *Asphodelus ramosus* and the specialist bug *Horistus orientalis* and its generalist herbivores.

System	Fire location	Year	Sampling years
U. parviflorus-E. fasciolatum	Cortes	2012	2014 & 2015
	Andilla	2012	2014 & 2015
***A. ramosus*-herbivores**	Cortes	2012	2014 & 2015
	Segorbe	2014	2014 & 2015

Seed predation on *Ulex parviflorus* was measured in 48 plots in two locations where large wildfires (of more than 20000 ha each) had occurred in the summer of 2012: Cortes de Pallás (hereafter, Cortes) and Andilla, both in the province of Valencia (Table 1.1). Before the fires, all plots were Mediterranean shrublands dominated by *Rosmarinus officinalis*, *Ulex parviflorus*, several *Cistus* species and *Quercus coccifera.* Field work was carried out between late March and June in 2014 and 2015 and corresponded to the first two postfire flowering years for the newly recruited individuals of *U. parviflorus*. The sampling included 34-35 burned plots and 13-14 plots in the adjacent unburned areas (see Table A1 in Appendix A details on plots at each fire location). At each plot, 400 mature pods were collected from 10 haphazardly chosen *U. parviflorus* plants (40 pods per plant), separated from each other by at least 5 m. The 400 pods from Andilla's burned plots in 2014 were collected from 20 plants (20 per plant) because fruit production per plant was lower in that location. We chose a fixed number of pods per plant to estimate the levels of seed predation as opposed to attempting to quantify whole-plant production, a difficult task given the massive and extended flowering period of *U. parviflorus*. Overall the total number of pods sampled was 18370 in 2014 and 19265 in 2015.

The content of each pod was observed at the laboratory under a stereo-microscope. The presence of the weevil at larva, pupa or adult stage was recorded in each pod. When a parasitoid wasp was observed inside the pod, it was also counted as predated, i.e., we assumed that wasps had emerged from a weevil larva (Barat et al. 2007). We used the proportion of predated pods as a measure of the effect of the seed predator on the fitness of the plant. This method directly estimates weevil predation within each pod and allows to differentiate their effect from other predispersal predators as mentioned above (Barat et al. 2007).

The study on *A. ramosus* was conducted in Cortes and in a second smaller wildfire that occurred in February 2014 in Segorbe (province of Castellón; Table 1.1). Sampling was performed during spring when *A. ramosus* was already in bloom, and included a total of 15 plots in 2014 (9 burned and 6 unburned plots) and 14 in 2015 (8 burned and 6 unburned plots, for details see Table A2 in Appendix A). At each plot, the presence and activity of the specialist bug *Horistus orientalis* and the most abundant herbivores (Cetoniinae and Pentatomidae) were recorded on 50 haphazardly chosen *Asphodelus* plants separated from each other by at least 5 m; censuses were conducted between 10:00 and 16:00 h. Other generalist herbivores were observed only rarely and were thus not included in the analyses. The number of branches, floral buds and flowers were also recorded for each plant. At the end of the flowering season (May-June) we collected ripening fruits from all plants and counted healthy seeds in the laboratory in all plots within the burned areas. The proportion of fruits in relation to the number of flowers produced (fruit set) was considered an indicator of reproductive success and was analyzed with respect to the presence of the seed predators on the plant (see below). We used fruit set as a proxy for reproductive success because it corrects for variation in plant size as opposed to using the absolute number of fruits or seeds produced per plant.

Although fruit set is not a direct measure of the plant damage, it may reflect the total effects of the different feeding habits of the insects including green parts of the plant and also fruits. In fact there is evidence of a strong negative relationship between plant fruit set and the bug's abundance in the closely related *Asphodelus aestivus*-*Capsodes infuscatus* interaction (Ayal and Izhaki 1993; Izhaki et al. 1996). Fires could also affect other factors linked to plant fruit set such as resource availability and pollination. We expect a limited effect of pollination, because this species has a generalized pollination system (Lifante 1996; Lázaro et al. 2016) and flying pollinators tend to recover quickly after fires (Potts et al. 2003).The increase in resources often associated with postfire environments could also have positive effects on fruit production. However, we did not find a difference either in the number of flowers nor in the absolute seed production between burned and unburned sites (see results), suggesting a limited relevance of the potential changes in resources.

Statistical analysis

To examine whether seed predation on *U. parviflorus* at the plant level differed between burned and unburned plots, we used a generalized linear mixed model (GLMM), with a binomial error distribution. For each year of sampling, the GLMM included the burned vs. unburned treatment as a fixed factor and plot nested within locality as random factor. The same approach was used to test whether seed predation varied between Edge and Center zones (i.e., within the burned area).

To test whether the number of specialist *Horistus orientalis* individuals differed between *A. ramosus* plants from burned and control plots, we used a similar GLMM model structure as above, in this case with a Poisson error distribution. We also used the same model structure to test for differences in the number of generalist herbivores (Pentatomidae plus Cetoniinae), in the total herbivores (*H. orientalis* and generalists together) and in the number of flowers and seeds produced per plant. We then tested for distance effects in the number of the three insect groups (specialist, generalist and total) by fitting a GLMM to the distance class variable (Edge vs Center). To evaluate to what extent the variability observed in herbivores in the burned zone correlates with the variability in plant fitness, we fitted the fruit set against the number of *Horistus* bugs using a GLMM with a binomial error distribution. For this, we used the number of *Horistus* bugs in relation to the number of flowers of each plant, and tested it with the nested design mentioned above to account for plot variability. We used a similar model with the number of total herbivores (also corrected by the number of flowers). For both studied interactions we also fitted a GLMM that included the combined data of both sampling years and the year as a random factor. Overdispersion was tested and corrected when necessary. All models were run with 'lme4' package in R (Bates et al. 2014).

RESULTS

Ulex plants from burned plots showed a much lower proportion of predated pods by their specialist seed predator (<5%) than the adjacent unburned plots (>15%; Table 1.2, Fig. 1.1 and Table A1 in Appendix A). The number of *Exapion* weevils was also lower in burned plots and, unexpectedly, decreased on the second sampling year (Table 1.2, Appendix A: Table A1). The predation of *Ulex* pods and the number of weevils decreased from the Edge to the Center of the burnt; this decrease was significant for 2014 and for the overall period, but not for 2015 (Table 1.2, Fig. 1.1 and Table A1 in Appendix A).

The number of specialist *H. orientalis* bugs as well the total number of herbivores on *Asphodelus* plants were significantly lower in burned than in the unburned plots on both years of sampling (Table 1.2, Fig. 1.2 and Appendix A: Fig. A1). However, the number of generalist herbivores did not differ neither between the two environments nor between the Edge and the Center (Table 1.2, Fig. 1.2). Neither the number of flowers nor seeds produced per plant showed significant differences between unburned and burned plots (N=1414, *P*=0.09 and *P*= 0.54

respectively). *Asphodelus* fruit set showed a significant negative relationship with the abundance of *Horistus* and also with the abundance of total herbivores when data from the two years were combined (see Fig. 1.3).

Table 1.2. Results from GLMMs of the effects of fire on seed predation and herbivory in two Mediterranean plants (*Ulex parviflorus* and *Asphodelus ramosus*). For each response variable and year of study, we first compared plants from Unburned vs Burned (U. vs B.) plots, and then for the burned plots, we compared Edge vs Center plots (E. vs C.). For *U. parviflorus*, the models test for differences in the incidence of seed predation by the weevil *Exapion fasciolatum* and the number of *E. fasciolatum* weevils. For *A. ramosus*, response variables were the number of *Horistus orientalis* (specialist bug), the number of generalist herbivores, and the total number of herbivores.

		2014			2015			2014 & 2015		
	Test	N	Est.	*P*	N	Est	*P*	N	Est.	*P*
Ulex parviflorus										
Predated pods	U vs B	578	-1.5	***	469	-1.7	***	1047	-1.6	***
	E vs C	480	-0.6	**	334	-	ns	814	-0.5	*
Specialist weevil	U vs B	578	-1.4	***	469	-1.4	***	1047	-1.4	***
	E vs C	480	-0.5	**	334	-	ns	814	-0.5	**
Asphodelus ramosus										
Specialist bug	U vs B	729	-1.9	***	686	-1.6	**	1415	-1.8	***
	E vs C	433	-	ns	400	-	ns	833	-	ns
Generalist herbivores	U vs B	729	-	ns	686	-	ns	1415	-	ns
	E vs C	433	-	ns	400	-	ns	833	-	ns
Total herbivores	U vs B	729	-0.4	*	686	-1.1	**	1415	-0.61	**
	E vs C	433	-	ns	400	-	ns	833	-	ns

The table shows the sample sizes (N) and, for each significant model, the estimated parameter for the fixed effects (Est. =Estimate) and the associated significance (*P*, n.s, not significant; *P < 0.05, **P < 0.01, ***P <0.001). Estimate refers to the coefficient of Burned (in relation to Unburned) and of the Center (in relation to the Edge).

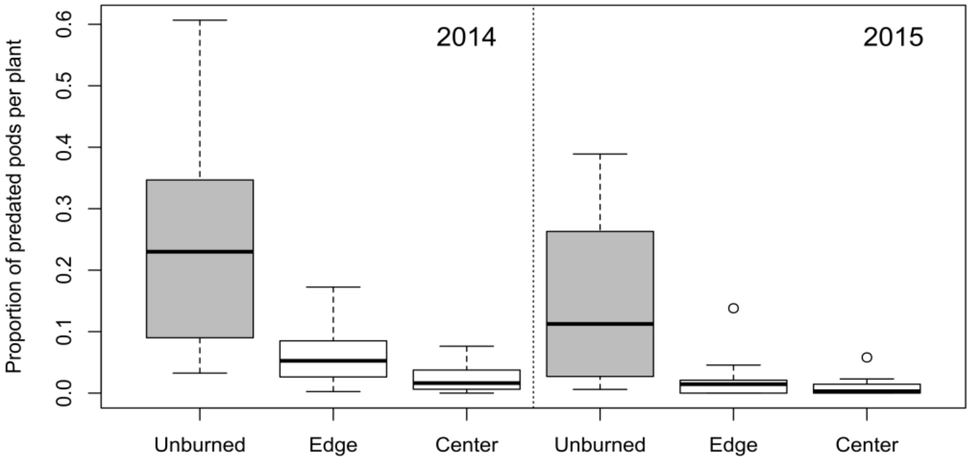

Figure 1.1. Predation rate of *Ulex parviflorus* pods in unburned plots (grey box) and burned plots (white boxes, "Edge" and "Center") for two years of sampling. N= 13 and 14 "Unburned" plots, and N= 15 and 14 "Edge" plots in 2014 and 2015 respectively. N= 20 for "Center" plots on both years.

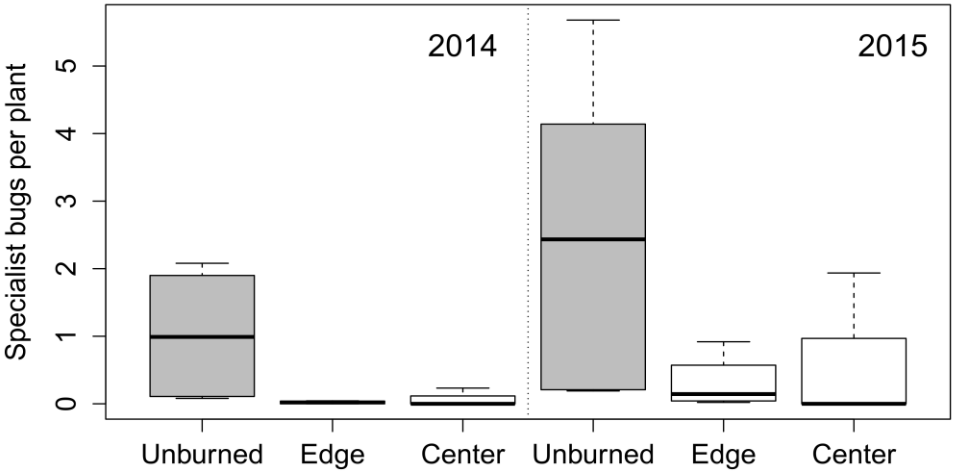

Figure 1.2. Number of specialist bugs *Horistus orientalis* on *Asphodelus ramosus* plants from unburned (grey box) and burned plots (white boxes in categories "Edge" and "Center") in two years. The number of individuals was estimated in 50 *A. ramosus* plants per plot (N= 6 unburned and N= 4 "Center" plots in the two studied years. N= 5 and 4 "Edge" plots in 2014 and 2015).

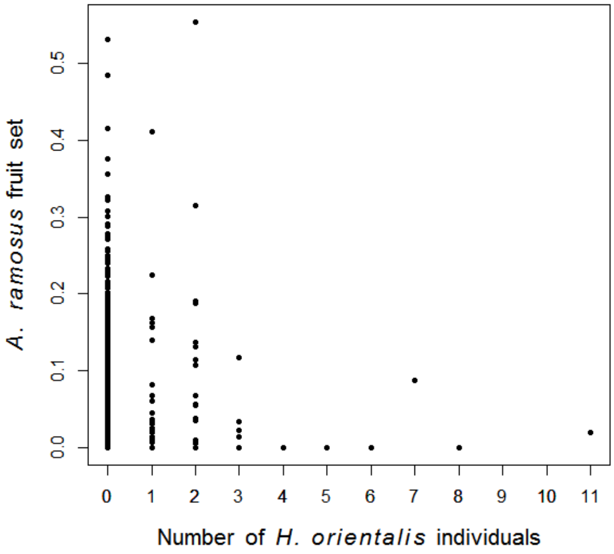

Figure 1.3. *Asphodelus ramosus* fruit set (proportion of fruits in relation to flowers) in relation to the number of the specialist bug *H. orientalis* in plants from burned plots. The relation is significant either considering *Horistus* only (N = 833, Estimate = −16.47, P = 0.018) or total herbivores (N = 833, Estimate = −12.57, P = 0.018) in a GLMM considering random effects and correcting for plant size.

DISCUSSION

Previous studies on the responses of insect herbivory to fire have shown an increment in herbivory related to a post-fire increase in herbivore abundance (Andersen 1988, Radho-Toly et al. 2001; Lopes and Vasconcelos 2011). Typically, these studies have focused on generalized interactions where the insects do not depend on the recovery of a specific plant to recolonize the burned areas. Our study, however, shows that fires can instead benefit plants by decreasing their herbivory pressure particularly from specialist insects (Auld and O'Connell 1989; Vickery 2002). Even three years after the fire, *Ulex parviflorus* plants experienced lower seed damage in burned plots than in control unburned sites (Table 1.2, Fig. 1.1). For *Asphodelus ramosus*, we found that the variability in fruit set in burned areas is significantly related to the presence of the specialist bug. That is, the disruption of the specialized interaction can affect plant fitness. In plant species with a quick recovery after fires, this disruption also coincides with an increase of resources and reduced competition after fire. Thus, the evidence suggests that the disruption of antagonistic interactions between plants and insects following a fire might be one mechanism contributing to plant success in fire-prone ecosystems.

Many insect populations decline immediately after a fire; furthermore, fire temporarily decreases the presence of the host plant required for insect development. Both the drop in insect populations and the reduction in food resources may have a stronger impact on specialist than on generalist insects (Swengel 1996, 1998, 2001). Generalist herbivores have access to a wider range of recovering plants, thus showing a faster re-colonization than the specialists. Consistently, in this study the presence of two specialist predators was much lower in plants from burned plots while generalist herbivores recorded on *A. ramosus* remained unaffected.

After three years of the wildfires we did not find a consistent effect of the distance from the perimeter of the fire on seed predation, herbivory pressures or plant fitness (Table 2). Several previous studies had shown a reduction of herbivory with the distance to unburned vegetation. For example, *Banksia* and *Eucalyptus* seedlings experienced a lower damage by generalist grasshoppers in large burned areas compared with small ones (Whelan and Main 1979). In a sandhill ecosystem, plants from the center of a burnt suffered half of the impact of insect herbivory compared to plants from the fire's edge (Knight and Holt 2005). The limited distance effect in the present study may be explained by the low postfire predation levels on *U. parviflorus* and the low number of *H. orientalis* individuals recorded in most burned plots. In fact, we did detect a significant decrease in *Ulex* predation towards the center of the burned zone in 2014 (Table 2), when the predation by the weevil was 5 times higher than in 2015. The causes behind the decreased densities in specialist predators are unknown, and seem to be unrelated to climate conditions, which did not show major differences between the two sampling years. Despite there is little knowledge on the population dynamics of the studied specialist insects, our results suggest that fires may have a great negative effect on their populations and it may last for several years while recolonization takes place.

The disruption of the interaction by fire is likely to have long-term benefits for the plant. *Ulex parviflorus* benefits from fire because the heat reached during a fire breaks seed dormancy and greatly stimulates germination from the soil seedbank (Paula et al. 2009; Moreira et al. 2010; Moreira and Pausas 2012), and thus the postfire population size is greater than in prefire populations. *Asphodelus* can also take advantage of the fire because the canopy gap opened allows this species to flower profusely, otherwise the high density of the shrubland limits sexual reproduction (Pantis and Margaris 1988; Pantis and Mardiris 1992). Here we show that fires can generate an additional benefit to the plant by creating a window of opportunity for reproduction under a lower predation pressure from their specialist herbivores. Although this release is likely temporary, it may have long-term effects because it ensures the quick refill of the seedbank after fire and thus the ability to massively recruit even under short fire intervals. For *Asphodelus*, it increases fruit set and promotes sexual reproduction under suitable postfire recruitment conditions. To what extent these beneficial effects can be generalized to other fire-adapted plant species reminds to be studied; previous research on this regard is limited to single populations or to prescribed fire regimes (Auld and O' Connell 1989; Vickery 2002).

Additionally to predispersal predation, fires can affect postdispersal seed predation which may also have implications on plant fitness (Andersen 1988; Ordoñez Retana 2004; Zwolak et al. 2010; Keeley et al. 2012). For example, seed predation on *Pinus* species from the Mediterranean increased after fires coinciding with a high presence of ants and rodents (Ordoñez and Retana 2004; Broncano et al. 2008). These changes on secondary seed predation are related with the dispersal season and the time since fire (Ordoñez and Retana 2004). Our two studied plants disperse seeds from spring (*Ulex parviflorus*) to summer (*Asphodelus ramosus*), when ants can be especially abundant after fires (Ordoñez and Retana 2004). Although ants are known as the main seed predators in burned zones (Rey 2002; Broncano et al. 2008), there is evidence that *Ulex parviflorus*, which has elaiosomes, can show higher germination rates after ant-dispersal (López-Vila and García-Fayos 2005). We are not aware of any information on seed predation by ants in *Asphodelus ramosus*. In any case, further studies depicting the relative role of ants as predators and dispersers (Auld and Denham 1999) on the two studied plants at burned areas would contribute to better understanding their success in burning ecosystems.

Despite the accepted key role of fire in many ecosystems, the responses of plant-insect interactions to fire are not well known (Dafni et al. 2012). This gap in the knowledge is even more remarkable for fire-prone ecosystems such as the Mediterranean ones, where there is evidence of fire-adaptive traits in many different species (Keeley et al. 2011). Our study, for which we monitored two plant-insect interactions across several years at different locations, indicates that when fire has disrupted specialized antagonistic interactions between insects and plants capable of quickly regenerating after fire, these plants can benefit from this for several years. This "cleaning" effect by fire might be one of the factors promoting the success of fire-adapted plants. It remains to be determined how wide-spread these effects are across different ecological settings. However, we feel that the fact that we observed beneficial effects for two plant species - interacting with different insects after multiple fires at two different locations in two consecutive years - suggests that it may be a general phenomenon. However, fires will not always benefit plants as they can also disrupt mutualisms (Dafni et al. 2012) and change the dispersal-predation balance of generalized interactions (Andersen 1988; Radho-Toly et al. 2001; Ordoñez and Retana 2004; Broncano et al. 2008; Lopes and Vasconcelos 2011). The current crisis of biotic interactions and the expected increase in fire size and frequency associated with anthropogenic activities, make understanding the effects of fire on plant-insect interactions an urgent need.

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CHAPTER II: Differential pollinator response underlies plant reproductive resilience after fires

Abstract

Assessing the resilience of plant–animal interactions is critical to understanding how plant communities respond to habitat disturbances. Most ecosystems experience some level of natural disturbance (e.g. wildfires) to which many organisms are adapted. Wildfires have structured biotic communities for millennia; however, the effects of fire on interactions such as pollination have only recently received attention. A few studies have shown that generalist plants can buffer the impact of fires by pollinator replacement, suggesting that the resilience to disturbance could depend on the level of specialization of the interactions. Here, we hypothesize that (1) fires could impose negative effects on plants with specialized pollination systems, and (2) in large wildfires, these negative effects will be stronger with increasing distance inside the burnt area because pollinators will need more time to recolonize. These questions were tested in the specialized pollination system of a widespread Mediterranean palm, *Chamaerops humilis*. The post-fire pollination resilience was assessed in replicated wildfires representing three post-fire ages by measuring the abundance of beetle pollinators and by estimating fruit set (i.e. the proportion of flowers setting fruits) in burnt and unburnt areas. To test for distance effects, plants were sampled along transects inside the burnt area. Despite a marked post-fire decline in the specialist pollinator, exacerbated by the distance from the fire's edge, the palm's fruit set was barely affected. The temporary replacement by a sap beetle at burnt sites – an effective pollinator that had not been previously recognized – provided post-fire reproductive resilience. Differential pollinator responses to disturbance can ensure plant success even in plants with only two functionally similar pollinators. This highlights the importance of pollinator

replacement and dynamics for the resilience of interactions and ultimately of plant reproduction in disturbance-prone ecosystems.

INTRODUCTION

One of the main challenges in community ecology is to get a better understanding of how plant–animal interactions respond to disturbance. Specifically, mutualistic interactions such as pollination play an essential role in the maintenance of biodiversity (Herrera and Pellmyr, 2002). There is evidence of negative impacts on plant fitness by the disruption of pollination interactions linked to recent human-induced disturbances such as habitat fragmentation (Aguilar et al. 2006), pesticides (Stanley et al. 2015) or species invasions (Chittka and Schürkens 2001; Traveset and Richardson, 2006). On a broader temporal scale, most ecosystems have experienced some level of natural disturbance to which many organisms are adapted (Elmqvist et al. 2003). Assessing the effects of natural disturbances (e.g. wildfires) on pollination interactions may contribute to understand their resilience, which is important in the current context of increasing anthropogenic perturbations.

Wildfires are common natural disturbances that have shaped communities for millennia (Pausas and Keeley 2009), resulting in the evolution of numerous adaptive traits and strategies that allow plants and animals from fire-prone regions to succeed under different fire regimes (Schütz et al. 1999; Keeley et al. 2011; He et al. 2012; Castellanos et al. 2015; Pausas and Parr 2018). Despite this long fire history in many terrestrial ecosystems, the way in which pollination interactions cope with fire has only recently received attention (Dafni et al. 2012; Brown et al. 2017), and most research on this topic has been focused on pollination by bees (Ne'eman et al. 2000; Potts et al. 2001; Moretti et al. 2006; Lazarina et al. 2016). Assessing the effects of fires on plant pollination is especially relevant given the current anthropogenic-driven disruptions of the natural fire regimes in different regions.

Fires affect plant and pollinator communities as well as their interactions (Potts et al. 2003; Lazarina et al. 2016; Ponisio et al. 2016). The time since the last fire (post-fire age) shapes the pollinator community because it alters vegetation structure, floral rewards and the pollinator's access to bare ground and nesting places (Pauw 2007; Moretti et al. 2009). During the first year after a fire, if the vegetation recovery is rapid, an increase in nesting sites and floral resources provided by fire estimulated plants, via resprouting or germination from the seed bank, can attract many pollinators into the burnt area. However, if post-fire recovery is slow, low availability of water and food resources (DeBano and Conrad 1978) can delay pollinator recolonization. This can be accentuated if pollinators are highly sensitive to fires (i.e. they do not survive, escape or move to unburnt refugia). In such cases, recently burnt areas would continue to have low pollinator richness and abundance, resulting in low levels of plant reproduction (Ne'eman and Dafni 1999; Ne'eman et al. 2000). Post-fire age can also interact with ecological and functional traits of pollinators, such as niche specialization, body size or life cycle, leading to differences in ability to recolonize after fire (Bradstock et al. 2002; Moretti et al. 2006). In addition, other factors characterizing the fire regime such as fire intensity and frequency may affect the postfire succession and ultimately pollinator responses.

For plant species with generalized pollination systems (i.e. a diverse set of floral visitors that are effective pollinators) the negative impact of fires can potentially be buffered if pollinators respond differently to fire (Bond 1994; Potts et al. 2001; Pauw 2007), as shown by studies on different disturbances (Ashworth et al. 2004; Aguirre and Dirzo 2008; Hallett et al. 2017). This is consistent with theoretical predictions of the advantages of generalized pollination (Waser et al. 1996), and could be explained, for example, if the different pollinators belong to a variety of functional groups that are differentially affected by disturbance (referred to as 'response diversity'; Ives et al. 1999; Walker et al. 1999; Bartomeus et al. 2013). However, fires can have stronger effects on plants with specialized interactions as we have previously shown for seed predation (García et al. 2016). For plants that rely on one or a few species of pollinators for reproduction, the loss of their interacting partners after fires will trigger a decrease in plant reproductive success, at least until the interaction is recovered. That is, for these plant species, the vulnerability to fire may be related to the resilience (i.e. the capacity of a system to maintain its function and identity after a change) of their mutualistic interactions. A variety of responses by pollinators with different nesting preferences or by plants and pollinators varying in their dispersal abilities may provide resilience to pollination systems under disturbance.

We hypothesize that plant species with specialized pollination systems will be negatively affected by fires due to impacts on their few pollinators that in turn affect the plant's reproduction. To test our hypothesis we used the pollination system of the dwarf palm *Chamaerops humilis* (Arecaceae). Current knowledge indicates that this dioecious palm is exclusively pollinated by the nursery weevil *Derelomus chamaeropis* (Curculionidae; Anstett 1999, Dufaÿ and Anstett 2004). The weevil feeds and develops inside persistent old palm inflorescences, which are burnt during fires and thus a strong decrease in pollinator abundance in burnt areas is expected. In addition to *D. chamaeropis*, small sap beetles (Nitidulidae) are also visitors of the palm's inflorescences (Anstett 1999; our per. obs.). Because sap beetles are important pollinators of other palm species (Henderson 1986; Anderson et al. 1988; Aguirre and Dirzo 2008; Barfod et al. 2011), we also predict that *Meligethinus pallidulus* (Nitidulidae) could contribute to the pollination of *C. humilis*. In addition, the strength of the interactions can vary along the distance inside the burnt area while recolonization takes place, as has been shown for herbivory and seed predation in large wildfires (Knight and Holt 2005; García et al. 2016). This may lead to stronger post-fire effects on pollinator abundance and fruit set levels with increased distance inside the burnt area.

In summary, we study the resilience of *C. humilis* pollination to wildfires by comparing the abundance of pollinators on the palm's inflorescences, and their consequences for fruit set, in burnt and in unburnt (paired) sites with different post-fire ages. We also test whether the effects of fire on the two beetle pollinators and on palm fruit set are stronger with increasing distance from the fire's edge.

MATERIAL AND METHODS

Study system

The Mediterranean dwarf palm *Chamaerops humilis* is a small dioecious palm native to the coastal shrublands of the western Mediterranean Basin. The plant resprouts quickly after fires and produces flowers the following spring (Paula et al. 2009). With or without fire, flowering occurs in early spring, with male anthesis starting 1 or 2 weeks before female anthesis (Anstett 1999). Although *C. humilis* can occasionally show polygamous individuals, we did not observe functional hermaphroditic flowers in the studied populations. New yellow greenish inflorescences emerge from the palm trunks while old brown inflorescences remain for years. Male and female individuals have branched inflorescences enclosed by two bracts (prophyll) that gradually open during flowering. Female flowers have three free carpels and develop into a polydrupe with 1–3 drupes. Each drupe was considered as a fruit as it acts as the dispersal unit containing the seeds.

Chamaerops humilis has a specialized nursery pollination system involving the weevil *Derelomus chamaeropis* (Curculionidae; Anstett 1999). During the winter, weevil larvae develop from eggs laid the previous spring inside the rachis of persistent old inflorescences (Dufaÿ and Anstett 2004; JácomeFlores et al. 2018). Adult *D. chamaeropis* (mean body length 2.9 ± 0.4 mm, excluding the rostrum, $n = 6$) emerge in early spring and are attracted to flowering plants by a chemical signal emitted by leaves during the flowering season (Dufaÿ et al. 2003). Female and male leaves produce a similar odour preventing the weevil from avoiding female palms, although the insect shows a preference for male individuals, where it feeds on pollen (Dufaÿ et al. 2003, 2004; Jácome-Flores et al. 2018).

Our field observations suggest that there is another common visitor on *C. humilis* inflorescences, the sap beetle *M. pallidulus* (Nitidulidae, mean length 1.7 ± 0.2 mm, $n = 6$) although its role on the palm's pollination is unknown. Occasionally honey-bees visit male inflorescences but we have never seen them on female flowers and thus do not consider them as potential pollinators. Ants are erratic and infrequent visitors and also unlikely to pollinate this dioecious plant. There have been suggestions that wind could also play a role in pollination of *C. humilis* (Herrera 1989; Jácome-Flores et al. 2016). Although most previous evidence does not support this possibility (Anstett 1999; Dufaÿ and Anstett 2004), we experimentally test it here (see Results).

Study areas

The study was carried out during 2016 and 2017 in four burnt sites in eastern Spain after wildfires (see Table 2.1 and Fig. 2.1 for details). Two sites (Dénia and Tivissa) were studied in both 2016 and 2017, and two other sites (Xàbia and Carcaixent) were studied in 2017 only. This design involved replicated sampling of sites during 1, 2 and 3 years post-fire (Table 2.1). All sites are located in coastal Mediterranean shrublands dominated by *Cistus monspeliensis* and *C. albidus* (Cistaceae) and Fabaceae species such as *Calicotome spinosa* and *Ulex parviflorus*. All fires were typical Mediterranean crown fires (Keeley et al. 2012), that is, of high intensity and fully affecting most plants (little unburnt islands, see Fig. 2.1).

Pollinator exclusion experiment: the role of the different pollinators

To investigate the role of *M. pallidulus* in *C. humilis* pollination and rule out the possible contribution of wind, we conducted a pollinator exclusion experiment during the flowering peak of *C. humilis* in 2017. We selected 12 female *C. humilis* plants from natural unburnt populations in Dénia. We chose four undehisced inflorescences per plant (in one or two stems) and assigned one inflorescence to each of four pollination treatments in which the inflorescences were either enclosed in mesh bags of different pore diameters or left as an unbagged open control. All bags were tied to the stems and the aperture sealed with silicone. We also added silicone to the base of inflorescences in the control treatment to control for possible effects of the experimental manipulation. The four treatments were: (1) pollination exclusion using a paper bag to exclude both wind and insect pollination; (2) potential wind pollination by enclosing the inflorescence in a bag with pore diameter 0.15 mm; (3) potential wind and small-insect (i.e. *M. pallidulus*) pollination, by enclosing the inflorescence in a bag with pore diameter 1.10 mm; and (4) unbagged control, in which wind, *M. pallidulus* and *D. chamaeropis* were able to pollinate.

We used the palm's fruit set as an estimate of female reproductive success by counting the flowers and fruits for each inflorescence in late June. We bagged the inflorescences and collected the fruits on the same day for all plants across treatments. We estimated fruit-set as the number of drupes produced in relation to the total potential drupes (i.e. the total number of flowers in the inflorescence multiplied by three carpels). For this, we collected all sampled inflorescences and counted all drupes produced and the scars left by aborted flowers on the inflorescence rachis. The number of flower scars is a good estimator of the potential fruit production (Pearson correlation between number of flowers in fully open inflorescences and flower scars in the same inflorescences was 0.95, $P < 0.001$, $n = 262$, tested in plants from burnt and unburnt areas during the sampling of the palm's fruit set, see below).

Table 2.1 Information on the study sites.

Sites	Province	Latitude	Longitude	Fire date	Sampling year	Fire ages	Burnt area (ha)
Dénia	Alacant	38.808054	0.160267	Sept. 2014	2016, 2017	2, 3	445
Tivissa	Tarragona	40.979691	0.693141	June 2014	2016, 2017	2, 3	890
Xàbia	Alacant	38.731141	0.169339	Sept. 2016	2017	1	800
Carcaixent	València	39.105267	-0.400584	June 2016	2017	1	2000

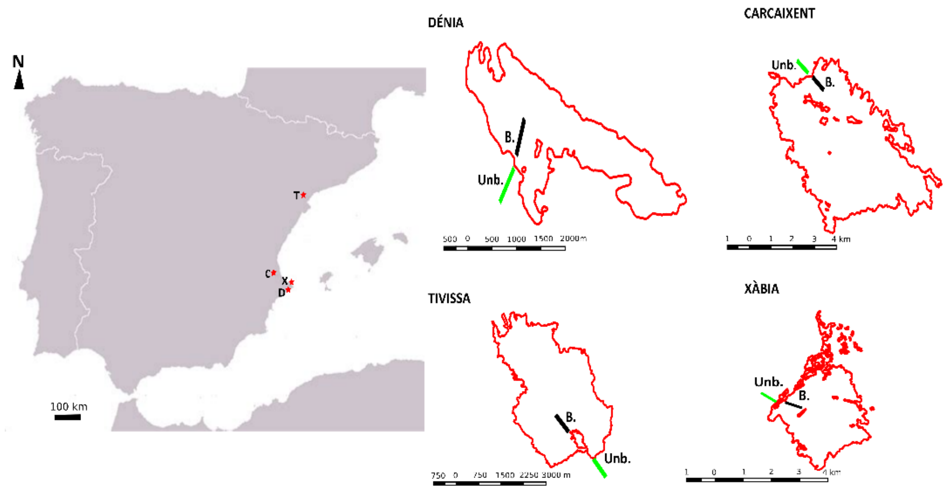

Figure 2.1. Location of the study sites in eastern Spain (left), and the burnt (B., in black) and adjacent unburnt (Unb., in green) areas sampled at each site (right). Red polygons denote the fire perimeter at each site. C=Carcaixent, D=Dénia, T=Tivissa, X=Xàbia.

To test the effectiveness of the bags used for excluding the flow of airborne pollen (treatments 1 and 2), we performed an additional experiment using the common anemophilous grass *Hyparrhenia hirta*. This species has hermaphroditic and staminate flowers with pollen grains of similar size (diameter 28.20 ± 1.82 µm, *n* = 10) to *C. humilis* pollen (diameter 20.45 ± 1.53 µm, *n* = 10). We bagged nonflowering shoots of ten *H. hirta* plants using one bag of both mesh size per plant. These shoots were surrounded by other flowering individuals of *H. hirta*, but were not directly touching any other flowers. Each bag contained two adhesive strips (1 cm^2) to retain wind dispersed pollen grains that entered the bag. After 1 week we dyed the adhesive strips with fuchsine jelly (Beattie 1972). We counted any pollen grains observed with the ImageJ software (Rasband 2007). The results suggested that bags of the wind pollination treatment did not reduce the amount of wind dispersed pollen [mean number of grains per adhesive strip: 186 ± 98 in 1.10 mm pore bags vs. 204 ± 115 in 0.15 mm pore bags, generalized linear model (GLM) with Poisson error distribution: estimate = 0.040 ± 0.033, z-value = 1.209, P = 0.22, n = 10 bags of each pore size], and thus the bags used were appropriate for the experiment.

Insect pollen loads

To test for differences in the numbers of pollen grains carried by *M. pallidulus* and *D. chamaeropis*, we haphazardly captured one individual of each species from each of 20 flowering *C. humilis* plants (ten per sex) at each study site in 2017. We individually kept the insects in Eppendorf tubes at −20 °C until a sample of the pollen loads was collected from the whole body surface of each individual using fuchsine jelly cubes. We melted the cubes on microscope slides and then identified the pollen loads from insects collected on female palms by comparing with a reference pollen library constructed by collecting anthers of *C. humilis* and 12 co-flowering plant species from the study sites. We dyed the pollen grains of each plant species with fuchsine jelly and identified them under a microscope (Leica DMR). To measure pollen size (of *C. humilis* and the co-flowering plant species) for the reference pollen library and count the number of *C. humilis* grains carried by the insects we used the ImageJ software (Rasband 2007) with a specific script developed for the counting analysis.

Postfire changes in pollinators and fruit set

To study post-fire changes in pollinators and fruit set at each site, we tagged palms within the perimeter of the burnt area and in adjacent unburnt (control) areas with conditions (soil type, topography and plant species composition) similar to those within the burnt area prior to the fire. Burnt and adjacent unburnt areas were embedded in the same vegetation matrix type (shrublands). We performed all sampling in mid-April at the peak of anthesis of male plants, and the beginning of female flowering. In each burnt and unburnt area, we sampled 98–197 *C. humilis* plants of both sexes separated from each other by at least 5 m. To test the effects of the distance from the edge on pollinator abundance and palm fruit set, plants were sampled along transects (of approx. 30–700 m) from the fire's edge to the interior (Fig. 2.1). We sampled and georeferenced a total of 744 plants in 2016 and 796 in 2017 (n = 1540 plants). At burnt areas, transects allowed us to investigate the effect of distance from the edge of the fire on the abundance of the two beetles and on the palm's fruit set; this distance was computed from the geographical coordinates with Quantum GIS v. 2.8 (Quantum GIS Team 2013) software.

For each male plant, we counted the number of inflorescences and, in one inflorescence at anthesis, the abundance of *D. chamaeropis* and the presence (2016) or number (2017) of *M. pallidulus* individuals during 3-min censuses. When part of the male inflorescence was not completely outside the prophyll, we carefully opened the prophyll to count all beetles. The insects are easily detected at the base of inflorescences, moving around the bracts that enclose them. We conducted the pollinator censuses between 0930 and 1600 h on sunny days with similar weather conditions across all sites. To assess whether the maturity of *C. humilis* inflorescences differed between burnt and control areas in a way that could affect other analyses, we classified the phenological stage of each sampled male inflorescence as either: (1) beginning of anthesis (many closed anthers and small amounts of pollen); (2) anthesis (yellow flowers producing pollen); and (3) end of anthesis (flowers turning brown with small amounts of pollen present). For female plants we counted the total number of inflorescences and tagged one of them (at anthesis) to estimate fruit set later in the season (see below). The number of *D. chamaeropis* and *M. pallidulus* on female inflorescences was also recorded over 3 min. We then classified the phenological stage of the female inflorescence as closed (including partially open inflorescences) or open (inflorescences with only their lowest part inside the prophyll). We estimated fruit-set in late June, when fruits were developing, in all tagged inflorescences and by using the same methodology as described in the pollinator exclusion experiment.

Statistical analysis

We investigated the effects of the three pollination exclusion treatments and control treatment on palm fruit set (the proportion of drupes in relation to potential drupes) as a response variable using a GLM with a quasi-binomial error distribution to control for overdispersion and the logit link function in the *stats* package in R (R Core Team 2017). Pollination treatment was included as a predictor variable and the number of experimental stems per plant (one or two) as a covariate. We then tested for differences in pollination treatments by post-hoc pairwise comparisons adjusted by Bonferroni's correction for multiple tests with the *multcomp* package in R (Hothorn et al. 2008). To explore potential differences in the pollen loads carried by *D. chamaeropis* and *M. pallidus* we fitted a GLM with number of pollen grains (with Poisson error distribution) as the response variable and insect species, plant sex and their interaction as predictors.

To test the effect of fire on *D. chamaeropis* abundance on *C. humilis* we used a GLM with a negative binomial distribution and a log link function. We included as predictor variables fire treatment (unburnt vs. burnt), number of inflorescences per plant, plant sex, site, and the interaction between fire treatment and site. To analyse the effect of distance from the edge of the fire on *D. chamaeropis* abundance, we ran a similar GLM in which the distance of each plant from the fire edge was included as a predictor variable. Only plants inside the burnt areas (*n* = 354 in 2016 and *n* = 401 in 2017) were included in the distance model, and interactions that did not contribute significantly were removed from the final model. To test whether the effect of distance to the edge varied when considering the post-fire age categories, an additional GLM grouping the sites by post-fire age (1 year vs. 3 years post-fire) was also fitted.

We ran equivalent models for *M. pallidulus* abundance on the plant in 2017. To test for differences in the presence of *M. pallidulus* beetles on *C. humilis* plants in 2016, when only presence data were available, we used GLMs with a binomial error distribution (presence vs. absence) and a logit link function. For *M. pallidulus* presence, the fire and distance from the edge models included both the same predictor variables and sample sizes as the abundance models.

Before analysing fruit set data, we checked for differences in the proportions of the developmental stages of inflorescences in our samples from the burnt and unburnt areas. No differences were detected in male ($\chi^2 = 1.83$, d.f. = 2, $P = 0.40$, $n = 808$ plants) or female inflorescences ($\chi^2 = 0.41$, d.f. = 1, $P = 0.55$, $n = 732$ plants).

To test whether fire affected *C. humilis* fruit set, we used a generalized linear mixed model (GLMM) with a binomial error distribution and a logit link function. To account for overdispersion we included an observation-level random effect (Harrison, 2015) by running a GLMM with individual plant as a random factor using the *lme4* package in R (Bates et al. 2015). We included as fixed factors fire treatment (unburnt vs. burnt), site and their interaction. We added the number of female inflorescences as a covariate in the model after checking its independence from the predictors. To test whether these models were congruent with the three post-fire age categories, we ran additional GLMMs of the effects of fire on fruit set where sites were grouped by post-fire age (1, 2 and 3 years post-fire, with plant and site as random factors).

To investigate the response of *C. humilis* fruit set to the distance from the fire's edge we ran a GLMM with female plants from burnt areas. We included the distance of each plant from the fire's edge and site as fixed effects, new produced inflorescences as a covariate and plant as a random factor.

Because of the differences in the number of studied sites (two in 2016 and four in 2017), we fitted fire (unburnt vs. burnt) and distance models separated for each sampling year. Prior to model fitting, the two continuous predictors, distance inside the burnt areas and number of inflorescences, were mean-centred. To test for differences of fire treatment (burnt vs. unburnt) among the study sites (in all models with a significant interaction term), we conducted post-hoc pairwise comparisons for multiple test as described above (Bonferroni-adjusted). All analyses and graphical treatments were performed in R software version 3.4.2 (R Core Team 2017).

RESULTS

Pollination exclusion experiment

The pollinator exclusion experiment confirmed that *C. humilis* is exclusively insect-pollinated, i.e. wind is not involved on its pollination (see also Jácome-Flores 2015). Inflorescences from the open controls produced a 12.15 % higher fruit set (28.30 ± 7.61 % mean fruit set, n = 12 plants) than any bagged treatment (Fig. 2.2, P < 0.01 in all comparisons, see Appendix B: Table B1 for details). In addition, *C. humilis* inflorescences from the wind and small-insect pollination treatment showed a higher fruit set (16.15 ± 10.41 % mean fruit set, n = 12 plants) than those in the wind pollination (1.19 ± 2.32 % mean fruit set, P < 0.001, n = 12 plants, Fig. 2.2) and pollination exclusion treatments (0.87 ± 0.75 % mean fruit set, P < 0.001, n = 12 plants, Fig. 2.2). No significant differences were observed between inflorescences with wind pollination only and complete pollination exclusion bags (P = 0.95, n = 12 plants, Fig. 2.2, Appendix B: Table B1). The number of sampled stems (one or two) did not affect the palm fruit set (estimate = 0.155 ± 0.224, *t*-value = 0.691 *P* = 0.49, *n* = 12 plants).

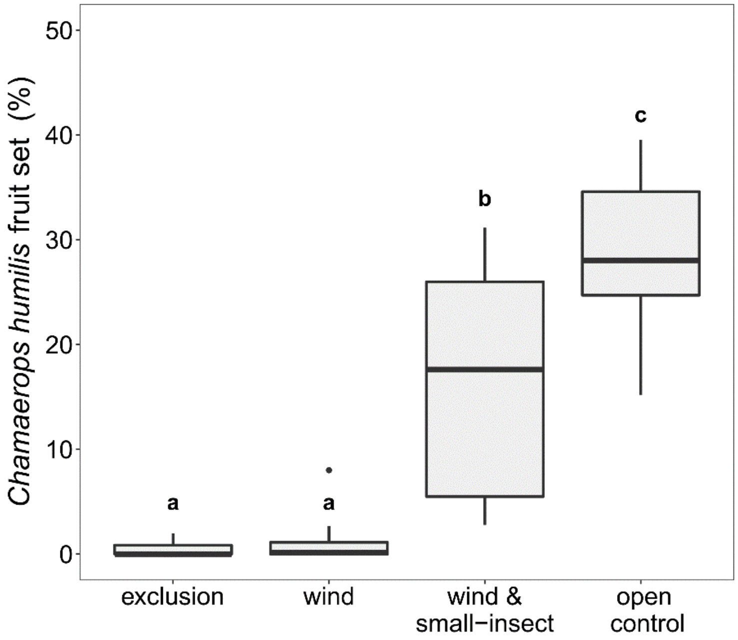

Figure 2.2. *Chamaeropis humilis* fruit set (proportion of developed drupes in relation to the potential drupes) of inflorescences with different pollinator exclusion treatments (pollination exclusion, wind pollination, wind and small-insect pollination, and open control). Different letters indicate statistically significant differences among treatments (for statistical values see Appendix B: Table B1). In all figures, boxplots show the median and interquartile range of each response variable. Outliers are represented by filled circles.

Insect pollen loads

Both *D. chamaeropis* and *M. pallidulus* carried pollen from *C. humilis* male plants to female plants (Fig. 2.3). Only a very small proportion of the pollen transported to female inflorescences was not from *C. humilis* (0.86 % of that on *D. chamaeropis* and 1.54 % of that on *M. pallidulus*). Insects collected at male inflorescences were carrying more grains than insects from female inflorescences (for *D. chamaeropis*: 4180 ± 2041.4 vs. 826 ± 207.6 mean grains per individual; for *M. pallidulus*: 803 ± 202.1 vs. 387 ± 91.2, estimate = 0.729 ± 0.007, *z*-value = 103.1, *P* < 0.001, *n* = 80 individuals per insect species, Fig S1). *Derelomus chamaeropis* carried more pollen grains than *M. pallidus* on both male and female inflorescences (estimate = 0.760 ± 0.007, *z*-value = 106.2, $P < 0.001$ $n = 80$, Appendix B: Fig. B1).

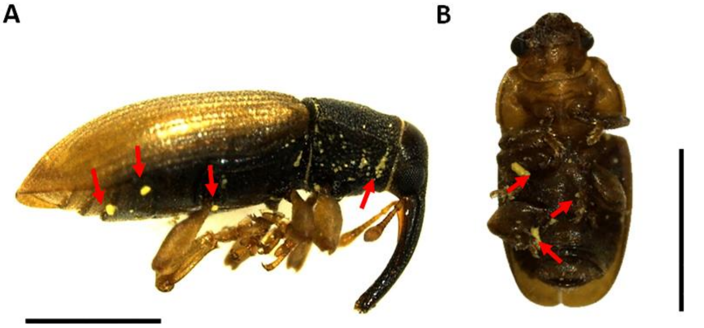

Figure 2.3. Pollen loads carried by the main flower visitors of *Chamaerops humilis*: (A) the weevil *Derelomus chamaeropis* (Curculionidae) and (B) the sap beetle *Meligethinus pallidulus* (Nitidulidae). Red arrows indicate pollen grains. Scale bars=1mm.

Post-fire changes in pollinators and fruit set

Burnt areas showed a marked reduction in *D. chamaeropis* abundance compared with unburnt areas, and the weevil was almost absent in the first and second year after fire (88 % and 74 % average reduction, respectively; Fig. 2.4). Weevil numbers were significantly lower inside the burnt areas in the two most recently burnt sites (Xàbia and Carcaixent), and in Tivissa 2 and 3 years after the fire (Fig. 2.4, Table 2.2; see Appendix B: Tables B2 and B3). Male plants had more weevils than female plants, both outside and inside the burnt areas (mean number of weevils per male inflorescence = 4.70 ± 5.93 at controls vs. 1.85 ± 3.12 at burnt areas, and 0.95 ± 1.67 at controls vs. 0.58 ± 1.34 weevils per female inflorescence at burnt areas; n = 808 males and n = 732 females; Table 2). Plants from burnt sites in 2017 showed a negative relationship between weevil abundance and distance from the fire's edge ($P < 0.001$, Table 2 and Appendix B: Table B3 for full details). However, the posterior model grouping of the sites by postfire age (1 year vs. 3 years) revealed that this effect occurred only in palms from recently burnt sites (interaction between distance and 1-year post-fire age, estimate = −0.009 ± 0.002, *t*-value = −4.25, $P < 0.001$, $n = 401$ plants at Xàbia and Carcaixent sites, Fig. 2.5).

Burnt and control areas showed similar numbers of *C. humilis* plants with *M. pallidulus* beetles (46.7 % in burnt and 51.4 % in unburnt areas). For 2017 (the year with quantitative data for both insect species), the number of *M. pallidulus* individuals per inflorescence was also similar after the fires (unburnt vs. burnt: $P = 0.33$, $n = 796$ plants, Appendix B: Table B3 and Fig. B2). That is, neither fire nor distance effects were detected on the sap beetle's abundance on *C. humilis* plants in 2017, nor on its presence in 2016 (Table 2.2, see Appendix B: Tables B2 and B3 for statistics).

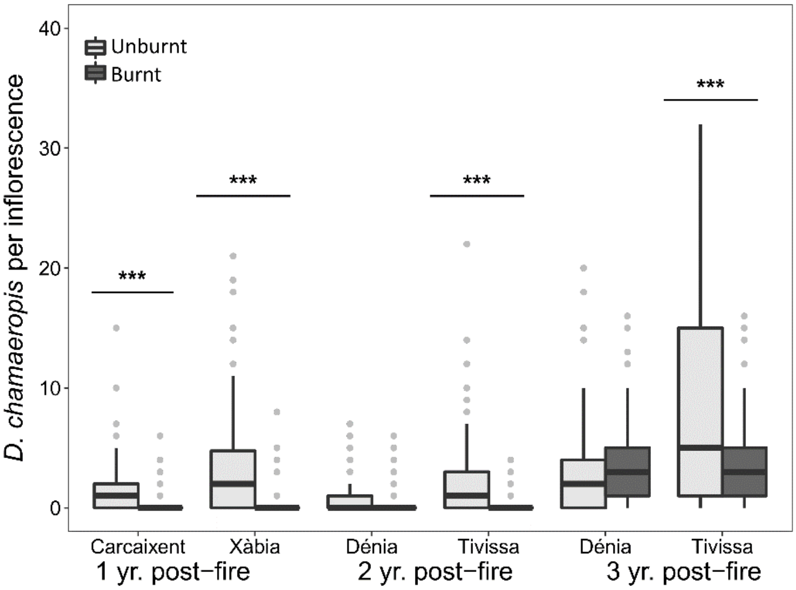

Figure 2.4. Number of *Derelomus chamaeropis* individuals per inflorescence in each study site in unburnt and burnt areas for three post-fire ages. Asterisks indicate a significant decrease of *Derelomus* individuals at the burnt area at that study site. ***P < 0.001.

Despite lower *D. chamaeropis* abundance, fruit set decreased only in the recently burnt Xàbia and, to a less extent, in Tivissa 2 years after the fire (Fig. 2.6,

Table 2.2, see Appendix B: Table B4 for details). We did not detect significant differences in fruit set 3 years after the fires, or any effect of distance from the fire's edge (Fig. 2.6, Table 2.2, see Appendix B: Tables B2 and B3 for statistics). The GLMMs on the effects of fire on fruit set in which sites were grouped by postfire age also showed that fruit set was only negatively affected 1 year post-fire (unburnt vs. burnt: estimate = 0.72 ± 0.175, *z*-value = 4.16, $P < 0.001$, $n = 196$ plants).

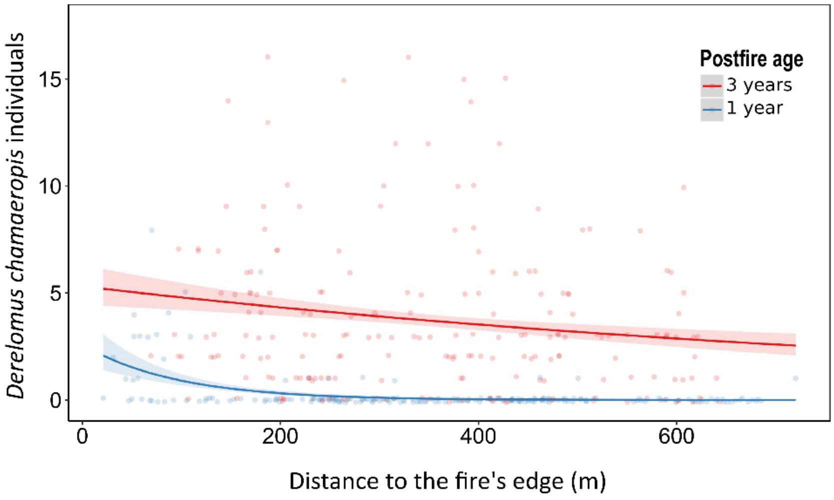

Figure 2.5. The relationship between *Derelomus chamaero*pis weevils on *Chamaerops humilis* with the distance to the fire edge at two different post-fire ages in 2017 (n = 401 plants). The negative effect of distance to the edge on the number of weevils per plant was significant only at 1 year post-fire (Xàbia and Carcaixent sites, blue solid line). Dark shading indicates 95 % confident intervals

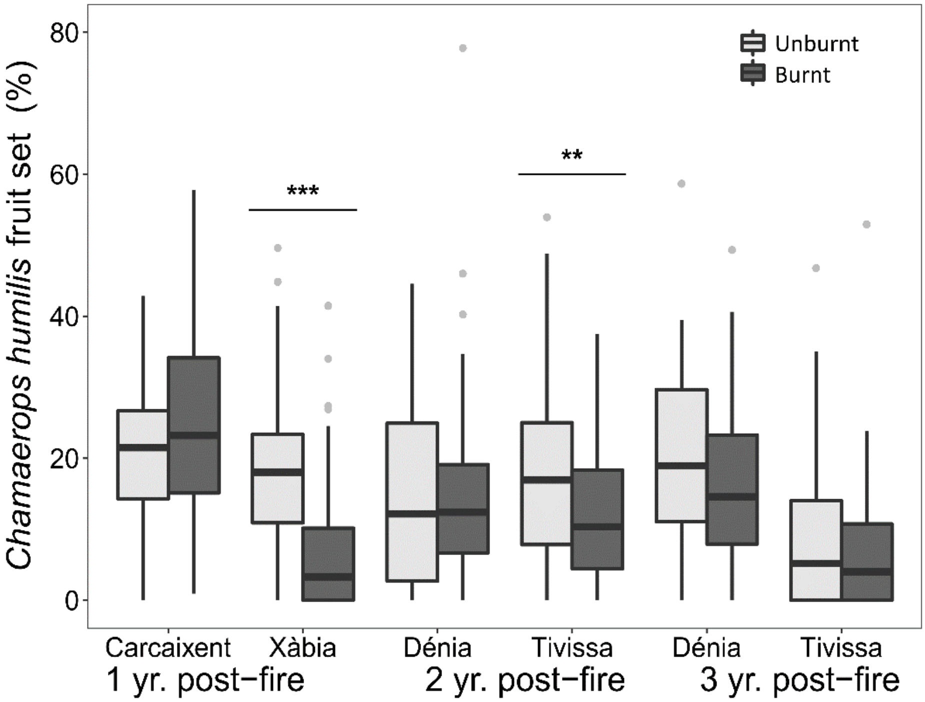

Figure 2.6. *Chamaerops humilis* fruit set (percentage of developed drupes in relation to total number of flowers produced, i.e. potential drupes) at each study site in burnt and unburnt areas during the two years of the study and the three post-fire ages. Asterisks indicate a significant decrease of *C. humilis* fruit set at the burnt area of that study site. **P<0.001.

Table 2.2. Effects of fire (Unburnt vs Burnt areas) and distance (to the fire edge) on the number of *Derelomus chamaeropis* weevils, *Meligethinus pallidulus* beetles, and *Chamaerops humilis* fruit set. For each response variable, the table shows the results of the GLM and GLMM models on the effects of fire or distance inside the fire. Full models for fire effects included the two way interaction ("x") between fire treatment (U. vs B. = Unburnt vs Burnt) and study site. All models included the number of inflorescences, site and plant sex (only for models on *D. chamaeropis* and *M. pallidulus* abundances) as predictor variables. Names in brackets represent the study site with statistically significant effects (T= Tivissa, X= Xàbia, C= Carcaixent). * *P* <0.05, ** *P* <0.01, *** *P* <0.001, ns= non-significant. For detailed statistics see Appendix B, Tables B2 (year 2016) and B3 (year 2017) and Table B4 for Post-hoc pairwise comparisons of the interaction between fire treatment and study site.

Sampling year	Response	Model	Predictor variables					
			U. vs B.	Distance	Inflorescences	Site	Plant sex	U. x Site vs B. x Site.
2016	*D.chamaeropis*	Fire	**	-	***	** [T]	***	***
		Distance	-	ns	*	** [T]	***	-
2016	*M. pallidulus*	Fire	ns	-	ns	***[T]	***	ns
		Distance	-	ns	ns	**[T]	***	-
2016	Fruit set	Fire	ns	-	ns	ns	-	**[T]
		Distance	-	ns	ns	ns	-	-
2017	*D.chamaeropis*	Fire	ns	-	*	***[X], **[C]	***	***[T], ***[X], ***[C]
		Distance	-	***	ns	***[X], ***[C]	***	-
2017	*M.pallidulus*	Fire	ns	-	**	ns	***	ns
		Distance	-	ns	*	ns	***	-
2017	Fruit set	Fire	ns	-	ns	***[T], ***[X]	-	***[X]
		Distance	-	ns	ns	***[T],***[X], *[C]	-	-

DISCUSSION

Our study highlights the importance of pollinator replacement as a way of providing resilience to disturbance in plant–pollinator interactions, even in a plant with a limited number of pollinators. Although we recorded a marked decline in numbers of the weevil pollinator after fires, *C. humilis* fruit set was barely affected. A temporary replacement by the sap beetle *M. pallidulus*, an effective pollinator that has not been previously recognized as such (Herrera 1989; Anstett 1999; Dufaÿ and Anstett 2004), explains the fast recovery. The abundance of this beetle was unaffected by the fires and provided resilience to the pollination process. As a result, fires did not alter the palm's reproduction in most study sites and fruit set showed a complete recovery in only 3 years.

In unburnt conditions, visits by the sap beetle *M. pallidulus* produced a mean 16.15 % fruit set compared to 28.30 % in the controls also visited by *D. chamaeropis*. Differences between these two treatments are probably explained by the higher amount of pollen carried by *D. chamaeropis*. This is consistent with the known importance of the weevil as a pollinator (Anstett 1999; Dufaÿ and Anstett 2004), although further research is needed to evaluate the possible differences in pollination efficiency between the two species. In contrast to the unburnt areas, our study suggests that at the most recently burnt sites (where the weevil was virtually absent), *C. humilis* pollination relies on the sap beetle (which was not affected by fire), and this replacement may last until the weevil recolonizes the burnt sites. In addition, pollen loads of both insects consisted mainly of *C. humilis*, suggesting a marked specialization (at least while the plant is flowering) which may avoid potentially negative effects of heterospecific pollen deposition (Thomson et al. 1982; Ashman and Arceo-Gómez 2013).

Fires had contrasting effects on the presence and abundance of the two beetle species, with a stronger negative effect on *D. chamaeropis* than on *M. pallidulus*. This striking weevil decline is consistent with earlier evidence on the negative effects of wildfires on other weevil species from temperate zones (Moretti et al. 2004). The life cycle of *D. chamaeropis* is completely dependent on old dry *C. humilis* inflorescences, within which female individuals lay their eggs and the weevil develops (Anstett 1999; Dufaÿ and Anstett 2004). Most old inflorescences burn in wildfires, and we did not detect any *D. chamaeropis* larvae in a preliminary sampling of the palm immediately after fire (unpubl. data). Experimental tests on a similar interaction between butterflies and cycads have shown that fire temperatures can kill all pupae growing inside fronds of the host plants (Thom et al. 2015). In all such cases, the burnt area must be recolonized from surrounding populations, which can result in spatial gradients in insect abundance and in turn in their interactions inside the burnt area (Knight and Holt 2005). Consistently, we observed a significant decline in weevil abundance on *C. humilis* plants with increasing distance to the edge of the burnt area in the first post-fire year, followed over the years by an increase in the number of weevils in the depleted parts of the burnt area. *Meligethinus pallidulus* also appears to be specialized on *C. humilis* pollen at least during the plant's flowering season. Little is known about the biology of this sap beetle, but its life cycle is likely to depend on *C. humilis* (Ponel and Lemaire 2012; Audisio et al. 2014). We have not detected any *M. pallidulus* larvae inside the palm's inflorescence: an examination of complete old inflorescences from 180 male plants at three sites only revealed the presence of *D. chamaeropis* and some Lepidoptera (data not shown). There are three possible explanations for the rapid post-fire recovery of *M. pallidulus*. First, *M. pallidulus* larvae may develop inside the palm's stem. Adults are often seen inside the stems (Appendix B: Fig. B3) where they could survive fires thanks to the protection by the fibrous bark-like structure (e.g. Brennan et al. 2011). The second possible explanation is that the higher densities of *M. pallidulus* on the plant, compared to the weevil in the unburnt sites, may allow faster recolonization.

This is consistent with a previous study where high numbers of sap beetles contributed to ensure a tropical palm set fruit even in highly fragmented zones

(Aguirre and Dirzo 2008). Finally, larger dispersal distances by the sap beetle might also be a mechanism explaining its fast recolonization from the surrounding areas (Saint-Germain et al. 2004). Further studies are needed to determine whether either of these routes to post-fire recovery is driving the sap beetle's response. Regardless, the fast recovery of *M. pallidulus* appears to maintain *C. humilis* pollination after fires. This, together with quick resprouting by the palm (Paula et al. 2009), and its ability to flower in the spring following a fire contribute to the high success of the palm in fire-prone environments. In addition, the quick availability of fruits at burnt sites may have broader implications for ecosystem resilience, such as maintaining frugivorous vertebrates and accelerating the post-fire recolonization of plants in fire-prone landscapes. Fruit dispersers such as badgers, foxes and deer can also transport seeds of other species (Herrera 1989; Fedriani and Delibes 2011; Castañeda et al. 2017) from the surrounding areas, which ultimately may promote the arrival of seeds in freshly burnt sites. However, fire did decrease palm fruit set in two sites. This reduction in the Xàbia site during the first post-fire year could be related to the marked significant reduction in weevil abundance together with the low numbers of the sap beetles (although not significant) at the burnt area (Table 2.2, Appendix B: Fig. B2). In Tivissa 2 years after the fire, weevil abundance was very low; *M. pallidulus* was present but we lack information on its abundance and thus we cannot fully explain the reduction of fruit set in this case. Abiotic factors not measured here, such as soil nutrient and water availability, could also alter the plant reproductive success after fire (Carbone and Aguilar 2017) and explain some of this variation. While fire may reduce *C. humilis* fruit set in some instances, this is not a general outcome, and only 3 years after the fires effects on fruit set were no longer detectable.

The frequent asymmetric nature of plant–pollinator interactions (specialist species interact with generalist ones) provides resilience to disturbance (Ashworth et al. 2004; Vázquez and Aizen 2004). This has led to the prediction that disturbances will have strong consequences on symmetric pollination interactions because of the reciprocal dependence between the mutualistic partners. However, empirical studies assessing the reproductive costs of disturbance for plants engaged in obligatory pollination systems are still scarce (Bronstein and Hossaert-McKey 1995; Lemke and Porembski 2013; Suchan et al. 2015). Some of these studies have shown that these highly specialized interactions can be also resilient if the species involved have traits that confer a rapid ability to respond (Bronstein and Hossaert-McKey 1995) or if the plant has additional (but overlooked) non-nursery pollinators at disturbed areas as we show here (Suchan et al. 2015).

This resilience could be more frequent in specialized interactions from disturbance-prone environments, such as fireprone ecosystems, as plants and animals in these areas have evolved persistent traits under recurrent disturbances (Schütz et al. 1999; Keeley et al. 2011; He et al. 2012; Castellanos et al. 2015; Pausas and Parr 2018). Yet only a few studies have assessed the effects of fire on specialized pollination interactions. For instance, the higher seed set levels at early post-fire ages in fire-stimulated flowering orchids depended on specialist oil-collecting bees for reproduction (Pauw 2007). In contrast, old fires were positively related to pollinator visitation in a specialist Australian orchid (Brown et al. 2016; Brown and York 2017a). These studies, together with our results, support the view that different species reach a reproductive optimum at different post-fire succession stages (Moretti et al. 2006, 2009; Lazarina et al. 2016). It is also noteworthy that other fire characteristics such as fire frequency or the diversity of fire histories at the landscape level ('pyrodiversity') can also alter the outcome of plant–pollinator interactions at different spatial scales (Brown et al. 2016; Ponisio et al. 2016; Brown and York 2017b; Carbone and Aguilar 2017). The success of highly specialized pollination systems in floras from different fire-prone regions (Gottsberger 1986; Johnson and Steiner 2003; Johnson 2010) calls for further research on the idea that resilience is common in such environments.

Conclusions

The resilience of plant communities to face disturbances may rely, at least in part, on the ability of reorganizing their mutualistic interactions, which can offset the indirect negative effects on plant reproduction. Previous studies suggested that a high diversity of interacting species may ensure a generalist plant species' success under fluctuating environmental conditions (Albrecht et al. 2012; Bartomeus et al. 2013). Such high diversity may allow for pollinator replacement and thus the resilience of the reproduction after disturbance (Potts et al. 2001). Here we provide field evidence of an unexpected pollinator replacement after fire in a specialized pollination system. To what extent post-fire pollination replacement is common in other specialized systems remains to be studied. Overall, the current fire regime changes in many ecosystems call for further research on the effects of fire on the dynamics of plant–animal interaction assemblages and ultimately on the implications for plant reproduction. Only with this research we can really evaluate the impact of future fire regimes on biodiversity.

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CHAPTER III: Do fire-induced changes in plant volatile organic compounds mediate pollinator switches?

Abstract

Volatile organic compounds (VOCs) conform chemical signals that mediate plant-pollinator interactions. In nursery pollination systems these signals are crucial because of the tight interdependence among the species. However, these systems can include non-nursery co-pollinators whose importance is context dependent, and who can potentially play an important role in plant success after disturbance. We explore the role of VOCs in pollination resilience after wildfires by asking whether the fire context (unburnt/burnt) can induce switches in the predominant pollinator. We studied the palm *Chamaerops humilis* and its nursery (dominant in unburnt sites) and non-nursery (dominant in burnt sites) pollinators. We tested whether fire altered plant VOCs and to what extent this affected attractiveness to pollinators. We analyzed the scent in burnt and unburnt areas and performed olfactory-bioassays with both pollinators. Fires modified the scent and both burnt and unburnt plants were similarly attractive to pollinators; thus, scent changes are unlikely to be mediating pollinator switches. The lack of changes in the main VOCs may explain the persistent attraction and the resilience of pollination. We show for the first time that wildfires can change chemical signals involved in pollination, and how the specificity between plant signals and pollinators provides resilience in disturbance-prone environments.

INTRODUCTION

Plants show a great diversity of volatile organic compounds (VOCs) produced in different plant tissues (i.e., leaves, flowers, roots) by a variety of biosynthetic routes (Peñuelas and LLusià 2001; Dudareva et al. 2013). VOCs conform chemical signals that mediate in plant-plant interactions (Runyon et al. 2006) and in interactions between plants and different organisms (Dudareva et al. 2013). Within plant-animal interactions, VOCs can play a relevant role in plant-pollinator (Raguso 2001), plant-parasite (Niinemets et al. 2013), or plant-herbivore interactions (Agrawal 1998). In some cases, these chemical signals mediate among a wide range of interacting species (Dötterl et al. 2012) while in others, such as highly specialized interactions, they mediate between a few species (De Moraes et al. 1998).

Nursery pollination mutualisms are well-known examples of these highly specialized interactions because of the frequent, tight mutual dependence of the interacting species for successful reproduction (Dufaÿ and Anstett 2003). Studies on plant VOCs in the context of nursery pollination have evidenced the relevance of these chemical signals emitted to attract a particular pollinator, while pollinators use them to specifically locate sites for breeding and develop on their nurse plants (Grison-Pigé et al. 2002; Dufaÿ et al. 2003; Hossaert-McKey et al. 2010; Svensson et al. 2010). However, plants with nursery pollination systems frequently show additional non-nursery co-pollinators (Thompson and Pellmyr 1992; Thompson and Cunningham 2002; Kephart et al. 2006; Cuautle and Thompson 2010; Hossaert-McKey et al. 2010). The predominance of each type of pollinator (nursery vs non-nursery) in the same plant species can be context-dependent, as in plants with different diurnal vs nocturnal pollinators (Prieto-Benítez et al. 2015, 2016; Chapurlat et al. 2018) or with pollinators varying geographically (Thompson and Cunningham 2002; Friberg et al. 2013).

An example that combines nursery and non-nursery co-pollinators is the fire-adapted Mediterranean dwarf palm *Chamaerops humilis* (Arecaceae). The palm is engaged in a nursery mutualism with the weevil *Derelomus chamaeropis* (Curculionidae), whose larvae develop inside the palm’s old inflorescences (Anstett 1999; Dufaÿ and Anstett 2004). During flowering, the palm leaves (and not the flowers) emit a strong blend that attracts the weevil to new inflorescences (Dufaÿ et al. 2003). Recently we showed that *C. humilis* has an effective co-pollinator, the sap beetle *Meligethinus pallidulus* (Nitidulidae), which does not develop inside the palm’s inflorescences (García et al. 2018). After a wildfire, there is a marked reduction in the weevil’s abundance on *C. humilis,* and a temporary replacement by the quickly recolonizing sap beetle ensures palm reproduction (García et al. 2018). That is, in the fire-prone landscape mosaics where the plant is native, the dominance of each pollinator can vary depending on the fire context: while the nursery (weevil) pollinator predominates in unburnt areas, the non-nursery co-pollinator (sap beetle) is dominant after recent fires.

Wildfires are natural disturbances that impose a myriad of changes in ecosystems by altering soil nutrient and water content or by changes in plant, soil-bacterial and herbivore communities. Therefore, wildfires may modify plant VOCs through changes in soil moisture (Burkle and Runyon, 2016), temperature (Farré-Armengol et al. 2014), plant-associated bacteria (Helletsgruber et al. 2017), and herbivory (Kessler et al. 2011; Burkle and Runyon 2016). Ultimately, changes on plant VOCs can lead to the disruption of plant-animal interactions (Farré-Armengol et al. 2016; Li et al. 2016). To our knowledge, the effects of fire on plant VOCs involved in pollination have not been yet addressed. Previous studies on the effects of fire on leaf volatiles have been conducted in the context of plant flammability (Alessio et al. 2008; Pausas et al. 2016) without considering their potential fire-induced changes in plant-animal interactions (but see Wheeler and Ordung 2006; Campbell and Taylor Jr 2007). Here we explore to what extent fires change VOCs emission in *C. humilis* and consequently modify pollinator attraction, promoting the switch to non-nursery type in recently burnt areas.

MATERIAL AND METHODS

Study system

Chamaerops humilis is a dwarf dioecious palm common in fire-prone shrublands of the western Mediterranean Basin. It shows quick postfire resprouting from surviving apical buds and can flower the spring following a fire (Tavsanoglu and Pausas 2018). Flowering occurs in early spring, with male individuals starting one week before female plants. Successful pollination depends on two pollen-feeding beetle species (Dufaÿ and Anstett 2004; García et al. 2018).

The weevil *Derelomus chamaeropis* (Curculionidae) and the palm have a nursery pollination interaction, where larvae develop inside the palm's old inflorescences during the winter and adults emerge in early spring coinciding with the flowering (Anstett 1999; Dufaÿ and Anstett 2004). Female palms offer significantly less reward to the weevil (no pollen), and the insect prefers male inflorescences to develop (Dufaÿ and Anstett 2004). This interaction is mediated by a chemical signal emitted by the leaves from both female and male palms during floral anthesis, when leaves produce the highest rates of VOCs emissions (Dufaÿ et al. 2003, 2004). Leaves are almost scentless to humans and produce much lower quantities of VOCs before flowering begins (Caissard et al. 2004). The weak blend produced by the flowers does not attract the weevil as shown by olfactometry bioassays (Dufaÿ et al. 2003, personal observation at field). Thus, despite being decoupled in space, the palm chemical signal (leaf scent) and the reward (in inflorescences) are temporally coupled during the flowering period.

The other pollinator is the sap beetle *Meligethinus pallidulus* (Nitidulidae) which is smaller than the weevil and carries less pollen, but it is an effective pollinator and relatively abundant in postfire conditions (García et al. 2018). Contrary to the weevil, *M. pallidulus* does not develop inside the palm inflorescences but is easily observed on the stem and inflorescences during blooming.

Scent collection and analysis

The study was carried out in 2017 during the palm flowering season (March to May) at two sites in eastern Spain (Carcaixent and Xàbia, separated by 90 km, Appendix C: Fig. C1). The sites had been affected by wildfires in the previous summer (June and September 2016, respectively). At each site, plants were sampled inside the burnt area and in an adjacent unburnt area (control; Appendix C: Fig. C1). Scent sampling was conducted between 10:00 and 15:00 hours on sunny days with similar temperature, humidity (low) and wind (calm). During the peak season of scent emission, *C. humilis* shows low VOC variation in composition and abundance along the day (Dufaÿ et al. 2004), thus, we carefully sampled all individuals during their peak phase of leaf odour production: in male plants, this occurs 9 days approximately since the floral bracts opens, while in female plants the peak occurs 4 days since floral bract opens (Dufaÿ et al. 2004). We selected plants with all inflorescences in the same stage of maturation.

Scent was collected in the field from a healthy leaf in 60 *C. humilis* plants using dynamic headspace adsorption. Specifically, in Carcaixent we sampled 8 individuals per sex at each burnt and control area (N=32 plants). The same was sampled in Xàbia but with 4 female plants inside the burnt (N=28 plants, see Notes S1 for details on scent collection). For the olfactory bioassays we sampled the leaf scent from four additional individuals per sex at burnt and control areas. To test the response specificity between the chemical signal emitted by *C. humilis* and the two pollinators (experimental bioassays below) we collected the floral scent of 8 common co-occurring plants (Appendix C: Notes C1 for details). Volatile compounds from the leaf scent were analysed and identified using gas chromatography-mass spectrometry (GCMS, details in Appendix C: Notes C1). The relative amount of each volatile compound was calculated using the MS compound peak areas for each scent sample and corrected by the volatile compounds present in the ambient controls.

Olfactory bioassays

Individuals of the two beetle species were collected from male inflorescences in unburnt and the surrounding areas of the two localities at the beginning of the flowering season. Beetles were kept in dark in the laboratory with a wet cotton to maintain humidity. To test if the two beetle species were attracted by the palm's scent and whether the attractiveness of the signal differed between i) male and female plants, and ii) burnt and control areas, we ran olfactory bioassays using a glass Y-tube olfactometer (Vidrafoc, Valencia Spain) as described in Dufaÿ et al. (2003, Appendix C: Fig. C2 and Notes C1 for details on olfactory bioassays). As mention before we also run olfactory tests with *C. humilis* scent and floral scent from eight co-occurring plants with the two beetle species to assess the specificity of the palm's signal (Appendix C: Notes C1 for details).

Statistical analyses

To study the differences in composition (relative abundance of each VOC) in *C. humilis* scent between sites, plant sex, and fire incidence (burnt vs unburnt) we defined the chemospace using an ordination with a nonmetric multidimensional scaling (NMDS) based on Bray-Curtis distances. Relative abundances were fourth-root transformed prior to ordination to reduce the effects of the most abundant compounds (Schlumpberger and Raguso 2008). NMDS analyses were run with the R package *vegan* (Dixon 2003). Compounds occurring in only one sample were excluded to simplify interpretation. N-compounds were all grouped and included as a group in the analysis because of the likely fire-induced changes in soil nitrogen content.

To test the effects of plant sex, site and fire treatment on *C. humilis* scent we used a model-based framework for compositional data; specifically we fitted multivariate generalized linear models (MGLMs) with binomial (for qualitative data, and using the "cloglog" link) and tweedie (for the semi-quantitative data, with many zeros using link "log" and a variance power of 1.3) family distributions. The final model included *C. humilis* VOCs as response variables and site, fire treatment (unburnt vs. burnt areas), plant sex, and the interaction between site and fire treatment as fixed factors. To control for differences in size between plants from burnt and unburnt areas we also included plant height (stem length in cm) of each individual as a covariate. Because of the significant differences in scent composition between sites (see results), we fitted separate MGLMs at each site to assess the effect of fire on VOCs composition based on the relative amount of each compound. We also ran additional MGLMs for the subsets of aliphatic, aromatic and terpene compounds. We visually inspected residuals to validate model assumptions. MGLMs were fitted with "manyany" and "manyglm" functions of the package *mvabund* in R (Wang et al. 2012, Appendix C: Notes C2 for details on MGLMs analysis).

We tested for choice differences of the two beetle species in the bioassays using binomial and Fisher exact tests (null hypothesis: 50:50 response) that included only those individuals that made a choice. Differences in choosiness (proportion of individuals that chose one of the arms vs. those that did not make a choice) between the two beetles and within each species were analysed with all tested individuals, and were considered an indicator of the potential variation in absolute VOCs emissions between treatments (Dufaÿ et al. 2003). To assess a potential bias of the insects choosing one arm of the olfactometer, directional preferences were analysed with binomial tests. Because of the low number of trials per plant species in the bioassays with the eight co-occurring plants, the number of choices for these plants were summed for this analysis. All analyses were run in R software version 3.4.2 (R Core Team 2017).

RESULTS

Scent composition

Leaves of *C. humilis* emitted 61 volatile compounds (Appendix C: Table C1), with the number of VOCs per plant ranging from 3 to 19 (8.9 ± 3.65 mean VOCs per sample). According to their biosynthetic origin, aliphatic fatty-acid derivatives were the most abundant compounds (39.7% mean abundance of the total scent) followed by terpenoid (30.6 %) and aromatic compounds (26.3 %). N-compounds (1 %) and a miscellaneous group (2.4 %) were the least abundant. The most common VOCs were the monoterpene β-Ocimene and a phenyl ester of pentanoic acid, Pentanoic acid, 5-hydroxy,2,4-di-t-butylphenyl ester (in 86% and 81% of the leaf samples) followed by another aromatic ester, bis (2-ethylhexyl) 1,3-Benzenedicarboxylic acid ester (in 57 % of the leaf samples).

C. humilis scent composition differed among the study sites but not among male and female individuals (Fig. 3.1). Fire changed the blend composition of the palm (burnt vs unburnt: $df_{res, diff}$= 56,1; Dev= 95.21; *P* =0.03) and its effect varied within each locality (significant interaction between study site and fire treatment; $df_{res, diff}$= 54,1; Dev= 48.65; *P* =0.01, Fig. 3.2). Similar models for the three subsets of VOCs (aliphatic, aromatic and terpenoids) revealed and effect of the study site on the three compound groups and of the fire treatment on aliphatic volatiles (Appendix C: Table C2).

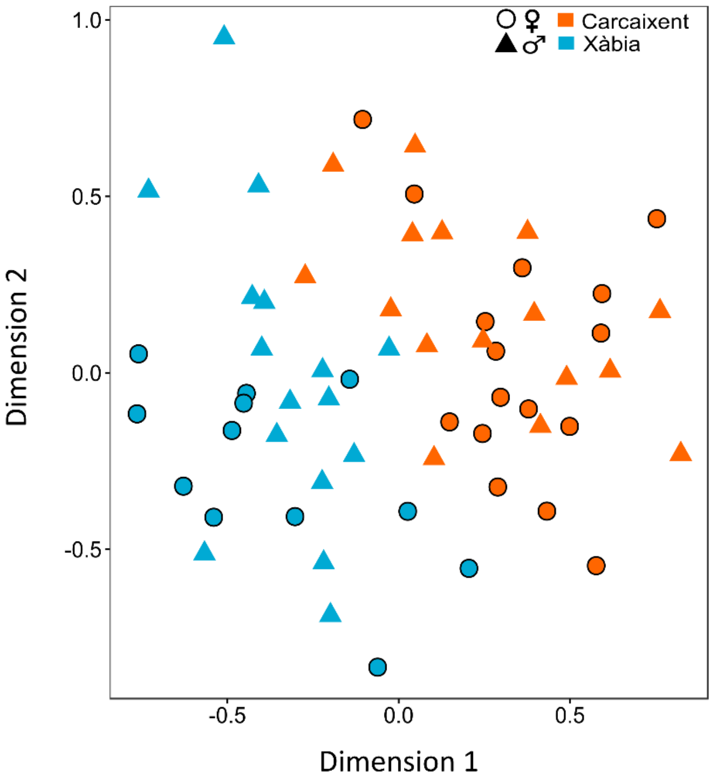

Figure 3.1. Distribution of female and male *Chamaerops humilis* plants (N= 60) in the NMDS chemospace (stress=0.19) at two study sites in Spain. The chemospace was significantly different between sites (site: $df_{res,\ diff}$= 58,1; Dev= 294.81; *P* <0.01) but not between plant sexes (plant sex: $df_{res,\ diff}$= 57,1; Dev= 73.70; *P* = 0.18).

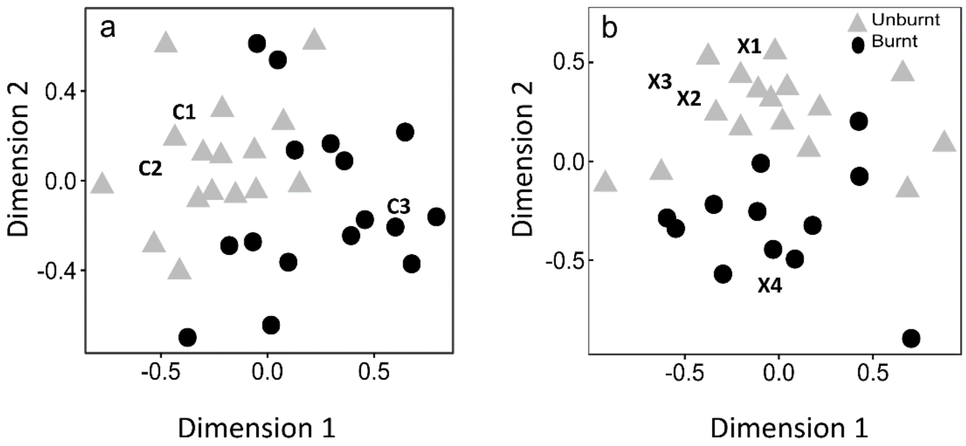

Figure 3.2. Distribution of *Chamaerops humilis* plants in the NMDS chemospace according to burnt (black dots) and unburnt (grey triangles) areas in the two study sites: a) Carcaixent (N=28 plants, stress=0.19) and b) Xàbia (N=32 plants, stress=0.18). Capital letters are some of the leaf volatiles with significant changes between the burnt and the unburnt area at each site: Carcaixent C1= 3,4 Dimethyl-benzaldehyde, C2= Dodecane,C3= Heptane; Xàbia X1= 2-Ethylhexyl methyl isopthalate, X2= Hexadecane, X3= Dodecane, X4= Linalool.

At the two sites fire changed VOCs composition (burnt vs control area: Carcaixent, Dev=66.30; $P = 0.003$; Xàbia, Dev=65.27; $P = 0.03$) and VOCs proportions in the palm's blend (burnt vs control area: Carcaixent, Dev=678.96; $P < 0.01$; Xàbia, Dev= 450.79; $P = 0.04$; Fig. 3.2). The proportion of VOCs that showed a significant change after fire was similar in both sites (8.5 % in Carcaixent and 11.6% in Xàbia, Table 1). The relative amount of the three most abundant compounds in the scent mentioned before did not change with fire ($P > 0.05$ in all cases at the two sites).

Table 3.1. Volatile organic compounds (VOCs) of *Chamaerops humilis* leaf scent that showed significant postfire changes at the two study sites (Carcaixent and Xàbia). Significance was tested with MGLMs based on semi-quantitative data of each VOC. FAD: Fatty acid derivative.

Site	**Compound**	**Group**	**Effect**	**Deviance**	***P***
Carcaixent	3,4 Dimethyl-benzaldehyde	Aromatic	Decrease	27.40	<0.01
	Dodecane	FAD alkane	Decrease	47.98	<0.01
	n-Tetracosane	FAD alkane	Decrease	151.04	<0.001
	n-Heptane	FAD alkane	Increase	62.65	<0.01
Xàbia	2-Ethylhexyl methyl isopthalate	FAD ester	Decrease	21.20	<0.05
	Dodecane	FAD alkane	Decrease	20.98	<0.05
	Hexadecane	FAD alkane	Decrease	33.75	<0.05
	Linalool	Terpenoid	Increase	57.29	<0.01
	n-Heptane	FAD alkane	Increase	25.01	<0.05

Olfactory bioassays

Insect choice did not vary between study sites, so results for the two sites were grouped. No directional preferences for the left or the right arm of the olfactometer were detected (χ_1^2= 1.6; P = 0.20). Both beetle species showed a clear preference (i.e. only beetles that made a choice) for *C. humilis* scent over the control arm (binomial test; weevil: χ_1^2= 90.9; P<0.001; sap beetle: χ_1^2=37.5; P<0.001, Fig. 3.3 see Videos C1 and C2, Appendix C). There were no differences in preference between VOCs from female and male plants (Fisher exact test; weevil: N= 91, P=0.55; sap beetle: N= 72, P=0.57). In addition, both beetle species responded in a similar way to the scent emitted by leaves from burnt and unburnt control areas (Fisher exact test; weevil: N=82, *P*=0.10; sap beetle: N=73, *P*=0.72, Fig. 3.3).

Regarding the choosiness (including all tested beetles), the two beetles made a choice more often in trials with VOCs samples than in control trials with only acetone in the two arms (weevil: χ_1^2= 65.83; *P*<0.001; sap beetle: χ_1^2= 24.68; *P*<0.001). We did not detect significant differences in choosiness in trials with VOCs from male and female plants (N=96; *P*=0.75 for the weevil and *P*= 0.59 for the sap beetle) nor with VOCs from unburnt vs. burnt areas (Fisher exact test; weevil: N=96; *P*= 0.65; sap beetle: N=96; *P*= 0.61). Overall, the weevil was choosier than the sap beetle in VOC trials (χ_1^2= 12.08; *P* <0.01) and in trials with only VOCs from the unburnt areas (χ_1^2= 5.01; *P* = 0.02). However, no differences were detected between beetle species when we only considered VOC trials from the burnt areas (*P*= 0.67). Moreover, while the weevil did not change its choosiness at trials with VOCs from the burnt areas (*P*= 0.72), the sap beetle chose more frequently in bioassays with scent from the burnt (*P*= 0.018).

Both beetles were significantly more attracted to *C. humilis'* leaf blend than to the floral VOCs from the group of the eight co-occurring plants (weevil: N=64; χ_1^2= 38.43; *P* <0.001; sap beetle: N=64; χ_1^2= 22.64; *P* <0.001, Fig. 3.4).

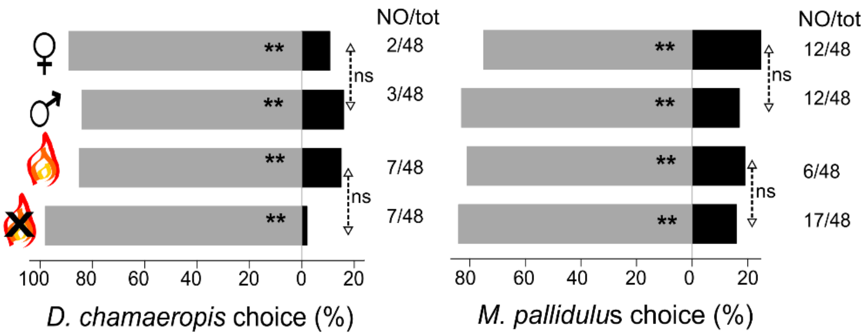

Figure 3.3. Response of the weevil *Derelomus chamaeropis* (left) and the sap beetle *Meligethinus pallidulus* (right) to *Chamaerops humilis* scent in Y-tube olfactometer bioassays. From top to bottom, the grey bars refer to preference for the treatment arm with VOCs from female plants, male plants, burnt areas, or unburnt areas; the black bars show response to the control arm. NO/tot indicates the number of individuals that did not make a choice out of the total (N= 48). Significant differences between the treatment and the control arm is denoted with asterisks (**$P < 0.01$). Dashed arrows show the results of the comparison between VOC treatments (ns: not significant).

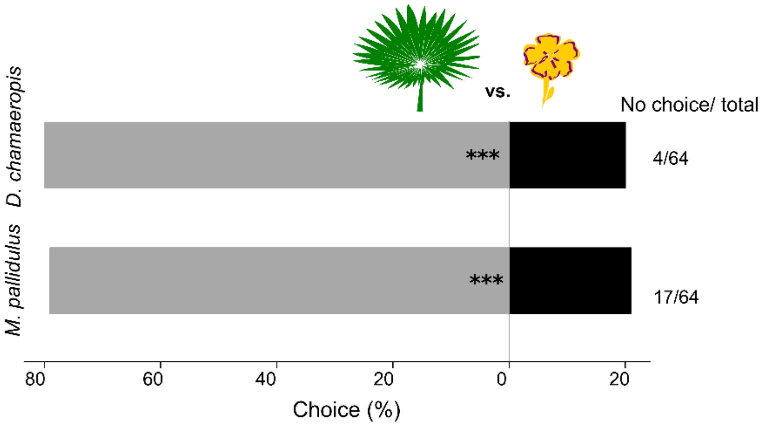

Figure 3.4. Response of *Derelomus chameropis* (upper bar) and *Meligethinus pallidulus* (lower bar) in Y-tube olfactometer bioassays with scent from *Chamaerops humilis* leaves (grey bars) and the floral blends of eight co-occuring plant species (black bars). No choice/total: individuals that did not make a choice out of the total individuals tested (N= 64). Significance levels are denoted with asterisks ***: $P < 0.001$.

DISCUSSION

Fires modified *C. humilis* scent composition, and VOCs from both burnt and unburnt similarly attracted the two beetle species. VOC changes were thus not responsible for the observed pollinator shift after fire; that is, fire did not disrupt the VOC-mediated pollination interaction. Low changes in the proportion of the most abundant compounds, and the different trends showed by the postfire-altered volatiles may explain the maintenance of the signal's attractiveness. Thus, contrary to our expectation, fire-mediated changes in scent did not favor the sap beetle (non-nursery pollinator), and its dominance in recently burnt areas is more likely a direct consequence of the higher fire sensitivity and slower postfire recovery of the weevil (nursery pollinator). Our study also showed a high specificity of the two beetle pollinators with *C. humilis* scent, providing further evidence for the important role of alternative pollinators in plants with nursery pollination systems in disturbance-prone ecosystems.

Scent composition

C. humilis scent composition in leaves varied among individuals and sites (see also Dufaÿ et al. 2004), and included compounds common in floral fragrances (Knudsen et al. 2006). Fatty acid derivatives (FADs) and particularly aliphatic hydrocarbons showed the greatest number of compounds in the scent. They are also frequent in floral blends involved in pollinator attraction (Knudsen et al. 2006), including other beetle-pollinated palm species (Knudsen et al. 2001) and nursery pollination systems (Bergström et al. 1991; Jürgens et al. 2002, 2003). However, the most abundant compounds, present in more than 80% of the scent samples, were a phenyl ester and the monoterpene β-Ocimene. Ester compounds are one of the most common functional groups of VOCs (Dudareva and Pichersky 2006) and predominate in the floral scent of other nitidulid-pollinated plants (Jürgens et al. 2000; Procheş and Johnson 2009), while the monoterpene β-Ocimene is a common generalist attractant (Farré-Armengol et al. 2017). Previous studies showed that β-Ocimene was more abundant in *C. humilis* samples collected by headspace absorption than in those from washed leaves, consistent with a function in pollinator attraction (Caissard et al. 2004).

We did not detect differences in scent composition between male and female palms (Dufaÿ et al. 2003) despite female plants offering significantly lower rewards. In other dioecious species, intersexual scent resemblance can occur during the receptive phases of the two plant sexes or when both sexes show flowering overlap, as in *C. humilis* (Proffit et al. 2007; Ashman 2009; Hossaert-McKey et al. 2016). Consistently, the two beetle pollinators did not show significant preferences for *C. humilis* scent collected from male or female leaves in the bioassays.

Postfire changes in scent emission and pollinator response

Fire changed VOCs from different biosynthetic pathways (aliphatic aromatic and terpenoid compounds) and these changes showed different trends (i.e. some VOCs increased and other VOCs decreased). The postfire increase in the proportion of the monoterpene alcohol linalool in the palm's scent resembles the increased terpene emissions under rising temperatures in other Mediterranean plants (Farré-Armengol et al. 2014). The relative abundance of other monoterpene alcohols also increased in *Juniperus picnhotti* leaves after prescribed fire (Campbell and Taylor Jr 2007). In fact, the increased volatilization, and thus emission, of plant VOCs in response to higher temperatures and CO_2 concentrations has led to hypothesize that global warming could enhance pollinator attraction (Farré-Armengol et al. 2013, Burkle and Runyon 2016). However, recent studies assessing the effects of gas emissions associated to global warming as ozone, on floral scents and pollinator behavior suggest a negative effect on pollinators (Dötterl et al. 2016; Farré-Armengol et al. 2016).

We found no evidence of changes in attraction as a response to the strong environmental fluctuations imposed by fire in any of the two beetle species, probably due to the lack of a fire effect on the most abundant compounds (β-Ocimene and two aromatic esters). The fact that these same compounds are dominant in the two sites emphasizes an important role of these volatiles in the chemical signal. In addition, because not all VOCs in the scent have a function in pollinator attraction (Schiestl et al. 1997; Friberg et al. 2013) a modest change in the scent composition (11.5 % of total VOCs showed significant changes) will not necessarily alter pollinator attraction. For instance, some of these VOCs may be also involved in other plant interactions such as defence against herbivores like other leaf VOCs.

The lack of effects of postfire scent changes on insect response might be explained by a potential adaptation to a dynamic odour landscape (Jürgens and Bischoff 2017). Under this hypothesis, organisms from landscapes that frequently experience changes in their plant VOCs emissions may be adapted to such dynamic environments (Endler 1992; Wilson et al. 2015; Jürgens and Bischoff 2017). That is, fire-prone ecosystems can be viewed as dynamic odourscapes where fire triggers changes in plant VOCs emissions by altering herbivory pressures and plant community structure. And, in the same way that plants and animals can show adaptive traits under certain fire regimes (Keeley et al. 2011; Pausas and Parr 2018), organisms from fire-prone ecosystems that depend on VOC signals might be adapted to a certain VOCs emission regime.

The experimental bioassays unambiguously demonstrate the role of foliar scent as pollinator attractant and the specificity of the interaction between the palm scent and the two beetles suggested by field observations and pollen loads analysis (García et al. 2018). Both the weevil *D. chamaeropis* and the sap beetle *M. pallidulus* preferred the palm's scent to the floral scent from the co-occurring plant species, as reported in other plant species by olfactometry studies with their specialist pollinators (Proffit et al. 2009; Friberg et al. 2014). This ultimately emphasizes the role of plant scents in promoting pollination specificity (Friberg et al. 2013, 2014).

Interestingly, we observed significant differences in choosiness for the two beetle species at burnt and control areas. While the weevil chose more frequently than the sap beetle in bioassays with *C. humilis* VOCs from the unburnt, the sap beetle increased its choosiness after fire to similar levels of the weevil. This could be related to the variation in the palm's blend composition, but also to differences in the total VOCs emission rates between burnt sites and unburnt areas (Dufaÿ et al. 2003); further research is needed to better understand this pattern.

Concluding remarks

Researchers have only recently started to assess the effects of anthropogenic-induced disturbances in plant VOCs emissions involved in pollination (Farré-Armengol et al. 2013, 2014, 2016; Burkle and Runyon, 2016, 2017; Jürgens and Bischoff 2017) and some of them have reported negative effects on pollinators (Farré-Armengol et al. 2016; Dötterl et al. 2016). Most of these studies were conducted under controlled conditions by artificially selecting the level of perturbation imposed. Here we provide, for the first time, field evidence of the effects of wildfires on chemical signals mediating plant-pollinator interactions. Despite the changes detected, the pollination interactions remained resilient to fire, even in this case of a specialized pollination system. Further studies on the effects of fires on VOCs involved in plant pollination emitted by floral tissues are still needed. Because natural fire regimes and pollinator services are changing worldwide (Pausas and Fernández-Muñoz 2012; González-Varo et al. 2013), understanding the mechanisms contributing to the resilience of pollination interactions is a priority in the current ecological agenda.

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CHAPTER IV: Plant-animal interactions contribute to the assembly of forest-savanna communities

Abstract

Community assembly studies have traditionally focused on the role of abiotic filtering and competition. Plant-animal mutualisms may influence plant species coexistence through biotic filtering and plant-plant facilitation/competition mediated by mutualists (e.g. pollinators, seed disperses). This may result in non-random community patterns of plant reproductive traits (e.g. floral, seed and fruit traits). In environments with contrasting habitat conditions, such as tropical forests and savannas that differ in the fire regime, the spatial variation in plant-animal mutualisms may generate varying patterns of reproductive traits across habitats (i.e. forest and savanna). We explore this idea in the Brazilian Cerrado by focusing on reproductive traits from forest and savanna plots (N= 98 plots) with contrasting fire histories. Most previous studies in these patchy ecosystems have focused on the abiotic filter exerted by fire on vegetative traits. Here, we measured the phenotypic and phylogenetic structure of forests and savannas based on 12 reproductive traits and compared it with patterns expected for the regional species pool (null models). Non-random patterns emerged in both community types, supporting the role of plant-animal mutualisms influencing the assembly of forest-savanna mosaics. While forests showed clustering patterns indicating functional similarity (lower functional diversity) in floral and fruit traits, savannas were functionally overdispersed (higher functional diversity) suggesting that plant-animal interactions may operate through different mechanisms in contrasting habitat conditions (e.g. canopy closure). Our study indicates that the community assembly of forest-savanna mosaics is the result of multiple processes acting on different traits and levels of disturbance, and that together with the fire, plant-animal mutualisms promote species coexistence.

INTRODUCTION

Ecologists have traditionally studied the assembly of biotic communities as the result of two main deterministic processes: abiotic filtering and competition (Diamond 1975; Inouye et al. 1980; Tilman 1994). Abiotic filtering limits species that can succeed through environmental barriers (e.g. drought, disturbance, poor soils), while competition by niche similarity excludes species that consume the same resources. More recently studies have also included facilitative plant-plant interactions as an additional force on the assemblage of plant communities (Kraft et al. 2008; Verdú and Valiente-Banuet 2011; Schöb et al. 2012). Most studies on community assembly use morphological and ecophysiological traits to assess how these forces result in plant communities with varying degrees of phenotypic similarity (Keddy 1992; Kraft et al. 2008). The different assembly processes can lead to communities showing non-random phenotypic patterns such as clustering, when coexisting species share traits more similar than expected by chance (related to habitat filtering, e.g. Pausas and Verdú 2008), or phenotypic overdispersion when coexisting species are less similar than expected for a given species pool (e.g. related to competition and facilitation, e.g. Webb 2000; Godoy et al. 2014). The incorporation of phylogenetic-based approaches that consider the evolutionary history of species can also help to understand community assembly processes. The combination of phylogenetic structure (clustering or overdispersion) and trait evolution (conserved or convergent traits) is often used for inferring the prevailing assembly force (Webb et al. 2002; Cavender-Bares et al. 2009; Jombart et al. 2010; Pausas and Verdú 2010; but see Gerhold et al. 2015).

Plant community assembly studies have paid much less attention on the role of plant-animal interactions in species coexistence (Sargent and Ackerly 2008; Pellissier et al. 2012). Plant-pollinator interactions can shape communities acting through biotic filtering and via facilitative and competitive mechanisms (de Jager et al. 2011; Briscoe-Runquist et al. 2016; Wolowski et al. 2017; Bergamo et al. 2018a, 2018b; Kemp et al. 2018). For example, pollinator-mediated filtering by color preferences of the locally dominant pollinator favors the coexistence of daisy communities (Asteraceae) with similar floral colors (i.e. functional clustering, Kemp et al. 2018). Alternatively, pollination interactions may promote the divergence of floral traits through plant-plant competition when the fitness costs imposed by sharing the same pollinators are higher than the benefits (de Jager et al. 2011; Muchhala et al. 2014). Like pollination and floral traits, different dispersal modes with varying dispersal abilities and linked to certain fruit and seed traits may also influence the spatial arrangement of plant communities (Seidler and Plotkin 2006; Beaudrot et al. 2013). For instance, Seidler and Plotkin found that dispersal mode (e.g. wing, small-bodied animals, larger-bodied animals) shaped the spatial pattern of tree species in a tropical forest from Malasya, resulting in a variety of species cluster sizes showing different dispersal syndromes (Seidler and Plotkin 2006). In fact, just as pollinators can impose selection on floral traits (Fenster et al. 2004), seed dispersers also contribute in shaping fruit traits (Jordano 1995; Valido et al. 2011). As these previous examples show, the study of community patterns in plant reproductive traits can provide evidence of the role of plant-animal mutualisms as drivers of species coexistence (Bergamo et al. 2018b). All this suggests that, when studying plant community assembly, a more integrative view of the mechanisms involved might be necessary by considering biotic interactions along with abiotic factors (Kraft et al. 2014; Bartomeus and Godoy 2018).

We focus here on the idea that the spatial variation in plant-animal interactions favored by particular habitat conditions can lead to different assembly outcomes across habitat types (Pellissier et al. 2010; Chalcoff et al. 2012; Maruyama et al. 2014; Koski and Ashman 2015; Pringle et al. 2016; Wolowski et al. 2017; Bergamo et al. 2018a, 2018b; Gray et al. 2018). Tropical forest-savanna mosaics are examples of highly diverse ecosystems with contrasted habitat conditions that provide ideal models to explore how plant-animal interactions influence community assembly. Previous studies in these ecosystems have so far focused on the abiotic filter imposed by fire that indeed shapes the assembly of savanna (open canopy) and forest (closed canopy) communities, promoting different sets of plant functional traits linked to contrasting fire responses (Hoffmann et al. 2003, 2012; Bond and Keeley 2005, Dantas et al. 2013a, 2013b, 2016; Lawes et al. 2013; but see Maruyama et al. 2014). This may result in clustered patterns of plant traits related to fire (e.g. bark thickness), as shown in other fire-prone ecosystems (Cavender-Bares et al. 2004; Verdú and Pausas 2007; Pausas and Verdú 2008). Plant-animal interactions can also be expected to play a role in structuring plant communities in forest-savanna mosaics, because in such complex ecosystems, multiple ecological processes could simultaneously shape the community assemblage by operating at different spatial scales and disturbance regimes, or on different plant traits (Kraft and Ackerly 2010; Ojeda et al. 2010; Ding et al. 2012; Spasojevic and Suding 2012; de Bello et al. 2013). For instance, plant-hummingbird assemblages in forest and savanna differ in their species composition and functional traits (e.g. Maruyama et al. 2014). Moreover, fire, which is frequent in savannas, may alter insect abundance and diversity (Swengel 2001; Vasconcelos et al. 2009), potentially inducing competition for floral resources.

Ding et al. (2012) found that abiotic filtering drove the assembly of highly disturbed communities while multiple processes including competition and biotic filtering at fine scale shaped the assembly of communities under low levels of disturbance. In addition to fire, forest-savanna mosaics also have marked variation in other abiotic factors such as the availability of nutrient, water, and light (de Assis et al. 2011; Dantas et al. 2013a, 2013b; Laureto and Cianciaruso 2015; Sfair et al. 2016; Maracahipes et al. 2018) that can also influence plant-animal interactions. Light availability affect the way in which pollinators perceive floral color and its contrast (Koski and Ashman 2015), and thus dense canopies may favor floral traits related to increase visibility in shaded conditions (Sargent and Ackerly 2008; Ostler and Harper 1978). In the case of seed dispersal, earlier studies have shown the predominance of fleshy fruits linked to zoochorous species in communities with closed canopies under moist conditions compared to more open and drier ones

(Ribeiro and Tabarelli 2002; Chazdon et al. 2003). More than half of the plants in the Cerrado region are dispersed by frugivorous birds, and both bird species composition and diversity may change along the canopy closure gradient from savanna to forest (Macedo 2002). In this study we use a multi-trait approach considering traits linked to plant-animal interactions to look for new insights on the mechanisms underpinning the assembly of forest-savanna mosaics. We expect the presence of different community patterns of plant traits related to mutualistic plant-animal interactions in forests and savannas; this would suggest that distinct processes contribute to the assembly of these tropical communities.

To evaluate this prediction, we assessed whether the functional structure of forests and savannas based on reproductive plant traits depart from the expected for the regional species pool (null model), and whether these patterns differed among forest and savanna habitats in the Brazilian Cerrado. We also assessed non-random phylogenetic community patterns that can be used to predict the predominant assembly processess in each habitat type. Finally, we explore whether different pollination and dispersal modes in the forests and savannas relate to the observed trait patterns.

MATERIAL AND METHODS

Study site and community closure index

The study was performed in Emas National Park located in the Cerrado ecoregion of the Brazilian Central Plateau ($17^{0}49'$-$18^{0}28'$ S and $52^{0}39'$-$53^{0}10'$ W). The climate in this region is tropical-humid with maximum precipitation between October and March. The park has an extension of 132000 ha and is mainly composed of wooded grasslands and savannas where fires are frequent, with patches of forests where fires are rare (Dantas et al. 2013a).

The plant trait dataset analyzed here is based on the species recorded in 98 5x5 m vegetation plots by Dantas et al. (2013a). This includes plots from open savannas to forests with dense canopies, conforming a canopy closure gradient, with a range of post-fire ages (from 1 to 31 years). Fieldwork was conducted during the rainy season of 2009 and 2010. The resulting list includes all woody plant individuals (stem diameter at the ground level ≥ 3cm) for a total of 98 species from 39 different families.

To determine the level of canopy closure along the forest-savanna transition, Dantas et al. (2013a) estimated a community closure index (CCI) for each plot from basal area and plant height of each sampled individual assuming a cone shape. This index varied from open communities (CCI values close to 0) to closed communities (CCI values close to 1). Different functional traits, soil variables and diversity indices showed a significant breakpoint along the canopy closure gradient (mean CCI value = 0.57 ± 0.009). This breakpoint revealed two clearly different states varying in their functional and diversity attributes as well in their fire histories: forests (CCI >0.57, n= 17 plots) and savannas (CCI<0.57, n= 81 plots, Dantas et al. 2013a); we use the same definition to differentiate the two habitats in the analysis below.

Reproductive traits

Our database of 12 reproductive traits includes all plant species in Dantas et al. (2013a) for which we were able to find reliable data (75 out of 98 species, see Table D1, Appendix D). Trait information was obtained through a combination of extensive literature review plus measurements in digital herbarium records from *Jardim Botânico do Rio de Janeiro*. We chose plant traits involved in reproduction and in plant-animal mutualisms, including six floral traits, pollination mode, sexual system, and four fruit traits. Floral traits were: *floral symmetry*, *size* (corolla length), *color*, *anthesis*, *reward* and *shape*. For each species we also included *pollination mode* and *sexual system*. Fruit traits were: *fruit size* (fruit length), *fruit type*, *number of seeds per fruit*, and *dispersal mode*. For the analyses we considered all traits as categorical factors, with fruit and floral sizes and seeds per fruit as ordered factors (Notes D1 in Appendix D, for full details on trait categories and database compilation).

Before the analyses of community structure and to rule out that reproductive traits in the two habitat types were related to other traits strongly influenced by fire, we first tested the association between relative bark thickness (i.e. bark thickness/diameter from Dantas et al. 2013a) and each of the reproductive traits. Relative bark thickness is a key plant trait related to fire resistance (Lawes et al. 2011a, 2011b; Dantas et al. 2013a, 2013b; Pausas 2015). We did not detect significant relationships between bark thickness and reproductive traits (except for wasp-pollinated plants, Table D2 in Appendix D for details).

Functional community structure

Community closure index and plant traits

To assess whether forest and savanna plots differ in their species trait similarity, we estimated their dissimilarity by using the mean pairwise distance of species traits in the study plots (hereafter Mean Functional Distance, MFD). We first obtained the trait distance matrix from the species traits by using the Gower transformation as it can handle mixed trait variables (i.e. ordinal and categorical in our case; Sneath and Sokal 1973). Then we used this trait distance matrix together with the original species co-occurrence (presence-absence) matrix ("Original", N=98 plots) to estimate the MFD. We conducted a non-metric multidimensional scaling ordination (NMDS) with the MFD to summarize the community trait dissimilarity. We tested the resulting NMDS axes against habitat type with a Wilcoxon *t*-test. We also tested the NMDS axes against the canopy closure index (CCI) with exponential regression models in R. In addition, we conducted a permutational multivariate analysis of variance (PERMANOVA) on the MFD in the study plots to test for changes with the habitat type (as predictor variable). We conducted the analyses in R using the packages *picante* (Kembel et al. 2010) and *vegan* (Oksanen et al. 2013).

Functional structure

We studied the effects of the habitat type on community functional structure by comparing the observed MFD (as defined above) with a null MFD derived from 1000 random matrices generated from the species pool. To generate the null distribution, we chose the independent-swap algorithm, which maintains the original species richness and their frequency of occupancy (Gotelli 2000). Then we computed the standardized effect-size of the MFD (sesMFD) as the difference between the observed MFD and the null MFD divided by the standard deviation of the null MFD (Webb et al. 2002). Positive sesMFD values indicate functional overdispersion (i.e. higher functional diversity), while negative values indicate functional clustering (i.e. lower functional diversity). Previously we tested for correlation between each pair of study traits (Cramer's V coefficient for discrete variables). Preliminary analyses on sesMFD excluding floral and fruit traits with higher correlation values (i.e. pollination and dispersal modes and fruit type) showed similar patterns of those detected for the whole set of 12 traits. For this reason, we run all the analyses for the complete set of reproductive traits.

To test for a possible effect of the differences in the number of forest and savanna plots in the original matrix (81 savanna vs 17 forest plots), we estimated the sesMFD for a subset of the co-occurrence matrix. This matrix ("Equal-size", N=34 plots) consisted in a random subset of 17 savanna plots taken from the original (N=81 savanna plots) plus the original 17 forest plots. This randomization was performed 1000 times, and thus we computed the sesMFD for 1000 equal-size matrices (i.e. 1000 matrices, N=34 plots).

For both the original and the equal-size sesMFD, we used Wilcoxon tests ($\alpha=0.05$) to evaluate whether the sesMFD within each habitat type (i.e. forest and savanna) showed a significant deviation from the null expectation (mean=0), as data were not normally distributed (Hultgren and Duffy 2012). We also used Wilcoxon tests to assess differences in sesMFD between the two habitats.

Effects of the habitat type on each trait

We individually explored for potential effects of the habitat type on each studied trait. To do that, we ran generalized linear models (GLMs) with the number of species with each particular trait category in each plot as the response variable and habitat (forest vs savanna) as the predictor. This was computed using the package *MASS* in R (Ripley et al. 2013) with negative binomial error distribution for count data with overdispersion.

Phylogenetic community structure

We first assembled a phylogenetic tree for the 75 study species using the function "S.Phylomaker" (Qian and Jin 2016) in R. This function takes as backbone an updated version of Zanne's phylogeny for vascular plants at the species level (Zanne et al. 2014; Qian and Jin 2016). We used the tree provided by the Scenario 3 in the function that follows the same methods of Phylocom and BLADJ to build the phylogeny. Three closely-related genera (*Myrcia*, *Eugenia* and *Psidium*) were manually collapsed because the function could not resolve accurately their relationship.

We then calculated the mean pairwise phylogenetic distance (MPD) for each plot as a measure of species phylogenetic community structure. We estimated the MPD based on the species co-occurrence matrix and the phylogenetic distance matrix obtained from the species phylogeny. As we did for the MFD, we estimated the MPD for the original (i.e. one matrix of N=98 plots) and for the equal-size co-occurrence matrices (i.e. 1000 matrices of N=34 plots) and used the independent-swap algorithm to estimate the null distribution. We also computed the standardized effect size of the MPD (sesMPD) with the same procedure used for the MFD but using the mean phylogenetic distance.

As for the MFD, we also conducted Wilcoxon tests (α= 0.05) to assess for differences between the sesMPD of each habitat type and the null distribution, and to test for differences in the sesMPD between the two habitats. In this case, positive sesMPD values are related with phylogenetic overdispersion, while negative values are related with phylogenetic clustering.

To assess the relationship between phylogenetic and functional patterns, we tested the correlation between the phylogenetic and the trait distance matrix after 1000 permutations in a Mantel test with the package *ade4* in R (Dray et al. 2007). We then evaluated the degree of trait conservatism among the study species by estimating the phylogenetic signal of each trait with the function "phylo.signal.disc" for discrete characters developed by Emilio Rezende (Universitat Autònoma de Barcelona) in R. The function is based on the procedure of Maddison and Slatkin (1991) that estimates whether the minimum number of transitions in a character-state of a particular phylogeny differs from the expected under a null model obtained from reshuffling 1000 times the species labels across the phylogeny. A lower number of observed transitions compared to the median of the null model results in a significant phylogenetic signal of the trait. Finally, we calculated a global phylogenetic signal for the study traits by estimating the *K* statistic (Blomberg et al. 2003) of the two axes of a NMDS ordination, in this case based on trait distances among the 75 species (Gower-transformed; see Kembel and Cahill 2011 for similar approach). K values significantly different from 1 indicate that traits depart from a Brownian motion process, while *K* values significantly different from 0 are related to phylogenetic signal of the study traits (Blomberg et al. 2003). To calculate the K statistic and its significance we used the package *picante* in R (Kembel et al. 2010).

RESULTS

Functional structure

Habitat type influenced the mean trait dissimilarity in the study plots (PERMANOVA: F 1, 96 = 7.51, $R^2=0.07$, $P = 0.002$,), as shown in the ordination analysis (Fig. 4.1). In addition, the first ordination axis of the NMDS (based on the MPD of reproductive traits at the study plots), showed significant changes with both the canopy closure index and habitat type (cci: $F_{1,96}= 14.62$, $R^2=0.14$, $P <0.001$; habitat: Wilcoxon, $P <0.001$; Fig. 4.2).

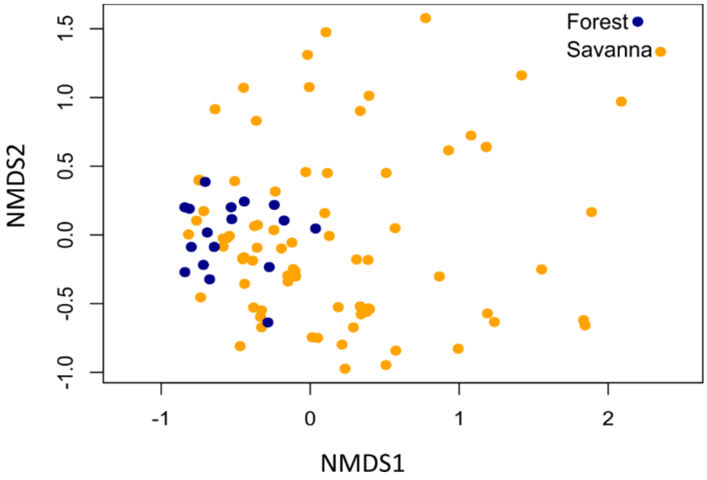

Figure 4.1. Distribution of the forest and savanna plots in the ordination space (NMDS) defined by their mean trait dissimilarity (measured as the Mean Pairwise Distance of species traits) based on 12 plant reproductive traits (stress=0.14).

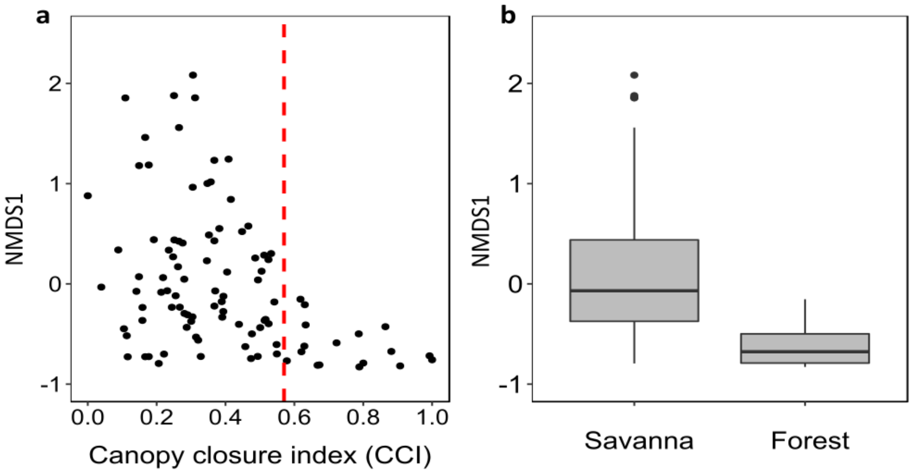

Figure 4.2. Association between mean trait dissimilarity in the study plots (summarized by the first axis of a NMDS ordination based on the mean pairwise distance of reproductive plant traits), and habitat characteristics in a forest-savanna mosaic a) Relationship between the mean trait dissimilarity in the study plots (MDS1 in Fig. 1) and the canopy closure index (cci). Red dashed lines indicate the canopy threshold separating the two habitat types (i.e. savanna vs forest, cci=0.57). **b)** Effect of the habitat type on mean trait dissimilarity (MDS1 in Fig. 1) in the study plots.

Regarding the functional structure of each habitat, forest communities showed significant trait clustering as reflected by their lower sesMFD compared to the expected under the null distribution, for both the original co-occurrence matrix and for the set of 1000 equal-size matrices (Fig. 4. 3). Savanna communities showed a random functional structure when we consider all plots because of the large variability detected; however, when considering the same number of plots in each community type, a significant overdispersed pattern emerged (Fig. 4.3). This overdispersion in plant reproductive traits from savanna plots contrasted with the clustering detected for the relative bark thickness, a key fire-associated trait among savanna woody species (see Fig. D1 in Appendix D). Forests and savannas showed significant differences in their sesMFD, with species co-occurring in forest plots having more similar reproductive traits than species in savanna plots (sesMFD between habitats for the original matrix: $P = 0.006$; for the same-size matrices: $P <$

0.001). sesMFD analyses conducted separately for the sets of floral and fruit traits showed similar results (Figs. D2 and D3 in Appendix D).

Forest and savanna communities differed in the number of species with certain floral, sexual and dispersal traits (Tables 4.1 and 4.2). Forest had significantly more species with actinomorphic (i.e. radial symmetry) flowers of easy access to pollinators (disk-bowl shaped), with white-cream colors, and very small sizes (Table 4.1). In addition, the number of species with flowers showing diurnal anthesis that offer nectar as main reward was greater in forests than in savanna plots, as was the number of hermaphrodite species with bisexual flowers (Table 4.1). We did not detect significant differences in the number of species with any of the pollination mode categories between the two habitats. Concerning plant dispersal traits, forests hold significantly more zoochorous species with fleshy, large fruits having few seeds than species in savannas (Table 4.2).

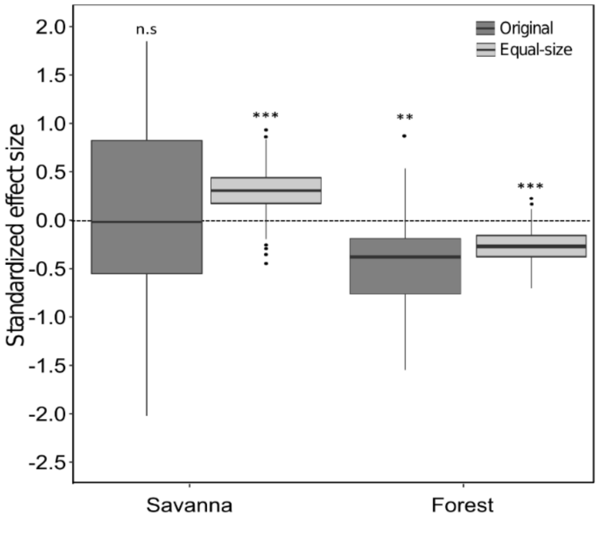

Figure 4.3. Functional structure of forest and savanna communities based on reproductive plant traits. Functional structure was measured as the standardized effect size of the mean functional distance (sesMFD). For each habitat type (i.e. forest and savannas), sesMFD was estimated for all plots (N=98 plots; 'Original'), and for the 17 forests and a

subset of 17 randomly selected savannas (N=34 plots; 'Equal size'). Boxplots show the interquartile range (IQR) with the median and the lower and upper quartiles, and whiskers extend to 1.5 times the IQR. Black dots are outliers of the sesMFD. The horizontal dashed line represents the null expectation (mean=0). Forest habitat: sesMFD original matrix Pseudo-median= -0.58, ***P* = 0.004; in equal-size matrices Pseudo-median= -0.28, ****P* < 0.001. Savanna habitat: sesMFD original matrix Pseudo-median= 0.09, *P* n.s (not significant); in equal-size matrices Pseudo-median=0.30, ****P* <0.001.

Phylogenetic structure

Phylogenetic structure in forest and savanna communities, measured as the sesMPD, showed opposite patterns to those detected for functional structure using reproductive traits. Species from forest communities were phylogenetically overdispersed, although this was only statistically significant when considering the same number of savanna and forest plots (Fig. 4.4). In contrast, species from savanna communities were phylogenetically clustered (Fig. 4.4). We detected significant differences in the phylogenetic patterns between the two community types with forests having fewer closely related species coexisting in close proximity than savannas in the equal-size matrices (sesMPD between forest and savanna communities: *P* < 0.001, Fig. 4.4).

Most of the study traits showed no significant phylogenetic signal (PS, Table D3 in Appendix D). Only floral reward, pollination mode and fruit type showed significant PS indicating that phylogenetically related species tend to offer similar floral rewards, attract similar pollinators and show the same fruit type (i.e. fleshy/non-fleshy). In addition, none of the two ordination axes summarizing trait distances among the study species showed a significant phylogenetic signal (NMDS1: K=0.08, *P* = 0.31; NMDS2: K=0.16, *P* = 0.06). In fact, the low K values (K<1) may indicate that in general, closely related species were less similar in reproductive traits than expected. Overall, there was no significant correlation between the phylogenetic and the trait distance matrices (Mantel test *P*= 0.38).

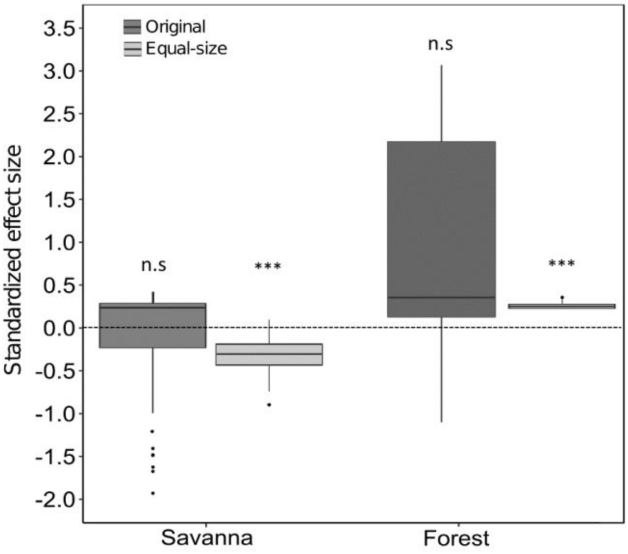

Figure 4.4. Phylogenetic structure of forest and savanna communities in the study. Phylogenetic structure was measured as the standardized effect size of the mean pairwise phylogenetic distance (sesMPD). For each community type (i.e. forest and savannas), sesMPD was estimated for all plots (N=98 plots; 'Original') and for the 17 forests and a subset of 17 randomly selected savannas (N=34 plots; 'Equal size'). Boxplots show the interquartile range (IQR) with the median and the first and third quartiles, and whiskers extend to 1.5 times the IQR. Black dots are outliers of the sesMPD. Horizontal dashed line represents the null expectation (median=0). *** P <0.001, n.s not significant. Forest habitat: sesMPD original matrix Pseudo-median= 0.73, P = n.s (not significant); in equal-size matrices: Pseudo-median=0.29, ***P < 0.001. Savanna habitat: sesMPD original matrix Pseudo-median= 0.02, P = n.s; in equal-size matrices: Pseudo-median= -0.40, ***P < 0.001.

Table 4.1. Effects of habitat type (forest vs savanna) on the number of species with each floral or sexual trait category (from negative binomial generalized linear models). Estimates are in relation to the 'forest', that is, positive values indicate predominance in forest while negative ones in savannas. Mo. & AM: monoecious with unisexual flowers and andromonoecious species.

Trait	Habitat type (Forest)		
	Estimate (SE)	*z*-value	*P*-value
Floral symmetry			
Radial	0.34 (0.13)	2.51	**0.012***
Non-radial	-0.12 (0.28)	-0.46	0.65
Floral size			
Very small	0.67 (0.16)	4.18	**<0.001*****
Small	-0.20 (0.32)	-0.63	0.53
Medium-large	-0.21 (0.26)	-0.79	0.42
Floral color			
White	0.76 (0.15)	4.90	**<0.001*****
Green	-0.27 (0.71)	-0.38	0.70
Yellow	-0.26 (0.71)	-0.37	0.70
Purple	-0.09 (0.95)	-0.10	0.92
Floral anthesis			
Diurnal	0.49 (0.13)	3.63	**<0.001*****
Nocturnal	-0.20 (0.30)	-0.66	0.50
Floral reward			
Nectar	0.62 (0.18)	3.44	**<0.001*****
Pollen	-0.24 (0.33)	-0.71	0.47
Pollen & Nectar	0.09 (0.22)	0.39	0.69
Oil	0.13 (0.50)	0.25	0.78
Floral shape			
Bell	-0.30 (0.44)	-0.67	0.50
Brush	0.07 (0.38)	0.20	0.87
Disk-bowl	0.55 (0.17)	3.24	**0.001****
Tube	0.20 (0.27)	0.74	0.46
Pollination mode			
Bee	-0.08 (0.26)	-0.29	0.76
Bird	0.13 (0.70)	0.17	0.86
Moth	-0.18 (0.32)	-0.56	0.57
Small-insects	0.42 (0.23)	1.76	0.08
Generalists	0.14 (0.29)	0.49	0.63
Others	0.28 (0.45)	0.62	0.53
Sexual system			
Hermaphrodite	0.30 (0.14)	2.07	**0.038***
Mo. & AM	0.15 (0.33)	0.45	0.64
Dioecious	-0.05 (0.31)	-0.18	0.85

Table 4.2. Effects of the habitat type (forest vs savanna) on the number of species with each fruit or dispersal trait category (from negative binomial generalized linear models). Estimates are in relation to 'forest', that is, positive values indicate predominance in forest while negative ones in savannas.

Trait	Habitat type (Forest)		
	Estimate (SE)	*Z*-value	*P*-value
Fruit size			
Small fruits	0.17 (0.18)	0.92	0.36
Large fruits	0.32 (0.15)	1.96	**0.04***
Fruit type			
Fleshy	0.51 (0.16)	3.25	**0.002****
Non-fleshy	-0.12 (0.20)	-0.60	0.54
Seeds per fruit			
Few	0.39 (0.14)	2.70	**0.007****
Medium	0.17 (0.25)	0.65	0.51
Many	-0.22 (1.04)	-0.21	0.82
Dispersal mode			
Zoochorous	0.54 (0.14)	3.79	**<0.001*****
Non-zoochorous	-0.17 (0.27)	-0.64	0.52

DISCUSSION

Forest and savannas showed contrasting (non-random) patterns of reproductive traits linked to plant-animal interactions. Specifically, savannas are more diverse in these traits (functional overdispersion) than forests (functional clustering; Fig. 4.3), and thus forests displayed only a subset of the savanna trait diversity (Fig. 4.1). This contrast with the results obtained for a fire-related trait (i.e. bark thickness; convergent in both forest and savanna; Appendix D: Fig. D1) and with the inferred predominant phylogenetic pattern for this habitat type (Fig. 4.4). Other studies have reported similar mismatches between phylogenetic and functional patterns when multiple ecological and evolutionary processes act on various traits or at different scales interfering with each other (Cavender-Bares et al. 2009; Swenson and Enquist 2009; Ding et al. 2012). This highlights the complementarity of trait- and phylogenetic- based approaches in studies on community assembly (de Bello et al. 2017). Thus, while habitat filtering by fire is the predominant factor assembling savannas-forests mosaics, other processes, such as plant-animal mutualisms, may also influence species coexistence in these complex communities.

Contrasting assembly patterns in forests and savannas

The functional clustering found in forests likely arises from biotic filtering imposed by plant pollinators and seed dispersers. Three complementary processes may have led to this clustered pattern in the case of pollinators as biotic filters in forests: i) the interplay between abiotic filtering and local pollinators, where the environment excludes floral traits other than those selected by the dominant pollinators; ii) facilitative plant-plant interactions via shared pollinators; and iii) selection of certain floral traits by locally dominant pollinators that can lead to the evolutionary convergence of coexisting species (Sargent and Ackerly 2008; Briscoe-Runquist et al. 2016; Kemp et al. 2018). Forest canopies show low light availability that can influence the presence of particular pollinators leading to the overrepresentation of certain floral patterns such as white corollas or small displays compare to open areas (Ostler et al. 1978; Kilkenny and Galloway 2008). In agreement with the first mechanism, we detected a greater number of species with small white flowers of easy access to pollinators in forest plots compared to savannas. These results suggest that shade can indirectly shape community patterns of floral traits through pollinator-mediated filtering. Our exploration of plant species with particular traits in the study plots did not detected significant differences in species pollinated by any pollinator group across habitat types. However, only field studies can accurately inform on the spatial variation of dominant pollinators in forest-savanna mosaics (Oliveira and Gibbs 2002; Maruyama et al. 2014; Gottsberger and Silberbauer-Gottsberger 2018). In addition, we cannot rule out the possibility that other biotic filters conformed by non-pollinating floral visitors such as florivores might also contribute in the functional patterns reported. However, as in other ecosystems our knowledge on florivory in the Cerrado is still very limited (Torezan-Silingardi 2007; Del-Claro and Torezan-Silingardi 2009).

In a similar way as with pollinators, seed-dispersers might also promote functional convergence of fruit traits in forests if, for example, certain animal groups with particular preferences predominate in this habitat type. For instance, seed-dispersers together with other biotic filters (i.e. herbivores and seed-predators) favored fleshy-fruit shrubs in a mosaic landscape from a Mediterranean mountain promoting functional similarity (Zamora and Matías 2014). Forest showed a significantly higher number of zoocorous plant species compared to savanna. In forests, high soil water content in shaded conditions may preferentially promote the presence of fleshy-fruited, zoochorous species with greater water demands (Riberito and Tabarelli 2002; Chazdon et al. 2003), contrasting with the higher isolation levels and drier soils in savannas. Moreover, the vertical habitat heterogeneity may simultaneously provide more diverse microhabitat for a higher diversity of potentially disperser species, as observed in birds (Macedo et al. 2002), and work as a barrier for wind, favoring zoochory as long distance dispersal mechanism. Further studies at field should assess whether this contrasting habitat conditions are impeding the movement of certain seed-disperal vectors across forest and savanna.

Despite the functional convergence in plant reproductive traits and in bark thickness in forests, we found an overdispersed phylogenetic pattern. Accordingly, other tropical forest showed patterns of phylogenetic overdispersion at fine scales (Swenson et al. 2007). Ding et al. (2012) also reported clustered patterns of functional traits and phylogenetic evenness in lightly disturbed forests (i.e. old growth). In that case, the resulting phylogenetic structure suggested a more important role of biotic interactions favoring the differentiation of phylogenetically conserved traits than of habitat filtering at fine scale in old growth forests (Ding. et al. 2012). In our study, most of the plant traits did not showed significant phylogenetic signal and, indeed, the signal detected for the whole set of traits suggested that close relatives were less similar than expected (i.e. K statistic close to 0). Both abiotic and biotic filtering (e.g. pollinators) may have contributed to the phylogenetic overdispersion detected in forest plots in our study scale, as long as the process select acting on distantly related species that share similar traits (adaptive convergence). In contrast, traits such as tree height, specific leaf area, wood density and leaf nutrient contents are phylogenetically conserved in both Neotropical forest (Kraft and Ackerly 2010) and savanna (Silva and Batalha 2010), and leaf carbon-to-nitrogen and tricome density are conserved in closed canopy woodlands composed of forest and savanna species (Loiola et al. 2012). These traits are often related to resource strategies and may be involved in resource competition and herbivory resistance (Dantas and Batalha 2012); both processes can lead to functional overdispersion and increase from savanna to forest (Neves et al. 2010; Dantas et al. 2013a).

In contrast to forests, savanna communities were functionally overdispersed when considering plant reproductive traits. In this case, competitive interactions among plants mediated by mutualistic animal vectors may act promoting the observed diversity of floral and fruit traits (Sargent and Ackerly 2008; Muchhala et al. 2014). Frequent fire may reduce arthropod abundance during certain periods in savannas (Vasconcelos et al. 2009, Neves et al. 2010). If this includes pollinators, fitness costs of sharing the same pollination resources in savanna could be high, limiting the scope for trait similarity. The overdispersed functional pattern contrasted with the clustering found for relative bark thickness, a trait related to fire-resistance. The latter, together with the emerging phylogenetic clustering suggests a predominant role of habitat filtering in the assembly of savannas at this scale, likely dominated by strong fire effects. This was shown by Dantas et al. (2013a) and supports previous evidence that recurrent fires filter a subset of close relatives from the regional species pool in fire-prone ecosystems (Verdú and Pausas 2007). Different vegetative traits also show strong clustering at individual and species level in other cerrado savannas (Laureto and Cianciaruso 2015) supporting the view of habitat filtering as the prevailing assembly force in this environment. This is consistent with a recent study suggesting that habitat filtering is the dominant process structuring community assembly at the global scale (Li et al. 2018).

Multiple processes acting on different traits

Our study illustrates how different forces may contribute to the assembly of forest-savanna mosaics by acting on traits linked to multiple ecosystem functions. Previous literature on community assembly has also shown how different processes can act on various functional traits (Swenson and Enquist 2009; Bernard-Verdier et al. 2012; Spasojevic and Suding 2012). However, most of these studies have focused on vegetative traits while much less have compared vegetative and reproductive trait patterns (e.g. Briscoe-Runquist et al. 2016; Junker and Larue-Konti'c 2018). The incorporation of floral and fruit traits to this field is importante because of their link to plant reproduction and different ecosystem functions, to species establishment and long term persistence, and to the diversity of pollinators and seed dispersers. Moreover, we show that the patterns detected in reproductive traits are not a by-product of filtering on fire-related traits (e.g. bark thickness) and may arise from additional assembly processes.

Our work adds to earlier studies highlighting the functional dichotomy of forest and savannas from these mosaic landscapes (Hoffmann et al. 2012; Dantas et al. 2013a, 2013b; Laureto and Cianciaruso 2015; Sfair et al. 2016; Maracahipes et al. 2018). It is also noteworthy that here we focused on woody species and only considered their presence in these mosaics. There is increased evidence of the role of fire triggering plant flowering in different fire-prone ecosystems, frequently associated with annual plants and geophytes recruiting from the seed-bank or resprouting from belowground organs (Lamont and Downes 2011; Conceição 2018; Pausas et al. 2018; Pilon et al. 2018). Studies including vegetation at the ground-layer in these forest-savanna mosaics that also take into account species abundance are needed. As previously mentioned our results rely on data from the literature and species phylogeny and are discussed in the light of previous studies on the role of plant-animal mutualisms shaping trait patterns. To our knowledge, there is no information at the community level in the study area on pollination and seed-dispersal interactions at field. Thus, our work calls for field studies that can provide relevant information on how pollinators and seed dispersers in addition to abiotic factors contribute to the spatial arrangement and the persistence of plant communities in these complex landscapes.

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GENERAL DISCUSSION

In this thesis I have shown that wildfires impact on plant-animal interactions through different fire-related factors and that the ultimate effects on plant performance may be influenced by the ability of these interactions to reestablish after the fire (*Chapters I* and *II*). The degree of specialization of the interacting organisms may also constrain the postfire recovery (*Chapter I*) but even in highly specialized mutualistic interactions, different postfire responses by a few interacting species (e.g. pollinators) can provide plant reproductive resilience (*Chapter II*). Fires may also alter the chemical signals that allow communication between plants and their pollinators, although in specialized pollination systems the high specificity between the signal and pollinators might remain invariant under disturbance (*Chapter III*). Because of the small number of species included and pairwise interactions assessed, further work involving different sets of species, and studies at the community level may provide valuable information on whether these findings are common in other systems and if they emerge at broader scales. Lastly, this thesis suggests that besides the role of fire as an important abiotic filter, other ecological forces such as plant-animal mutualisms may participate in the assembly of plant communities from tropical forest-savanna mosaics (*Chapter IV*).

In fire-prone shrublands from the Mediterranean basin, where many plants display fast responses to fires (e.g. recruitment from seed-bank, resprouting from belowground organs, fire-induced flowering), they may also obtain beneficial effects through the postfire disruption of antagonistic interactions (García et al. 2016). Particularly, plants may experience lower pressures from specialist seed predators who compared to generalists depend not only on the presence of their host plants at the burnt, but on certain plant life stages (e.g. adult plants with green fruits to oviposit and develop, García et al. 2016). These particular requirements may increase the time needed by specialists to reach the burnt and in turn to interact again with their plant hosts. These results are consistent with previous evidence of indirect effects of disturbances on plant performance through changes in their antagonistic interactions (Vickery et al. 2002; Knight and Holt 2005; Elderd 2006; Elderd and Doak 2006), which in some instances can result in positive outcomes for plant reproduction (Knight and Holt 2005; García et al. 2016). Ultimately, this temporal advantage together with the nutrient flush or light gaps opened by fires might have implications on plant population dynamics (i.e. abundance and distribution patterns), which advocate for future research covering multiple burnt sites and longer postfire periods. It is also noteworthy that we have only focused on herbivory and pre-dispersal seed predation while fires can also modify post-dispersal predation (e.g. Ordóñez and Retana 2004; Broncano et al. 2008; Puerta-Piñero et al. 2010). The overall postfire variability in different interactions may influence the ultimate effects on plant reproduction. Therefore, integrative studies exploring how wildfires shape the balance among multiple plant-animal interactions are needed.

Our results also provide insights on the role of fires creating spatio-temporal patterns in the strength of pre-dispersal predation interactions (García et al. 2016). Pre-dispersal seed predators can impose selective pressures on disparate plant traits such as flowering phenology, floral, fruit and seed size, among others (Gómez and Zamora 1994; Mezquida and Benkman 2004; see Kolb et al. 2007 for a review; Paynter et al. 2016). These traits are often also under pressures by pollinators, nectar robbers or herbivores that can generate patterns of opposing selection when they exert pressures in different directions (Strauss and Whitall 2006; Gómez 2008; Perez-Barrales et al. 2013; Sun et al. 2016). Moreover, fires can also act as selective agents on seed and fruit traits (e.g. Escudero et al. 2000; Castellanos et al. 2015). All this calls for further research exploring fire landscapes with varying fire histories (e.g. different postfire ages and fire frequencies) in the context of conflicting selection pressures (e.g. Benkman and Siepielski 2004) and mosaics of phenotypic selection (Thompson 2005).

I have reported some examples of the mechanisms providing resilience in specialized pollination systems from fire-prone environments (García et al. 2018; *Chapter III*). The postfire pollinator replacement found in *Chamaerops humilis* pollination is consistent with earlier evidence on plants with generalized pollination systems, where different pollinator responses can counterbalance the negative effects on most vulnerable pollinator species (Potts et al. 2001; Campbell et al. 2007; van Nuland et al. 2013; Lybbert et al. 2018). We detected that even one pollinator species showing a quick postfire response can ensure plant pollination in a highly specialized system (García et al. 2018). This also points out the importance of additional pollinators in nursery pollination systems (Thompson and Pellmyr 1992; Thompson and Cunningham 2002; Kephart et al. 2006; Hossaert-McKey et al. 2010), especially in environments under frequent disturbances (García et al. 2018; *Chapter III*). Whether a fast pollinator recovery is common in other specialized pollination interactions from fire-prone environments needs further attention. In addition, because fire modifies vegetation structure and plant species densities, it would be interesting to test for postfire changes in patterns of pollen deposition (i.e. inter- and intraspecific pollen) that may alter plant reproductive success (Ashman and Arceo-Gómez 2013).

In *C. humilis* system, despite the lack of differences in fruit set levels among most of the burnt and unburnt sites, we still detected a significant decline in one of the specialist pollinators three years after the fires (García et al. 2018). Previous studies have also found slow recovery rates in specialist pollinators depending on particular subsets of plant species or those with marked nesting preferences (e.g. Lazarina et al. 2016, Peralta et al. 2017). At the community scale, the impact of wildfires on the structure of a plant-visitor nework can result in low modularity and high generalization levels at early postfire ages (Peralta et al. 2017). Such shifts in network properties may be influenced by increasing abundances of generalist bee species and the decline of specialists at recently burnt sites (Peralta et al. 2017). A modular structure with subsets of preferentially interacting species, can provide network stability to further disturbance delaying the spread of perturbation effects across the entire community (Olesen et al. 2007; Stouffer and Bascompte 2011; Grilli et al. 2016). Under a future scenario of larger fire sizes and higher fire frequencies in different regions (Lavorel et al. 2007; Pausas and Paula 2012), these changes might lead to more vulnerable plant-pollinator communities (Peralta et al. 2017). Network studies on different disturbances have reported reduced levels in network nestedness that may be also associated with less robust and resilient communities (e.g. Vanbergen et al. 2014; Revilla et al. 2015; Traveset et al. 2018). On the contrary, generalized and modular networks may arise when moderate disturbance pressures enhance the number of interacting species increasing network size and modularity (e.g. Lázaro et al. 2016). Moreover, the postfire changes on network structure might be exacerbated through the interactive effects of fire and other disturbances such as drought or invasive species (Brooks et al. 2004; Koerner and Collins 2014; Pyšek et al. 2012; Guthrie et al. 2016). Studies exploring how these interacting networks respond to different fire-regime characteristics (i.e. intensity, age, frequency, severity) as well as to heterogeneous fire landscapes (e.g. Ponisio et al. 2016) might thus provide valuable information on the assembly/disassembly dynamics of biotic communities and on their robustness to disturbance. From an evolutionary approach, Nuismer et al. (2018) have recently proposed the study of mutualistic communities with different fire histories to address the potential role of the coevolutionary history among interacting species in delaying the effects of disturbance.

Another way in which wildfires may alter pollination interactions is through shifts in plant scents involved in pollinator attraction (*Chapter III*). A small number of studies have found signs of the impacts of different anthropogenic-driven disturbances (e.g. ozone, increased drought and temperatures) on floral scent emissions (Farré-Armengol et al., 2013, 2014, 2016; Burkle and Runyon, 2016, 2017; Jürgens and Bischoff 2017). Such effects may modify scent attractiveness and, ultimately, pollinator attraction although evidence is still scarce (e.g. Farré-Armengol et al. 2016; Dötterl et al. 2016). Here, we reported only slightly changes on scent composition and a strong specificity between the palm's signal and pollinators that provide resilience to disturbance (*Chapter III*). Our results are in agreement with the fine-tuned olfactory systems detected in other specialist pollinator species including nursery pollinators (e.g. Hossaert-McKey et al. 2010; Svensson et al. 2010). A recent study has proposed that pollinators from fluctuating environments where changes in chemical emissions are common may be adapted to such dynamic odour landscapes (Jürgens and Bischoff 2017). This idea comes from evidence on pollinator-mediated selection on floral bouquets (Schiestl 2010, 2015), the habituation to olfactory background noise found in several insect species (Schröder and Hilker 2008; Wilson et al. 2015; Jürgens and Bischoff 2017) and the importance of odour learning in floral visitors (Wright and Schiestl 2009; Lawson et al. 2018). In this sense, it would be interesting to explore whether fire-prone ecosystems can act as dynamic odourscapes where pollinators respond to certain "scent regimes" (*Chapter III*). In addition, because of the particularities of *C. humilis* system, where the plant's leaves instead of flowers emit the scent that attracts the pollinators (Dufaÿ et al. 2003), whether our findings can be generalized to other pollination interactions medited by floral signals remain to be studied. However, postfire-induced changes on floral scents are expected, since fires modify soil bacterial communities, temperature, water content, and herbivory pressures that indeed can change floral emissions (Kessler et al. 2011; Farré-Armengol et al. 2014; Burkle and Runyon 2016; Helletsgruber et al. 2017). Moreover, as floral scents are involved in defense against non-beneficial visitors (e.g. nectar robbers, florivores and herbivores, Galen et al. 2011; Schiestl et al. 2011; Jürgens and Bischoff 2017), postfire changes on floral fragances might also alter these plant-animal relationships. In this sense, our findings advocate the incorporation of olfactory cues together with other traits under selection by pollinators (e.g. morphological and visual floral traits) to better understanding of plant-pollinator communities in changing environments and their ability to face disturbance (Junker and Parachnowitsch 2015; Schiestl 2015; Filella et al. 2013; Jürgens and Bischoff 2017; Opedal 2018). This integrative perspective is also reinforced by a recent study by Kantsa et al. (2017) showing the presence of integrated floral patterns in terms of color and scent as perceived by bee pollinators in plant communities from Mediterranean scrublands.

Finally, this thesis suggests that multiple ecological processes including fire and plant-animal mutualisms may drive species coexistence in forest-savanna mosaics by acting on different plant traits (i.e. fire-related vegetative traits and reproductive traits). This is consistent with previous research showing how multiple assembly forces act through different spatial scales, plant traits or levels of disturbance (Swenson and Enquist 2009; Bernard-Verdier et al. 2012; Ding et al. 2012; Spasojevic and Suding 2012). Furthermore, our work adds to the body of knowledge on the functional distinction of forest and savanna formations from these patchy ecosystems (Hoffmann et al. 2003; 2012; Dantas et al. 2013, 2016; Laureto and Cianciaruso 2015; Maracahipes et al. 2018). Particularly, we showed that plant communities from savannas exhibit higher functional diversity levels contrasting with forest communities where patterns of functional similarity emerged (*Chapter IV*). Field studies assessing for differences in plant-pollinator and seed-dispersal interactions in forests and savannas are needed to unveil the specific mechanisms (e.g. biotic filtering, plant-plant competition/facilitation mediated by pollinators and seed-dispersers; Sargent and Ackerly 2008; Pellissier et al. 2012) resulting in the non-random patterns observed. Likewise, as we only focused on woody species, further research should try to extend our study to the herbaceous layer that might be particularly relevant because of its link to frequent fires in savanna communities (i.e. herbs and geophytes with fire-induced flowering, Conceição 2018; Pilon et al. 2018). Lastly, this chapter highlights the importance of combining both trait- and phylogenetic-based approaches in order to understand the different forces shaping species co-occurrence patterns (Cavender-Bares et al. 2009; Pausas and Verdú 2010; Kraft and Ackerly 2010; de Bello et al. 2017).

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CONCLUSIONS

1. Wildfires can disrupt antagonistic plant-animal interactions resulting in beneficial effects on plants with fast postfire responses, until interaction recovers. These postfire effects may be stronger on interactions with specialists that need more time to establish inside the burnt.

2. Different pollinator responses after fires can provide plant reproductive resilience even in highly specialized pollination systems from fire-prone ecosystems.

3. Wildfires can create spatio-temporal patterns of variation in plant-animal interactions.

4. Wildfires can modify plant chemical signals mediating in pollination interactions. In specialized pollination systems, the limited changes in scent composition and the high specificity between the signal and pollinators may provide resilience under disturbance.

5. Besides the abiotic filter imposed by fire, plant-animal mutualisms such as pollination and seed-dispersal interactions may drive species-coexistence in forest-savanna mosaics by acting on reproductive plant traits.

6. The functional distinction of forest and savanna formations in Cerrado mosaics is reinforced by reproductive plant traits, with savannas showing higher diversity levels (i.e. functional overdispersion) than forests where functional similarity is promoted (i.e. functional clustering).

APPENDIX A

Fires can benefit plants by disrupting antagonistic interactions

Table A1. Number of *Ulex parviflorus* pods predated by the specialist *Exapion fasciolatum*, plus the total pods collected at each plot, plus the number of *E. fasciolatum* individuals per plot and the distance class (“Unburned”, “Edge” and “Center). Predation and number of weevils were estimated from 400 pods at each plot in two fire locations (Segorbe and Cortes) the two years of sampling (2014 and 2015). In 2014, predation at “Cortes” unburned plots was estimated from 300 pods.

Location	Plot	Year	In/out	Distance class	Predated pods	Total pods	*Exapion fasciolatum*
Cortes	FA	2014	Unburned	Unburned	73	300	73
Cortes	FB	2014	Unburned	Unburned	102	300	108
Cortes	FC	2014	Unburned	Unburned	176	300	177
Cortes	FD	2014	Unburned	Unburned	38	300	43
Cortes	FE	2014	Unburned	Unburned	21	300	21
Cortes	FF	2014	Unburned	Unburned	31	300	31
Cortes	FG	2014	Unburned	Unburned	182	300	187
Cortes	FH	2014	Unburned	Unburned	116	300	117
Cortes	DCA	2014	Burned	Edge	69	400	70
Cortes	DCB	2014	Burned	Center	16	400	16
Cortes	DCC	2014	Burned	Center	0	400	0
Cortes	DCD	2014	Burned	Edge	27	400	27
Cortes	DCE	2014	Burned	Edge	8	400	8
Cortes	DCF	2014	Burned	Center	7	400	7
Cortes	DCG	2014	Burned	Center	0	400	0
Cortes	DCH	2014	Burned	Edge	13	400	4
Cortes	DCI	2014	Burned	Edge	6	400	6
Cortes	DCJ	2014	Burned	Edge	41	400	41
Cortes	DCK	2014	Burned	Edge	38	400	38
Cortes	DCL	2014	Burned	Edge	1	400	1
Cortes	DCN	2014	Burned	Center	8	400	8
Cortes	DCO	2014	Burned	Center	0	400	0
Cortes	DCP	2014	Burned	Edge	21	400	20
Cortes	DCQ	2014	Burned	Edge	30	400	13
Cortes	DCR	2014	Burned	Center	6	400	6
Cortes	DCS	2014	Burned	Center	17	400	18
Cortes	DCT	2014	Burned	Center	23	400	19
Cortes	DCU	2014	Burned	Center	7	400	5
Cortes	DCV	2014	Burned	Edge	21	400	5
Cortes	DCW	2014	Burned	Edge	3	400	2
Andilla	FAA	2014	Unburned	Unburned	92	400	41
Andilla	FAS	2014	Unburned	Unburned	22	400	16
Andilla	FAC	2014	Unburned	Unburned	13	400	10
Andilla	FAN	2014	Unburned	Unburned	99	400	48

Andilla	FAT	2014	Unburned	Unburned	36	400	17
Andilla	DAA	2014	Burned	Edge	109	400	49
Andilla	DAB	2014	Burned	Edge	30	400	14
Andilla	DAC	2014	Burned	Center	39	400	28
Andilla	DAD	2014	Burned	Center	62	400	16
Andilla	DAE	2014	Burned	Center	12	400	5
Andilla	DAF	2014	Burned	Center	4	400	1
Andilla	DAG	2014	Burned	Center	14	400	16
Andilla	DAH	2014	Burned	Center	2	400	1
Andilla	DAI	2014	Burned	Center	11	400	7
Andilla	DAJ	2014	Burned	Edge	45	400	25
Andilla	DAK	2014	Burned	Center	9	400	4
Andilla	DAL	2014	Burned	Center	6	400	5
Andilla	DAM	2014	Burned	Center	28	400	14
Cortes	FCA	2015	Unburned	Unburned	119	400	81
Cortes	FCB	2015	Unburned	Unburned	104	400	91
Cortes	FCC	2015	Unburned	Unburned	139	400	80
Cortes	FCD	2015	Unburned	Unburned	139	360	113
Cortes	FCE	2015	Unburned	Unburned	41	400	39
Cortes	FCF	2015	Unburned	Unburned	19	160	3
Cortes	FCG	2015	Unburned	Unburned	124	400	42
Cortes	FCH	2015	Unburned	Unburned	16	400	4
Cortes	FCI	2015	Unburned	Unburned	92	400	96
Cortes	DCA	2015	Burned	Edge	5	400	6
Cortes	DCB	2015	Burned	Center	4	400	4
Cortes	DCC	2015	Burned	Center	0	400	0
Cortes	DCD	2015	Burned	Edge	7	400	8
Cortes	DCE	2015	Burned	Edge	2	400	1
Cortes	DCF	2015	Burned	Center	8	400	7
Cortes	DCG	2015	Burned	Center	6	400	6
Cortes	DCH	2015	Burned	Edge	0	400	0
Cortes	DCI	2015	Burned	Edge	6	400	6
Cortes	DCJ	2015	Burned	Edge	55	400	51
Cortes	DCK	2015	Burned	Edge	16	360	18
Cortes	DCL	2015	Burned	Edge	1	400	1
Cortes	DCN	2015	Burned	Center	0	400	0
Cortes	DCO	2015	Burned	Center	5	400	5
Cortes	DCP	2015	Burned	Edge	0	400	0
Cortes	DCR	2015	Burned	Center	23	400	17
Cortes	DCS	2015	Burned	Center	0	400	0
Cortes	DCT	2015	Burned	Center	0	400	0
Cortes	DCU	2015	Burned	Center	0	400	0
Cortes	DCX	2015	Burned	Edge	7	400	3
Cortes	DCY	2015	Burned	Edge	0	400	0
Andilla	FAA	2015	Unburned	Unburned	4	400	5
Andilla	FAS	2015	Unburned	Unburned	14	400	7
Andilla	FAC	2015	Unburned	Unburned	2	400	2
Andilla	FAN	2015	Unburned	Unburned	10	400	3
Andilla	FAT	2015	Unburned	Unburned	2	400	1
Andilla	DAA	2015	Burned	Edge	15	400	7
Andilla	DAB	2015	Burned	Edge	8	400	3
Andilla	DAC	2015	Burned	Center	1	400	1

Andilla	DAD	2015	Burned	Center	9	400	12
Andilla	DAE	2015	Burned	Center	1	400	1
Andilla	DAF	2015	Burned	Center	2	400	2
Andilla	DAG	2015	Burned	Center	1	400	1
Andilla	DAH	2015	Burned	Center	1	400	2
Andilla	DAI	2015	Burned	Center	0	400	0
Andilla	DAJ	2015	Burned	Edge	0	400	0
Andilla	DAK	2015	Burned	Center	0	400	0
Andilla	DAL	2015	Burned	Center	1	400	3
Andilla	DAM	2015	Burned	Center	7	400	8

Table A2. Total number of *Horistus orientalis* bugs, generalists and total herbivores recorded on *Asphodelus ramosus*, mean fruit set and total seeds produced at each plot. The distance class ("Unburned", "Edge" and "Center") is also included. The number of individuals was estimated in 50 *A. ramosus* plants per plot in two fire locations (Segorbe and Cortes) during the two years of sampling (2014 and 2015).

Location	Plot	Year	In/out	Distance class	Specialist	Generalists	Total	Fruit set	Seeds
Segorbe	Edge1	2014	Burned	Edge	0	37	37	0.02	215
Segorbe	Central1	2014	Burned	Edge	2	36	38	0.03	262
Segorbe	Central2	2014	Burned	Edge	0	14	14	0.03	364
Segorbe	Edge2	2014	Burned	Edge	1	9	10	0.04	359
Segorbe	Ranas	2014	Unburned	Unburned	104	21	125	0.01	50
Segorbe	Coronel	2014	Unburned	Unburned	80	9	89	0.003	18
Segorbe	Mirador	2014	Unburned	Unburned	19	27	46	0.002	9
Segorbe	Porta	2014	Unburned	Unburned	5	26	31	0.002	11
Cortes	Canyes1	2014	Burned	Center	0	26	26	0.02	170
Cortes	Canyes2	2014	Burned	Center	0	12	12	0.12	1747
Cortes	Fulfa1	2014	Burned	Edge	2	15	17	0.02	2
Cortes	Fulfa2	2014	Burned	Center	10	37	47	0.001	136
Cortes	Cazador	2014	Burned	Center	0	27	27	0.002	20
Cortes	Muela	2014	Unburned	Unburned	95	0	95	0.03	261
Cortes	Chiva	2014	Unburned	Unburned	4	3	7	0.02	123
Segorbe	Central1	2015	Burned	Edge	1	17	18	0.13	1405
Segorbe	Central2	2015	Burned	Edge	11	8	19	0.15	1821
Segorbe	Edge2	2015	Burned	Edge	45	10	55	0.12	1279
Segorbe	Coronel	2015	Unburned	Unburned	154	0	154	0.10	1037
Segorbe	Mirador	2015	Unburned	Unburned	8	30	38	0.03	315
Segorbe	Porta	2015	Unburned	Unburned	78	0	78	0.05	593
Cortes	Canyes1	2015	Burned	Center	0	4	4	0.07	1370
Cortes	Canyes2	2015	Burned	Center	0	2	2	0.06	1085
Cortes	Fulfa1	2015	Burned	Edge	3	0	3	0.05	733
Cortes	Fulfa2	2015	Burned	Center	91	2	92	0.01	106
Cortes	Cazador	2015	Burned	Center	0	1	1	0.02	223
Cortes	Muela	2015	Unburned	Unburned	207	43	250	0.02	208
Cortes	Calica1	2015	Unburned	Unburned	10	28	38	0.14	2197
Cortes	Calica2	2015	Unburned	Unburned	284	2	286	0.17	3024

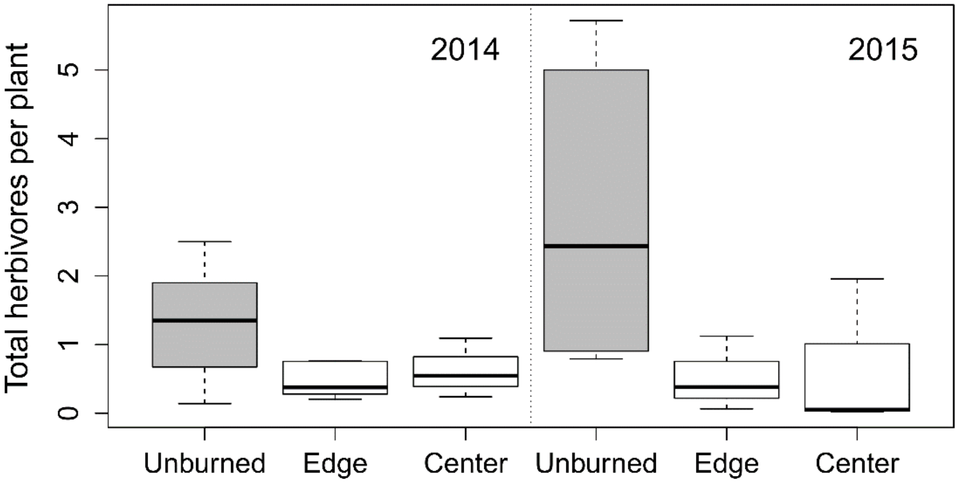

Figure A1. Number of total herbivores on *Asphodelus ramosus* plants from unburned (grey box) and burned plots (white boxes in categories "Edge" and "Center") in two years. The number of individuals was estimated in 50 *A. ramosus* plants per plot (N= 6 unburned and N= 4 "Center" plots in the two studied years. N= 5 and 4 "Edge" plots in 2014 and 2015).

APPENDIX B

Differential pollinator response underlies plant reproductive resilience after fires

Table B1. Post-hoc pairwise comparisons of regression coefficients among pollinator exclusion treatments (the GLM also included the number of palm stems as a covariate) on *Chamaerops humilis* fruit set (response variable).

Pairwise comparison	Estimate ± SE	*z* value	*P* value
wind pollination – pollination exclusion	0.54 ± 1.12	0.48	0.95
wind and small-insect pollination – pollination exclusion	3.48 ± 0.94	3.70	**0.001**
open control – pollination exclusion	4.29 ± 0.94	4.58	**<0.001**
wind and small-insect pollination – wind pollination	2.94 ± 0.65	4.50	**<0.001**
open control – wind pollination	3.75 ± 0.65	5.80	**<0.001**
open control – wind and small-insect pollination	0.81 ± 0.22	3.62	**0.002**

N=12 plants, SE: standard error. Significant *P* values are in bold.

Table B2. Detailed results of the GLM and GLMM models of the effects of fire (Unburnt vs Burnt) and distance to the fire edge on the number of *Derelomus chamaeropis,* the presence of *Meligethinus pallidulus* and *Chamaerops humilis* fruit set in 2016. For *D. chamaeropis* the models test for differences in the number of weevils. For *M. pallidulus* the models test for differences in its presence n *C. humilis* plants. For *C. humilis* fruit set the model test for differences in the drupes produced in relation to the potential drupes per plant.

Response	**Model**	**Predictors**	**N**	**Estimate**	**±SE**	***t/z***	***P***
Fruit set	Fire	Intercept (Burnt/Dénia)	338	-2.05	0.13	-15.2	***
		U. vs B.	338	_	_	_	ns
		Tivissa vs Dénia	338	_	_	_	ns
		No. inflorescences	338	_	_	_	ns
		Interaction U. x site vs B x Site	338				*
		Plant (random) 1.62 ± 1.26					
Fruit set	Distance	Intercept (Dénia)	172	-2.02	0.11	-18.3	***

		Distance	172	_	_	_	ns
		Tivissa vs Dénia	172	_	_	_	ns
		No. Inflorescences	172	_	_	_	ns
		Plant (random) 1.32 ± 0.95					
D. chamaeropis	Fire	Intercept (Burnt/Dénia/Female plants)	744	-2.21	0.19	-11.4	***
		U. vs B.	744	0.57	0.18	3.2	**
		Tivissa vs Dénia	744	-0.78	0.24	-3.2	**
		No. inflorescences	744	0.17	0.04	4.3	***
		Male vs female plants	744	1.94	0.16	11.5	***
		Interaction U. x site vs B x Site	744				*
D. chamaeropis	Distance	Intercept (Dénia/ Female plants)	354	-2.40	0.32	-7.3	***
		Distance	354	_	_	_	ns
		Tivissa vs Dénia	354	-0.79	0.25	-3.1	**
		No. Inflorescences	354	0.17	0.07	2.5	*
		Male vs female plants	354	2.23	0.35	6.3	***
M. pallidulus	Fire	Intercept (Burnt/Dénia/Female plants)	744	-2.64	0.31	-8.5	***
		U. vs B.	744	_	_	_	ns
		Tivissa vs Dénia	744	0.90	0.26	3.5	***
		No. inflorescences	744	_	_	_	ns
		Male vs female plants	744	2.30	0.21	10.7	***
		Interaction U. x site vs B x Site	354	_	_	_	ns
M pallidulus	Distance	Intercept (Dénia/ Female plants)	354	-2.02	0.46	-4.3	***
		Distance	354	_	_	_	ns
		Tivissa vs Dénia	354	0.82	0.25	3.2	**
		No. Inflorescences	354	_	_	_	ns
		Male vs female plants	354	2.063	0.28	7.3	***

Models of fire and distance effects on fruit set include the plant as random factor (GLMM) for which the variance and standard error (SE) are shown. Full models for fire effects included the two way interaction between fire treatment (U. vs B. = Unburnt vs Burnt) and study site. Both, fire and distance models also included the number of inflorescences, fire site and plant sex (only for insect models) as predictor variables. For model intercepts, the table shows the reference level of each categorical predictor. The table shows the sample sizes (N), the estimated parameter of fixed effects and the standard error (Estimate ± SE). *z/t* represent the z-test statistic of the GLMM (fruit set) and the Student's t-test of the GLM models (*D. chamaeropis* and *M. pallidulus*). Interactions are indicated with "x" between predictor variables. *P* value; *ns*: no significant, * *P* <0.05, ** *P* <0.01, *** *P* <0.001.

Table B3. Detailed results of the GLM and GLMM models of the effects of fire (Unburnt vs Burnt) and distance to the fire edge on the number of *Derelomus chamaeropis,* the number of *Meligethinus pallidus* and *Chamaerops humilis* fruit set in 2017. For *D. chamaeropis* and *Meligethinus* pallidulus the model test for differences in the number of individuals. For *C. humilis* fruit set the model test for differences in the drupes produced in relation to the potential drupes per plant.

Response	**Model**	**Predictors**	**N**	**Estimate**	**± SE**	***t /z***	***P***
Fruit set	Fire	Intercept (Burnt/ Dénia)	394	-1.90	0.18	-10	***
		U. vs B.	394	_	_	_	ns
		Tivissa vs Dénia	394	-1.38	0.27	-5.0	***
		Carcaixent vs Dénia	394	_	_	_	ns
		Xàbia vs Dénia	394	-1.47	0.26	-5.6	***
		No. inflorescences	394	_	_	_	ns
		Interaction U. x site vs. B x site	394				*
		Plant (random) 1.44 ± 1.12					
Fruit set	Distance	Intercept (Dénia)	199	-1.99	0.21	-9.5	***
		Distance	199	_	_	_	ns
		Tivissa vs Dénia	199	-1.34	0.32	-4.2	***
		Carcaixent vs Dénia	199	0.70	0.30	2.3	*
		Xàbia vs Dénia	199	-1.48	0.29	-4.9	***
		No. Inflorescences	199	_	_	_	ns
		Plant (random) 1.77 ± 1.32					
D.chamaeropis	Fire	Intercept (Burnt/ Dénia/ Female plants)	796	0.29	0.13	2.3	*
		U. vs B.	796	_	_	_	ns
		Tivissa vs Dénia	796	_	_	_	ns

		Carcaixent vs Dénia	796	-2.37	0.24	-9.7	***
		Xàbia vs Dénia	796	-2.93	0.29	-10	***
		No. inflorescences	796	0.04	0.02	2.3	*
		Male vs female plants	796	1.51	0.09	15.3	***
		Interaction U. x site vs B. x site	796				*
D.chamaeropis	Distance	Intercept (Dénia/ Female plants)	401	0.44	0.13	3.4	***
		Distance	401	-0.01	0.001	-6.2	***
		Tivissa vs Dénia	401	_	_	_	ns
		Carcaixent vs Dénia	401	-2.54	0.23	-11	***
		Xàbia vs Dénia	401	-2.85	0.27	-10	***
		No. Inflorescences	401	_	_	_	ns
		Male vs female plants	401	1.13	0.14	8.3	***
M. pallidulus	Fire	Intercept (Burnt/ Dénia/ Female plants)	796	0.44	0.16	2.7	**
		U. vs B.	796	_	_	_	ns
		Tivissa vs Dénia	796	0.74	0.20	3.4	***
		Carcaixent vs Dénia	796	_	_	_	ns
		Xàbia vs Dénia	796	_	_	_	ns
		No. inflorescences	796	0.07	0.03	2.6	**
		Male vs female plants	796	1.64	0.11	14.8	***
		Interaction U. x site vs B. x site	796	_	_	_	n.s
M. pallidulus	Distance	Intercept (Dénia/ Female plants)	401	0.54	0.19	2.7	**
		Distance	401	_	_	_	ns
		Tivissa vs Dénia	401	0.77	0.26	2.9	**
		Carcaixent vs Dénia	401	_	_	_	ns
		Xàbia vs Dénia	401	_	_	_	ns
		No. Inflorescences	401	_	_	_	ns
		Male vs female plants	401	1.35	0.18	7.6	***

Models of fire and distance effects on fruit set include the plant as random factor (GLMM) for which the variance and standard error are shown. Full models for fire effects included the two way interaction between fire treatment (U. vs B. = Unburnt vs Burnt) and study site. Both, fire and distance models also included the number of inflorescences, fire site and plant sex (only for insect models) as predictor variables. For model intercepts, the table shows the reference level of each categorical predictor. The table shows the sample sizes

(N), the estimated parameter of fixed effects and the standard error (Estimate ± SE). *z/t* represent the z-test statistic of the GLMM (fruit set) and the Student's t-test of the GLM models (*D. chamaeropis* and *M. pallidus*). Interactions are indicated with "x" between predictor variables. *P* value; *ns*: no significant, * *P* <0.05, ** *P* <0.01, *** *P* <0.001.

Table B4. Post-hoc pairwise comparisons of regression coefficients of the interaction term between fire treatment (Unburnt vs Burnt) and study site from the models of fire effects on a) *Chamaerops humilis* fruit set and b) *Derelomus chamaeropis* abundance. SE: standard error, N: number of *Chamaerops humilis* plants. Signifcant values are in bold.

Sampling year	**Response variable**	**Pairwise comparison**	**N**	**Estimate ± SE**		**Zvalue**	***P* value**
2016	Fruit set	Dénia unburnt-Dénia burnt	338	-0.36	0.19	-1.89	0.231
		Tivissa unburnt-Tivissa burnt	338	0.87	0.20	3.27	**0.009**
	D.chamaeropis	Dénia unburnt-Dénia burnt	744	0.57	0.18	3.19	**0.008**
		Tivissa unburnt-Tivissa burnt	744	2.29	0.22	10.17	**<0.001**
2017	Fruit set	Carcaixent unburnt-Carcaixent burnt	394	-0.10	0.17	-0.63	1.000
		Dénia unburnt-Dénia burnt	394	0.30	0.11	2.92	0.342
		Xàbia unburnt-Xàbia burnt	394	1.66	0.001	1241.6	**<0.001**
		Tivissa unburnt-Tivissa burnt	394	0.19	0.001	148.6	0.100
		Carcaixent unburnt-Carcaixent burnt	796	1.38	0.25	5.42	**<0.001**
	D.chamaeropis	Dénia unburnt-Dénia burnt	796	-0.24	0.16	-1.45	1.000
		Xàbia unburnt-Xàbia burnt	796	2.63	0.29	9.01	**<0.001**
		Tivissa unburnt-Tivissa burnt	796	0.58	0.15	3.84	**0.004**

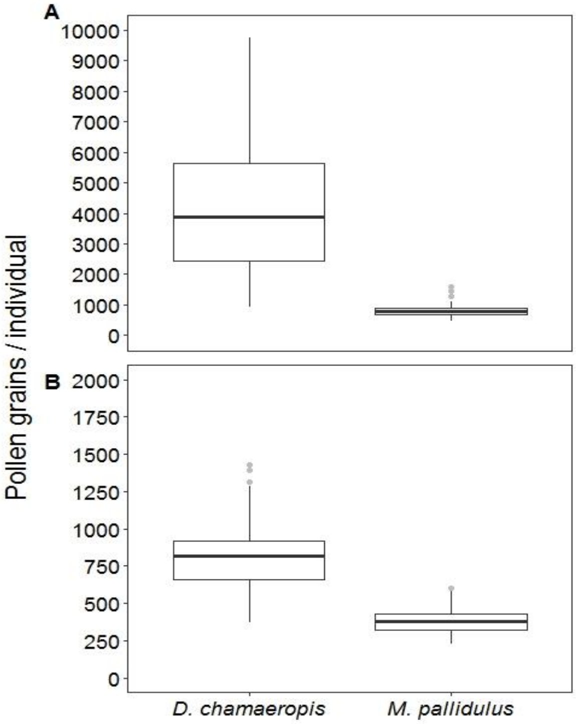

Figure B1. Number of *Chamaerops humilis* pollen grains carried per individual by the two pollinator species, *Derelomus chamaeropis* and *Meligethinus pallidulus*, on male (A) and female (B) *Chamaerops* inflorescences. N= 80 individuals per insect species.

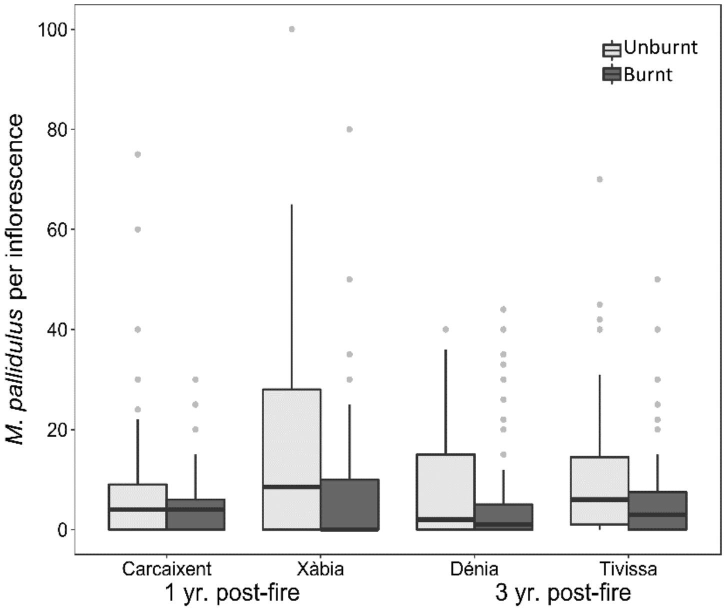

Figure B2. Number of *Meligethinus pallidulus* individuals per inflorescence at each study site in uburnt and burnt areas at 2017 including 2 postfire ages (1-3 years post fire).

Figure B3. *Meligethinus pallidulus* (Nitidulidae) inside the prophyll of a male inflorescence of *Chamaerops humilis* from one of the burnt areas. White arrows indicate *M. pallidulus* individuals moving around the palm stem.

APPENDIX C

Do fire-induced changes in volatile organic compounds promote pollinator switches?

Table C1. *Chamaerops humilis* VOCs detected, and **Videos C1 and C2** on *Derelomus chamaeropis* and *Meligethinus pallidulus* responses in the Y-tube olfactometer, are available at: **https://github.com/Yedra/Thesis-AppendixC**

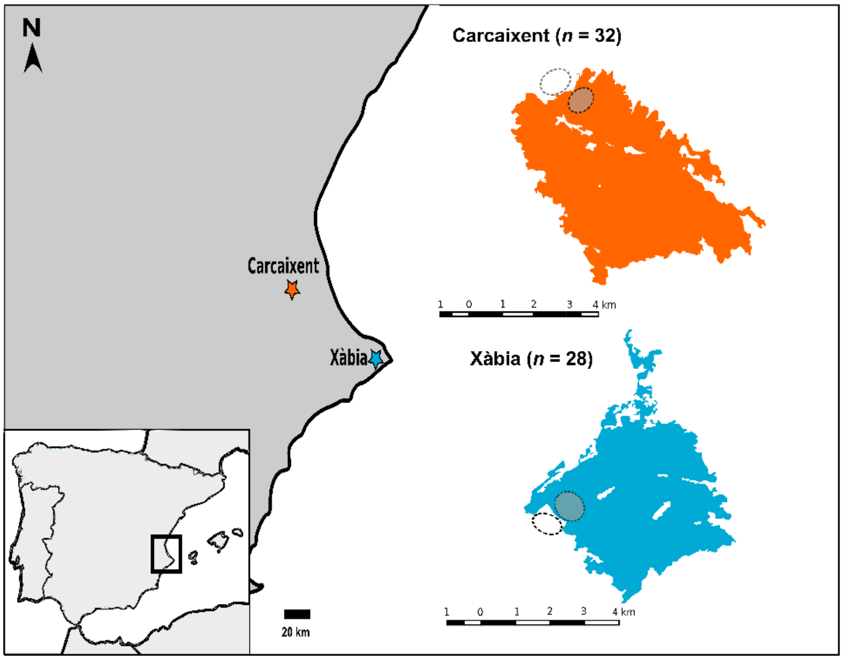

Figure C1. Location of the two study sites, Carcaixent and Xàbia, in eastern Spain (left) and the fire perimeters (right) with the paired unburnt (dashed circles) and burnt (dashed grey circles) sampling areas.

Figure C2. Y-tube olfactometer used in the experimental bioassays (Vidrafoc ®, Valencia Spain). The tube inner diameter was 1.5 cm, the basal branch was 8 cm long and each of the Y-branches 6 cm long. Compressed air entered at a constant flow rate through a glass flask with activated charcoal connected with a second flask with distilled water. Teflon tubes were used to connect the system (Labbox, Barcelona Spain).

Notes C1. Scent collection, analysis and olfactory bioassays.

Scent collection

Leaf scent was collected in the field from *C. humilis* plants separated from each other by at least 5 m, and from the fire's perimeter by at least 50 m. In each plant, the leaf was enclosed in a polyethylene terephthalate bag (40 cm width x 49.5 cm length) for 10 minutes and then the volatiles were trapped for 5 minutes using scent traps connected by a Teflon tube to a 9V portable membrane pump (constant flow rate 200 ml min^{-1}). The scent traps were made with modified Teflon tubes (id= 6 mm, Labbox, Barcelona, Spain) previously washed with acetone and dried. Each trap contained a mixture 1:1 of 2.5 mg Tenax-TA (mesh 20-40) and 2.5 mg Carbotrap (mesh 60-80) adsorbents (Supelco® Sigma Aldrich, St. Louis, USA) between silanized glass wool (Panreac, Applichem). Ambient blanks were collected in parallel in burnt and unburnt areas. Samples were eluted with 200 µl of dichloromethane (Sigma Aldrich) and stored at -20ºC before GC-MS analysis.

For the olfactory bioassays, the leaf scent from four additional *C. humilis* individuals per sex at burnt and unburnt areas was sampled during 10 minutes in scent traps filled with a mixture 1:1 of 25 mg of each adsorbent. Ambient controls were also collected at field.

To assess the specificity between the palm signal and the beetle pollinators we also collected the scent of flowers of eight co-occurring plant species (*Cistus salvifolius*, *Genista scorpious*, *Gladiolus illyricus*, *Iris sisyrinchium*, *Lavandula angustifolia*, *Minuartia hybrida*, *Muscari neglectum* and *Rosmarinus officinalis*). In this case, one inflorescence or five flowers per individual (for species with solitary flowers) of each plant species were collected in the field and stored at -20ºC until the bioassays. Then, the scent from two individuals of each species was collected by diluting the flowers of each individual in 1ml high-grade acetone (Chromasolv ®) and stored at 4º C for 12 hours before the olfactometry bioassays. Leaf VOCs samples from *C. humilis* inside the scent traps were also diluted in acetone and stored in the same conditions. For each trial, 10 µl of each VOC sample type (floral samples diluted in acetone and *C. humilis* leaf samples diluted in acetone) were applied to a small strip of filtered paper and deposited in the glass flasks connected to the arms of the Y- tube olfactometer.

GCMS analysis

The analysis of the leaf VOCs was carried out in the Mass Spectrometry Section of The Experimental Research Support Service (SCSIE) of the University of Valencia using an Agilent 7890B gas chromatograph (GC) coupled with a Agilent 5977A mass spectrometer (MS) and separated on a HP-5 MS capillary column (30 m x 0.25 mm inside diameter, 0.25 μm film thickness). Helium was used as the carried gas. Oven temperature was held at 60 ºC for 5 min and increased by 5 ºC min^{-1} until 180 ºC, and then by 25 ºC min^{-1} until 280 ºC under a flow of 1.1 ml min^{-1}. Tentative identification of volatile compounds was made by comparison with mass spectral databases from NIST 11 and Willey 9 libraries.

Olfactory bioassays.

We followed a similar procedure as in Dufaÿ et al. (2003), except that we used VOCs samples collected from *C. humilis* leaves in the field instead of directly using leaves at the laboratory, to avoid for potential changes in VOCs after leaf cutting. Humidified clean air entered into the two arms of the olfactometer through two glass flasks containing a strip of filtered paper with i) 10 μl of *C. humilis* VOCs eluted in acetone, or ii) the control with acetone only. Before each trial, the acetone was allowed to evaporate from the paper strips.

Each sample was tested during six trials alternating three individuals of each beetle species and *D. chamaeropis* sex. VOC site (leaf origin) and plant sex were changed after six VOCs trials. Control trials with acetone in the two arms were alternated with scent trials. Insects were not used more than twice in the bioassays and always on a different day. Response of each individual was monitored for 3 min (preliminary tests showed this was long enough to get a response in both beetle species), and the choice was recorded when a beetle entered in one of the arms of and remained for at least 10 seconds. We ran 384 trials ([24 with VOCs + 24 controls] x 2 plant sex x 2 beetle species x 2 study sites).

For the olfactory bioassays with *C. humilis* scent and floral scent of the co-occurring plants, we putted a sample of floral VOCs in one arm of the olfactometer and in the other arm a VOCs sample from *C. humilis* leaves (alternating burnt and unburnt areas from the two study sites). For each co-occurring species, eight VOCs trials plus eight alternated control trials were conducted with each beetle pollinator. For this round of the bioassays, insects were collected from a third *C. humilis* population (Tivissa; 40°58’47” N, 0°41’35” E) that started flowering later. After each trial, the olfactometer and the glass flasks were washed with an odorless detergent (Labkem A106, Labbox) and acetone and oven-dried at 130 ºC for 5 min. All bioassays were conducted under red light conditions (20W-1000 Lumen-1P66, Matel ®) to avoid visual cues.

Notes C2. Multivariate generalized linear models (MGLMs) on the scent matrix.

The MGLM approach fits a GLM to each response variable (each VOC in the scent matrix) with a set of predictors and uses a resampling method of rows of the data matrix. To test for differences in the estimated multivariate (overall scent composition) and univariate (each VOC in the samples) deviances the method uses 999 bootstrap interactions by probability integral transform (PIT-trap) residuals. MGLMs are appropriate for compositional data with skewed distributions as was our case. Compared to distance-based approaches, MGLMs are robust detecting effects on less abundant species or VOCs (with low variance) and include a mean-variance relationship assumption (Wang et al. 2012). As in recent studies on floral VOCs, this approach yielded more conservative results than those obtained by permutational multivariate analysis of variance (Kantsa et al. 2017, 2018).

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Table C2. Results of MGLMs analysis, testing for differences in composition of aliphatic, terpenoid and aromatic volatile organic compounds (VOCs) among *C. humilis* leaf samples (N=60) according to the study site (Carcaixent vs. Xàbia), plant sex (female vs. male plants), fire treatment (burnt vs. unburnt areas), stem length and the interaction between site and fire treatment.

Response	**Predictors**	**Df** $_{\text{res, diff}}$	**Deviance**	***P* value**
Aliphatic	Site	58, 1	178.44	**0.001**
	Fire treatment	57, 1	67.16	**0.01**
	Plant sex	56, 1	28.54	0.86
	Stem length	55,1	31.71	0.84
	Site*Fire treatment	54, 1	27.40	0.08
Terpenoid	Site	58, 1	17.07	**0.01**
	Fire treatment	57, 1	8.58	0.21
	Plant sex	56, 1	9.79	0.18
	Stem length	55,1	5.39	0.47
	Site*Fire treatment	54, 1	9.98	0.07
Aromatic	Site	58, 1	24.64	**0.003**
	Fire treatment	57, 1	8.42	0.29
	Plant sex	56, 1	14.34	0.07
	Stem length	55,1	2.27	0.92
	Site*Fire treatment	54, 1	10.49	0.08

Df $_{\text{res, diff}}$ = Degrees of freedom. Significant differences are in bold.

APPENDIX D

Plant-animal interactions contribute to the assembly of forest-savanna communities

1) Table D1. Database for 75 plant species sampled in a forest-savanna mosaic from Emas National Park in Brasil based on 12 reproductive traits, **2) References**, and **3) Phylogenetic tree** of the 75 plant species studied. Available at **https://github.com/Yedra/Thesis-AppendixD**

Notes D1. Details on species trait compilation.

Floral traits considered: *floral symmetry* (radial, non-radial), *size* (corolla length: very small ≤ 3 mm, small >3 and ≤10mm, medium-large > 10 mm), *color* (white-cream, green, yellow, red, purple and pink), *anthesis* (diurnal, nocturnal), *reward* (no reward, nectar as main reward, pollen as main reward, pollen & nectar, oil; Girão et al. 2007; Warring et al. 2016) and *shape* (bell, brush, disk-bowl-shaped, tube and other that included a few inconspicuous, papilionoid and chamber-shaped species, Faegri and van der Pijl 1979; Olesen 2007). Information was obtained from the literature (see Table D1), the webpage of Flora Brasiliensis (http://florabrasiliensis.cria.org.br/), and when possible, direct measures in digital herbarium records from Jardim Botânico do Rio de Janeiro (http://jabot.jbrj.gov.br/v2/consulta.php) to confirm the flower size category assigned by previous literature. In addition, *pollination mode* was classified into 8 categories (bat-pollinated, bee-pollinated, bird-pollinated, moth-pollinated, wasp-pollinated, small insect-pollinated, generalist species, and others that included a few wind and butterfly pollinated species plus one not visited species) and *sexual system* into 3 categories (hermaphrodite: with bisexual flowers, Mo. & AM: monoecious with unisexual flowers plus andromonoecious species, and dioecious species) based on field studies and previous literature (see Table D1).

Fruit traits considered were: *fruit size* (small: length ≤ 2cm, large: length >2cm; from the literature), *fruit type* (fleshy, non-fleshy), number of *seeds per fruit* (few: <10 seeds, medium: ≥10 and ≤20 seeds, many: > 20 seeds), and *dispersal mode* (zoochorous, non-zoochorous including anemochorous and autochorous species). To confirm the information found in the literature review for each fruit size category, we measured the fruit length in 5 fruits of the study species in digital herbarium records from Jardim Botânico do Rio de Janeiro.

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Table D2. Relationship between relative bark thickness (response) and 12 reproductive traits of 75 plant species from a savanna-forest mosaic.

Trait	Trait category	Estimate	SE	t-value	*P*-value
Floral symmetry					
(radial)	non-radial	16.37	22.04	0.74	0.46
Floral size					
(small)	medium	12.98	13.15	0.96	0.34
	large	-2.79	16.73	-0.17	0.86
Floral color					
(white)	green	0.58	28.31	0.02	0.98
	pink	-1.66	70.62	-0.024	0.98
	purple	25.04	32.87	0.76	0.44
	red	-78.60	36.40	-2.15	0.06
	yellow	42.95	23.40	1.83	0.07
Floral anthesis					
(diurnal)	nocturnal	-34.27	24.31	-1.41	0.16
Floral reward					
(absence)	nectar	-23.75	52.07	-0.45	0.64
	pollen	24.99	54.29	0.44	0.65
	nectar & pollen	-3.03	51.70	-0.05	0.95
	oil	37.12	54.29	0.68	0.50
Floral shape					
(bell)	brush	30.68	28.91	1.061	0.29
	disk-bowl	-14.09	22.53	-0.62	0.53
	tube	-48.90	33.09	-1.47	0.14
	others	-43.92	40.36	-1.08	0.28
Pollination mode					
(others)	bat	-3.34	47.69	-0.07	0.94
	bee	3.03	37.70	0.08	0.93
	bird	59.63	39.38	1.51	0.14
	generalist	54.84	36.13	1.52	0.14
	moth	45.46	43.54	1.04	0.30
	small insects	-6.80	47.69	-0.14	0.88
	wasp	130.89	51.52	2.54	**0.02***
Sexual system					
(dioecious)	hermaphrodite	49.20	24.30	2.05	0.06
	Mo. & AM	44.42	36.68	1.21	0.23
Fruit size					
(small)	large	-9.80	11.79	-0.83	0.40
Fruit type					
(non-fleshy)	fleshy	-7.83	16.91	-0.46	0.64
Seeds per fruit					
(few)	medium	35.95	20.22	1.78	0.08
	many	28.10	19.92	1.41	0.16
Dispersal mode					
(non-zoochorous)	zoochorous	-25.74	18.70	-1.37	0.17

SE: standard error. The level of reference for each trait category is in parenthesis. Mo. & AM: Moenoecious with unisexual flowers and andromonoecious species.

Table D3. Phylogenetic signal (PS) of the study traits. The table shows the number of character-state transitions observed for each trait in the species phylogeny and the median transitions from a null model obtained of 1000 random trees by species labels reshuffling. Significant PS are in bold.

Trait	Observed transitions	Median (null) transitions	*P*-value
Floral symmetry	12	13	0.47
Floral size	33	34	0.41
Floral color	28	27	0.22
Floral anthesis	10	10	0.99
Floral reward	**34**	**40**	**0.01**
Floral shape	31	33	0.13
Pollination mode	**38**	**44**	**0.002**
Sexual system	16	16	0.99
Fruit size	23	26	0.12
Fruit type	**20**	**25**	**0.02**
Seeds per fruit	18	19	0.22
Dispersal mode	17	18	0.30

Figure D1. Functional structure of forest and savanna communities based on plant relative bark thickness (bark to diameter, log10-transformed data from Dantas et al 2013b). Functional structure was measured as the standardized effect size of the mean pairwise distance (here mean functional distance, sesMFD). For each community type (i.e. forest and savannas), sesMFD was estimated for all plots (N=98 plots; 'Original'), and for the 17 forests and a subset of 17 randomly selected savannas (N=34 plots; 'Equal size'). Savanna habitat: sesMFD original matrix Pseudo-median= -1.01, ****P* <0.001; in equal-size matrices Pseudo-median= -1.48, ****P* <0.001. Forest habitat: sesMFD original matrix Pseudo-median= -1.16, ***P* <0.01; in equal-size matrices Pseudo-median= -1.81, ****P* < 0.001.

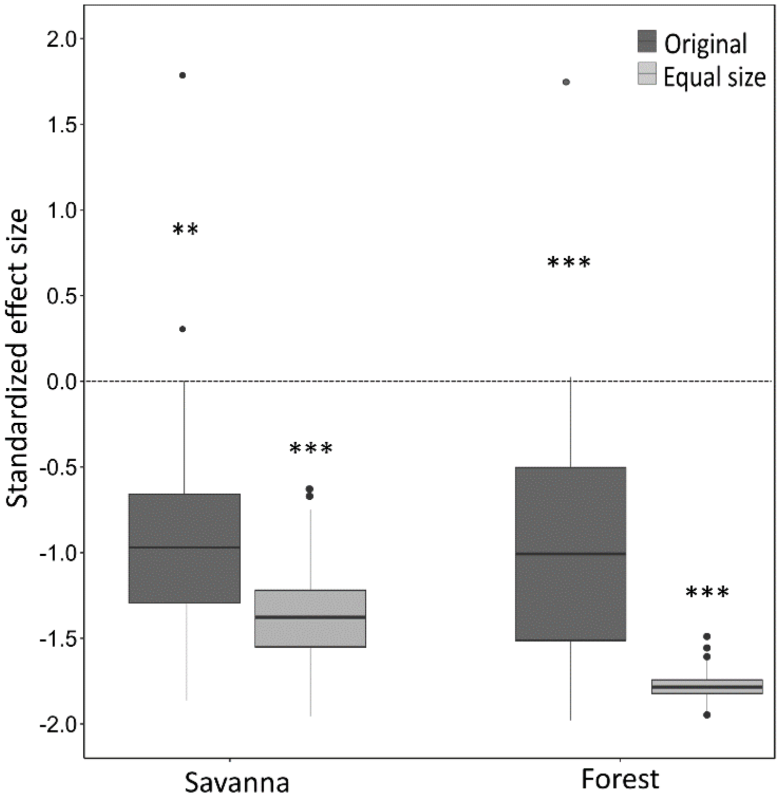

Figure D2. Functional structure of forest and savanna communities based on 8 floral traits including pollination mode. Functional structure was measured as the standardized effect size of the mean pairwise distance (sesMFD). For each community type (i.e. forest and savannas), sesMFD was estimated for all plots (N=98 plots; 'Original'), and for the 17 forests and a subset of 17 randomly selected savannas (N=34 plots; 'Equal size'). Savanna habitat: sesMFD original matrix Pseudo-median= 0.07, *P* n.s (not significant); in equal-size matrices Pseudo-median= 0.18, ****P* <0.001. Forest habitat: sesMFD original matrix Pseudo-median= -0.30, *P* n.s (not significant); in equal-size matrices Pseudo-median= -0.20, ****P* < 0.001.

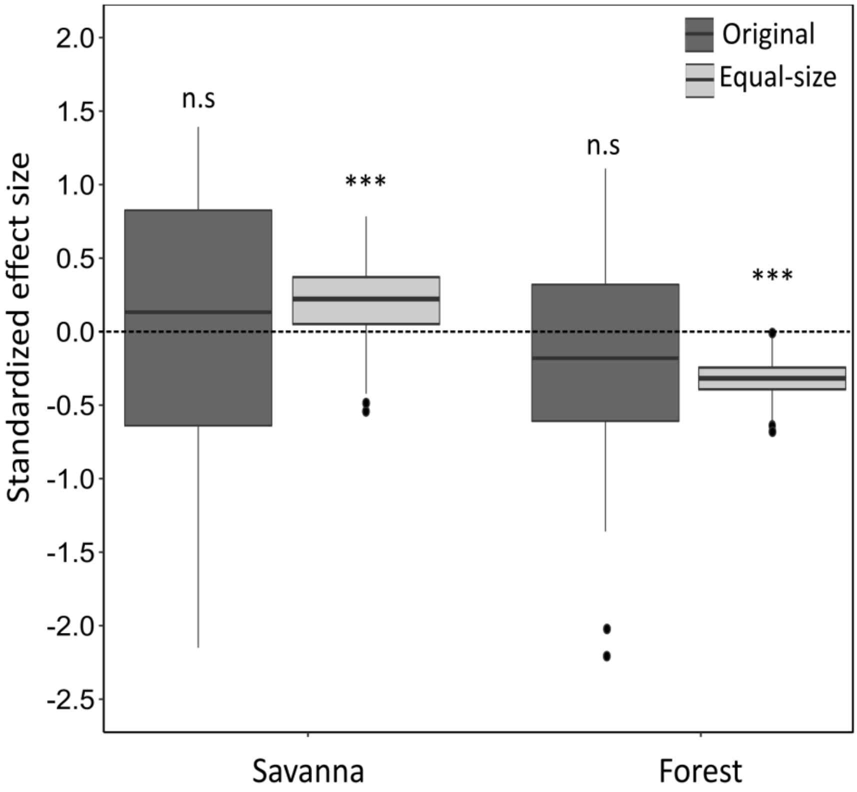

Figure D3. Functional structure of forest and savanna communities based on 4 fruit traits including dispersal mode. Functional structure was measured as the standardized effect size of the mean pairwise distance (here mean functional distance, sesMFD). For each community type (i.e. forest and savannas), sesMFD was estimated for all plots (N=98 plots; 'Original'), and for the 17 forests and a subset of 17 randomly selected savannas (N=34 plots; 'Equal size'). Savanna habitat: sesMFD original matrix Pseudo-median= 0.07, *P* n.s (not significant); in equal-size matrices Pseudo-median= 0.35, ****P* <0.001. Forest habitat: sesMFD original matrix Pseudo-median= -0.30, ***P* <0.01; in equal-size matrices Pseudo-median= -0.23, ****P* < 0.001.