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TESIS DOCTORAL:

Facilitation in Mediterranean mountains: engineering role of *Juniperus sabina* L. at community, population and individual levels

Presentada por Ana Isabel García-Cervigón Morales para optar
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Dirigida por:
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A Mikel y Sara, con los que he compartido esta
etapa de crecimiento

A Helios, maestro y amigo

A Pilu, por no rendirse

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NOTE TO READERS

This PhD thesis is based on five original works, each one representing one chapter. All of them are going to be published in scientific journals, including the introductory chapter which is written in Spanish. The current status of each chapter is listed below.

- **Chapter 1.** Incluyendo el tiempo en el estudio de la facilitación. Una visión general de la tesis.
  Ana I. García-Cervigón. Reduced version submitted to *Ecosistemas*.

- **Chapter 2.** Colonization in Mediterranean old fields: the role of dispersion and plant-plant interactions.
  Ana I. García-Cervigón, Eduardo Velázquez, Thorsten Wiegand, Adrián Escudero, José M. Olano. In preparation.

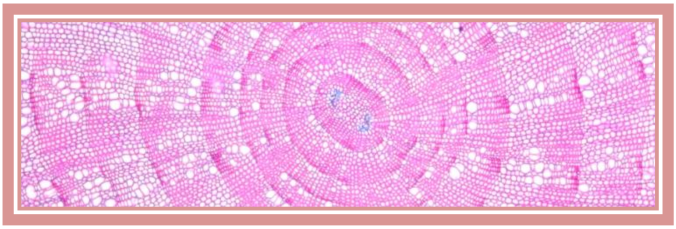
- **Chapter 3.** Intraspecific competition replaces interspecific facilitation as abiotic stress decreases: the shifting nature of plant-plant interactions.
  Ana I. García-Cervigón, Antonio Gazol, Virginia Sanz, J. Julio Camarero, José M. Olano. Published in *Perspectives in Plant Ecology, Evolution and Systematics* 15, 226-236 (2013).

- **Chapter 4.** Facilitation promotes changes on leaf economics traits of a perennial forb.
  Ana I. García-Cervigón, Juan Carlos Linares, Pablo Aibar, José M. Olano. Accepted in *Oecologia*.

- **Chapter 5.** Deconstructing facilitation along the life cycle: impacts of plant-plant interactions at vegetative and reproductive stages in a Mediterranean forb.
  Ana I. García-Cervigón, José M. Iriondo, Juan Carlos Linares, José M. Olano. Submitted to *Annals of Botany*.

Capítulo 1

Incluyendo el tiempo en el estudio de la facilitación. Una visión general de la tesis.



Dejarse llevar suena demasiado bien
Jugar al azar y nunca saber dónde puedes terminar... o empezar

Vetusta Morla

Incluyendo el tiempo en el estudio de la facilitación. Una visión general de la tesis.

Antecedentes

Las interacciones positivas entre plantas son uno de los principales factores que controlan la estructura y diversidad de las comunidades (Brooker et al. 2008, Cavieres et al. 2014, McIntire y Fajardo 2014). Las plantas nodriza, es decir, aquéllas que tienen un efecto neto positivo sobre otras plantas, modifican las condiciones bióticas y abióticas a través de mecanismos directos e indirectos. Entre los directos se encuentran la creación de nuevos hábitats o la mitigación de diversos tipos de estrés (Stachowicz 2001). Entre los indirectos, las plantas nodriza pueden promover cambios en las redes de interacciones entre las especies subordinadas, por ejemplo a través de la propagación de efectos competitivos (negativos) que en definitiva originan un resultado neto positivo sobre alguna de estas especies (Soliveres et al. 2011). Al ampliar los rangos de tolerancia de las especies a las que afectan, las interacciones de facilitación pueden modificar la estructura de las comunidades a través de su efecto en la dinámica de poblaciones (Eckstein 2005) y en las estrategias funcionales de estas especies (Schöb et al. 2012, Gross et al. 2013), expandiendo así sus rangos de distribución, aumentando la riqueza local de especies y permitiendo la persistencia de las comunidades en ambientes altamente estresantes (Le Bagousse-Pinguet et al. 2014, Soliveres y Maestre 2014). Los impactos de la facilitación pueden tener también consecuencias evolutivas en términos de selección de grupo (McIntire y Fajardo 2011) o de estructura filogenética de las comunidades (Valiente-Banuet y Verdú 2007). De hecho, la facilitación incluso se ha propuesto como parte de la teoría evolutiva (Kikvidze y Callaway 2009) al ser considerada como el mecanismo subyacente a las principales transiciones evolutivas como por ejemplo las explicadas por la teoría de la endosimbiosis serial (Margulis et al. 2000).

Como regla general, la facilitación predomina en ambientes estresantes, mientras que en ambientes más benévolos prevalecen las interacciones de competencia (Bertness y Callaway 1994, He et al. 2013, aunque hay trabajos que lo matizan, p.ej. Maestre et al. 2005, 2009, Soliveres et al. 2011). Sin embargo, los niveles de estrés cambian con el tiempo debido a dos fuentes principales de variabilidad temporal: el clima (Kitzberger et al. 2000, Sthultz et al. 2007) y la ontogenia de las plantas (Miriti 2006). Las condiciones climáticas varían a lo largo de un ciclo anual en el que las diferentes estaciones determinan los períodos adecuados para el crecimiento y desarrollo vegetal, pero también cambian a lo largo de los años como resultado de distintos fenómenos que ocurren a escala global y generan tendencias a largo plazo. En relación con la ontogenia, las plantas adultas pueden inicialmente favorecer el establecimiento y crecimiento de otros individuos de la misma o diferentes especies, pero a medida que los individuos facilitados crecen esta relación puede convertirse en negativa (Callaway y Walker 1997, Rousset y Lepart 2000, ver Soliveres et al. 2010). Los cambios temporales en los niveles de estrés pueden inducir cambios transitorios en la intensidad y dirección de las interacciones bióticas a diferentes escalas, por lo que se hace necesario incluir una perspectiva temporal en su estudio (Callaway y Walker 1997, Holzapfel y Mahall 1999).

Para detectar la existencia de interacciones entre plantas y evaluar su intensidad y dirección, se suelen analizar las asociaciones espaciales entre individuos de distintas especies

(McIntire y Fajardo 2009) así como los efectos de la nodriza en la riqueza y abundancia de especies (Tirado y Pugnaire 2005) y en las tasas de establecimiento, supervivencia, crecimiento y fitness de las plantas protegidas (Sthultz et al. 2007, Fajardo y McIntire 2011). Estas variables se miden habitualmente en momentos puntuales o a lo largo de cortos períodos de tiempo que pueden incluir de una a varias estaciones de crecimiento (aunque hay excepciones, p.ej. Miriti 2006). Los cambios en las interacciones en función de la ontogenia también se incorporan de modo puntual, seleccionando individuos que pertenecen a distintos estadios vitales (Núñez et al. 2009). Sin embargo, el modo más adecuado de incluir el tiempo en los trabajos sobre facilitación sería monitorizar las plantas a lo largo de sus ciclos vitales completos. Esto requiere largos períodos de estudio previos a alcanzar algún resultado concluyente, particularmente en el caso de especies longevas como los árboles o los cojines de alta montaña. Ante estas limitaciones, el estudio retrospectivo de la información contenida en los anillos de crecimiento es una alternativa válida para cuantificar cambios pasados en el crecimiento en especies de larga vida (Fritts 1976) y evaluar cambios en las interacciones bióticas a lo largo del tiempo en plantas perennes (Soliveres et al. 2010). También supone un modo mucho más preciso de incluir la ontogenia, puesto que permite conocer la edad exacta de cada planta. A pesar de que esta aproximación dendroecológica tiene sus limitaciones –por ejemplo no permite evaluar las tasas de mortalidad–, supone un complemento que puede proporcionar gran cantidad de información al combinarse con los estudios habituales sobre facilitación.

Los anillos de crecimiento registran las condiciones ambientales existentes en el momento de su formación. Pueden contener información sobre recurrencia de plagas (Esper et al.

2007) o incendios (Iñiguéz et al. 2008), registran la emisión de metales pesados (Sheppard et al. 2007) y el ratio isotópico de los elementos constituyentes de la madera (carbono, hidrógeno, oxígeno y nitrógeno; Guerrieri et al. 2009, Olano et al. 2014), y también son un registro de las condiciones climáticas (Büntgen et al. 2010) y de las interacciones bióticas. El estudio de las interacciones bióticas a través de los anillos de crecimiento se ha desarrollado sobre todo en dinámica forestal, donde las variaciones en la anchura de anillos se han utilizado como un registro de la variación en los niveles de competencia entre árboles vecinos a la hora de entender los efectos de distintos métodos de manejo en el crecimiento secundario de especies maderables (Linares et al. 2011, Molder y Leuschner 2014), como un registro de la presión ganadera y su evolución a lo largo del tiempo (Olano et al. 2008) o para estudiar la respuesta de los bosques ante eventos climáticos extremos en cuanto a cambios en dominancia competitiva entre diferentes especies (Calvin et al. 2013). Sin embargo, los anillos de crecimiento apenas se han utilizado para el estudio explícito de las interacciones de facilitación, ni con árboles ni con otras formas de vida en plantas (ver Soliveres et al. 2011). Los árboles no son las únicas plantas con anillos de crecimiento, sino que muchos arbustos y herbáceas perennes dicotiledóneas también forman anillos (**Fig. 1**). La presencia de anillos de crecimiento en herbáceas y arbustos y su potencial como herramienta en estudios ecológicos se conoce desde hace tiempo (Petersen 1908, Dietz y Ullmann 1997), pero sólo en los últimos años ha comenzado a extenderse su uso en ecología.

Un ejemplo de su aplicación en el estudio de las interacciones entre plantas con arbustos es el trabajo de Soliveres et al. (2010) en el que estudian la relación entre la gramínea *Stipa tenacissima* (esparto, planta nodriza) y el pequeño arbusto

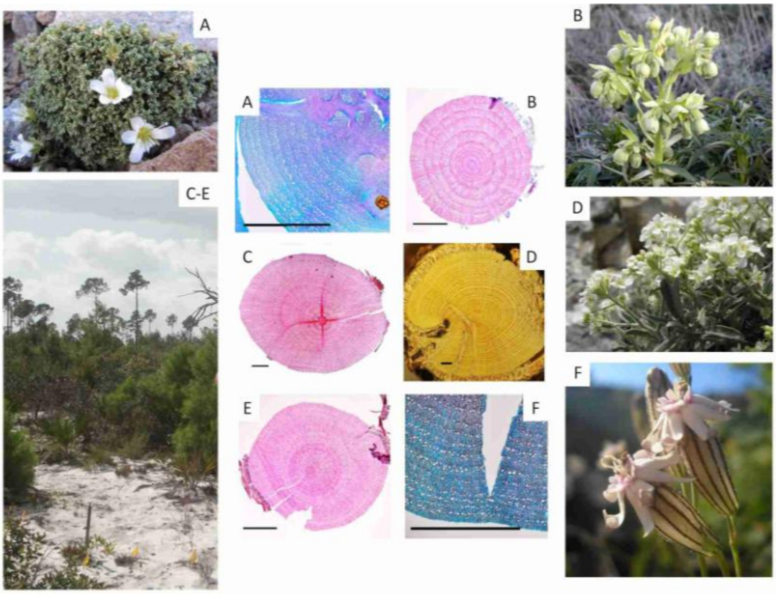


Figura 1. Diversas especies herbáceas y arbustivas con anillos de crecimiento. A, *Arenaria tetraquetra* subsp. *amabilis* (Bory) H. Lindb (Caryophyllaceae); B, *Helleborus foetidus* L. (Ranunculaceae); C, *Ceratiola ericoides* Michaux (Ericaceae); D, *Hormathophylla spinosa* (L.) P. Küpfer (Cruciferae); E, *Hypericum cumulicola* (Small) P. Adams (Guttiferae); F, *Silene ciliata* Pourr. (Caryophyllaceae). Las plantas A, B, D y F viven en ambiente de alta montaña mediterránea. Las plantas C y E forman parte del matorral de Florida (*Florida scrub*), que se muestra en la imagen C-E. Las barras negras representan 1 mm. Fotografías: Gonzalo Juste (cortes B, C y E); Iván Almería (cortes A y F); Ana I. García-Cervigón (madera D, plantas B y D, imagen C-E); José Miguel Olano (plantas A y F).

Lepidium subulatum. En este trabajo, los autores combinan variables clásicas (variables reproductivas y relacionadas con los niveles de reservas de *Lepidium*, relaciones espaciales entre ambas especies) con el análisis de los anillos de crecimiento de *Lepidium*. Utilizan la información contenida en los anillos sobre la ontogenia de los individuos, ya que permiten conocer con exactitud la edad de cada planta, y sobre la variabilidad climática interanual, ya que diferentes anchuras de anillo se relacionan con las condiciones climáticas existentes en el año de formación de cada uno. A través del cálculo de índices relativos de interacción (Armas et al. 2004) y del análisis de las anchuras de anillo en función de la edad de las plantas considerando la lluvia de marzo como variable predictora del grado de estrés abiótico, son capaces de detectar la existencia de múltiples cambios ontogenéticos entre facilitación y competencia durante el ciclo vital de *Lepidium*. También observan que los cambios espacio-temporales en el estrés abiótico modulan los cambios ontogenéticos en las interacciones entre plantas. A pesar del potencial que este estudio muestra, las técnicas dendrocronológicas han tenido un desarrollo muy escaso en estudios de facilitación.

Los trabajos desarrollados en esta tesis doctoral incorporan las estructuras de edades, crecimientos radiales (relacionados con la distribución de recursos) y edad de las plantas (ontogenia) en estudios clásicos sobre facilitación en ecosistemas de alta montaña mediterránea con la sabina rastrera (*Juniperus sabina* L.) como planta nodriza (**Fig. 2**). En estos ecosistemas las plantas están sujetas a dos períodos de estrés climático a lo largo del año: por una parte, las bajas temperaturas invernales retrasan el inicio de la estación de crecimiento, mientras que la sequía estival supone una fuerte limitación para el crecimiento durante el período térmicamente óptimo (García-Cervigón et al. 2012, Olano et al. 2013a). Las plantas nodriza, incluyendo cojines y arbustos rastreros, juegan un importante papel en estos ambientes, puesto que están relacionadas con suelos más profundos y ricos que alivian el estrés hídrico estival (Verdú y García-Fayos 2003, Schöb et al. 2012, García-Cervigón et al. 2013).

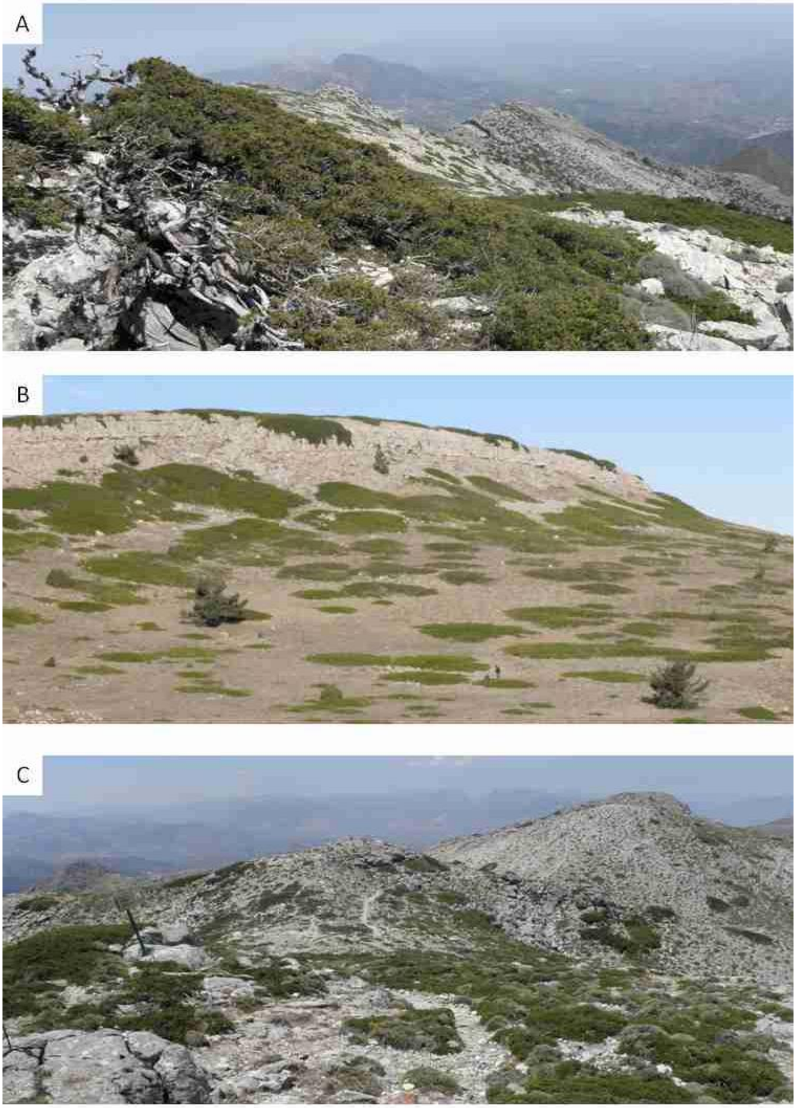


Figura 2. Ecosistemas de alta montaña mediterránea en la península Ibérica. A, Sierra de las Nieves (Málaga); B, Sierra de Javalambre (Teruel); C, Sierra Mágina (Jaén). Fotografías: Ana I. García-Cervigón.

Objetivos

El objetivo principal de esta tesis doctoral es evaluar el papel de la sabina rastrera como elemento clave en la organización de las comunidades, la estructura de las poblaciones y el funcionamiento de los individuos de varias especies arbustivas y herbáceas perennes en diferentes ambientes de montaña mediterránea en la península Ibérica, incluyendo una perspectiva temporal a través del análisis de los anillos de crecimiento. Además de aspectos clásicos como la estructura espacial o el crecimiento radial de los individuos facilitados, se evalúan aspectos novedosos en el estudio de las interacciones de facilitación, como son la variación en los rasgos funcionales de las hojas relacionados con la adquisición e inversión de recursos o la evaluación simultánea de los efectos de la planta nodriza en distintos estadios vegetativos y reproductivos a lo largo del ciclo vital. Los trabajos de investigación desarrollados en esta tesis se estructuran en cuatro capítulos adicionales a este capítulo introductorio cuyos objetivos específicos son:

Cap. 2 Estudiar la relevancia de los procesos de dispersión y de facilitación en la recolonización de cultivos abandonados y su variación a lo largo del tiempo, poniendo especial énfasis en el papel de la sabina rastrera como elemento estructurador del paisaje y de la comunidad al actuar como facilitadora para el establecimiento y supervivencia de individuos de otras especies leñosas, principalmente enebro común *Juniperus communis* L. (Cupressaceae) y pino albar *Pinus sylvestris* L. (Pinaceae), en el Parque Natural del Alto Tajo (Guadalajara).

Cap. 3 Evaluar el efecto de la sabina rastrera en la estructura espacial y las características de los individuos de una población de *Hormathophylla spinosa* (L.) P. Küpfer (Cruciferae) bajo condiciones ambientales contrastadas en dos sitios muy próximos en la Sierra de Javalambre (Teruel).

Cap. 4 Analizar el modo en que la presencia de sabina rastrera modifica los rasgos funcionales relacionados con la adquisición e inversión de recursos en las hojas de *Helleborus foetidus* L. (Ranunculaceae). Se hace hincapié en la variabilidad intraespecífica de dichos rasgos funcionales y en las estrategias económicas que representan, comparando dos poblaciones sometidas a diferentes grados de estrés abiótico en el Parque Natural de la Sierra de las Nieves (Málaga).

Cap. 5 Evaluar el modo en que la sabina rastrera modula la estructura y dinámica de las poblaciones de *Helleborus foetidus* y cómo varía dicho efecto en función del nivel de estrés. Para ello se evalúa el impacto en diferentes estadios vegetativos y reproductivos a lo largo del ciclo vital en el mismo sistema del capítulo 4.

Metodología

La metodología empleada para desarrollar esta tesis doctoral combina la dendrocronogía con técnicas clásicas utilizadas en ecología para obtener información sobre indicadores diversos del funcionamiento de las especies, incluyendo la estructura de las poblaciones, el crecimiento secundario de los individuos, el contenido foliar de nutrientes o los niveles de carbohidratos no estructurales, entre otros. Los datos se analizan empleando una panoplia de técnicas estadísticas que incluyen herramientas de diferentes niveles de sofisticación: modelos lineales generales y generalizados, tests chi-cuadrado, modelos lineales mixtos, análisis multivariados, análisis de patrones de puntos, modelos aditivos generalizados o modelos de ecuaciones estructurales.

Diseño de los trabajos

En el **capítulo 2** se estudia el proceso de recoloniza-ción de antiguos cultivos abandonados en el Parque Natural del Alto Tajo (Guadalajara, España). Las aproximaciones clásicas para estudiar procesos de sucesión comprenden el análisis de patrones de puntos para incluir la perspectiva espacial (Getzin et al. 2006) y la utilización de cronosecuencias (o sustituciones de “tiempo por espacio”) para incluir la perspectiva temporal (Walker et al. 2010). En este trabajo se utiliza el análisis de patrones de puntos, pero se combina el estudio de fotografías aéreas históricas con la estimación de las edades a través de técnicas dendrocronológicas para incluir el tiempo. Las tres especies más abundantes en la zona son la sabina rastrera (*Juniperus sabina*), el enebro común (*J. communis* L.) y el pino albar (*Pinus sylvestris* L.). En este capítulo se analizan tres parcelas de entre 4 y 14 ha en las que se estudian las relaciones espaciales entre los individuos de estas tres especies organizados por cohortes. Tras mapear todos los individuos presentes en las parcelas combinando la fotointerpretación de la ortofotografía más reciente de la zona de estudio con trabajo de campo, se establecen relaciones tamaño-edad en una submuestra de los individuos de cada una de estas especies, lo que permite estimar la edad del resto de individuos presentes en las parcelas de estudio a partir de sus tamaños medidos en campo. Esto permite clasificar a todos los individuos actualmente presentes en las parcelas en grupos de edad de 10 años, lo que posibilita, por una parte conocer la estructura de edades de las poblaciones y, por otra, organizarlas en cohortes y reconstruir la situación en diferentes momentos temporales en el pasado sin necesidad de trabajar en parcelas que fueron abandonadas en diferentes momentos (**Fig. 3**). Los datos se analizan de forma similar en diferentes momentos temporales, empleando técnicas de análisis espacial de patrones de puntos bivariados (Wiegand y Moloney 2004) y de patrones de puntos para objetos de tamaño definido (Wiegand et al. 2006).

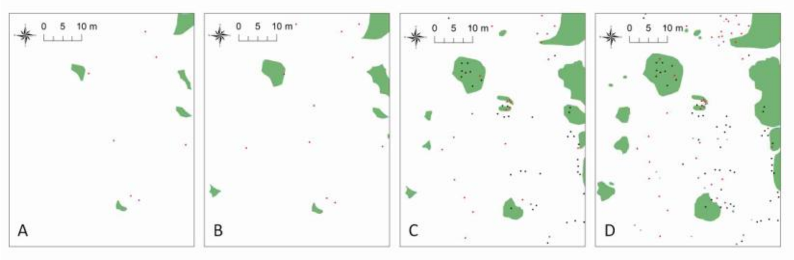


Figura 3. Reconstrucción de los patrones espaciales de sabinas rastreras (áreas en color verde), enebros (puntos rojos) y pinos (puntos negros) a partir de las edades individuales estimadas en la actualidad. Se muestran los individuos presentes en 1960 (A), 1980 (B), 2000 (C) y 2014 (D).

En el **capítulo 3** se estudia la relación de *H. spinosa* con la sabina rastrera en dos sitios próximos con niveles de estrés contrastados: un fondo de valle y una ladera cercana. Se incluyen el análisis de los anillos de crecimiento para considerar las edades relativas, tanto de la planta facilitada como de la nodriza, y los patrones de crecimiento secundario de la primera en función de la presencia de la segunda. En primer lugar se analizan los patrones espaciales de *H. spinosa* en relación con la sabina rastrera en dos transectos de 5 x 50 m en cada sitio. La relación se evalúa con tests de la chi-cuadrado y con análisis de patrones de puntos univariados (Wiegand y Moloney 2004). Para entender el origen de estos patrones espaciales se comparan las edades de las plantas de *H. spinosa* con las de las ramas de sabina más cercanas a cada individuo y se analizan las estructuras de edades de la población. Por último se comparan el tamaño, la forma, el crecimiento secundario y los niveles de carbohidratos no estructurales de plantas de *H. spinosa* que crecen en áreas abiertas y en áreas cubiertas por sabina rastrera en los dos sitios de estudio utilizando modelos lineales y modelos lineales mixtos (Zuur et al. 2009).

En el **capítulo 4** se estudia el efecto de la sabina rastrera en los rasgos funcionales de las hojas de *Helleborus foetidus*. Se analizan plantas que crecen en áreas abiertas y en zonas cubiertas por sabina rastrera en dos sitios con diferente grado de estrés abiótico, uno cercano a la cima del pico más alto de la zona (Torrecilla, 1890 m s.n.m.) y otro menos expuesto a los vientos dominantes con suelos más profundos y húmedos situado a menor altitud (1700 m). En ambos sitios se realizan análisis de suelos para determinar la variación en distintos parámetros (pH, profundidad del suelo, materia orgánica, nutrientes inorgánicos) en función del sitio y de la presencia de sabina rastrera. Los rasgos funcionales estudiados están relacionados con la economía de las hojas (*leaf economics spectrum*, Wright et al. 2004) y sujetos a variación individual debida a la ontogenia (Mason et al. 2013). Se analizan el área de las hojas, el área específica foliar (SLA, área de la hoja dividida por el peso seco), el ratio limbo/peciolo, el contenido foliar en nitrógeno y fósforo, el ratio entre ambos nutrientes y la eficiencia de uso del agua (iWUE) medida a través de los isótopos estables del carbono. Los datos se analizan con modelos lineales mixtos (Zuur et al. 2009) incluyendo la edad de cada individuo como covariable, lo que permite controlar el efecto de la ontogenia en la variación de estos rasgos funcionales. En el caso de arbustos y herbáceas de corta vida la determinación de la edad exacta es de gran utilidad porque evita tener que agrupar a los individuos en clases de edad o tamaño para tener en cuenta su estado ontogenético.

Para finalizar, en el **capítulo 5** se estudia el efecto de la sabina rastrera en la estructura de las poblaciones y dinámica de los individuos de *Helleborus foetidus* del capítulo IV. Para evaluar el impacto de la sabina rastrera en diferentes estadios vegetativos y reproductivos a lo largo del ciclo vital de *H. foetidus* se comparan diversos rasgos vegetativos (crecimiento secundario, número de hojas y altura de las plantas) y reproductivos (número de flores, número de carpelos por flor y producción total de semillas) entre individuos que crecen en áreas abiertas y en áreas cubiertas por sabina rastrera en las dos localidades. Se utilizan modelos lineales y un modelo aditivo generalizado (GAM) para analizar el crecimiento secundario y modelar su relación con la edad de las plantas de forma no lineal. Se analizan también las estructuras de edades, densidad de individuos y asociaciones espaciales con la sabina rastrera en dos transectos por localidad de 10 m de ancho por entre 30 y 80 m de longitud en función de la densidad de individuos de *H. foetidus*. La inclusión del crecimiento secundario y de las estructuras de edades permite ampliar el rango de variables vegetativas y complementar la evaluación de los parámetros reproductivos de modo inmediato, sin necesidad de emplear varios años –o al menos una estación de crecimiento completa– para evaluar las variaciones en el crecimiento vegetativo. Por último, se ajusta un modelo de ecuaciones estructurales (SEM, Grace 2006) a los datos de cada uno de los sitios de estudio, para analizar si el efecto de la sabina rastrera en diferentes momentos de la fase reproductiva, desde la floración hasta la producción final de semillas, varía entre sitios.

Descripción de las principales técnicas y análisis estadísticos empleados en esta tesis doctoral

Mapeado de individuos utilizando SIG

Existen diferentes alternativas para determinar las coordenadas de los objetos ecológicos en la ventana de observación (Wiegand y Moloney 2014). Así, se puede establecer una rejilla en el campo y posicionar los objetos midiendo distancias y direcciones a los nodos de la cuadrícula. También se puede determinar la localización de los objetos utilizando dispositivos GPS o equipos de sondeo. Por último, es posible posicionar objetos de modo indirecto a partir de fotografías aéreas o imágenes de satélite, lo que potencialmente posibilita muestrear áreas mucho más amplias (p ej. Nelson et al. 2002, Gil et al. 2013). Los grandes arbustos postrados como la sabina o el enebro rastreros cubren superficies discretas que pueden llegar a alcanzar los 1000 m^2, lo que facilita el uso de esta última técnica. En fotografías aéreas con la suficiente resolución, los individuos de sabina rastrera se identifican como superficies de color verde que se distinguen del color parduzco de las zonas abiertas circundantes, y se diferencian fácilmente de otras especies arbóreas o arbustivas no postradas porque no presentan sombra asociada al no tener tronco y estar sus ramas a pocos cm del suelo. Esto permite trabajar con Sistemas de Información Geográfica (SIG) para mapear grandes superficies de estudio, aunque sigue siendo imprescindible completar y verificar en el campo la correcta interpretación de las ortofotografías.

En el capítulo 2 se emplea esta metodología. Primero se realizó trabajo de gabinete para delimitar las parcelas de estudio y posicionar las sabinas rastreras, los enebros comunes y los pinos albares sobre la ortofotografía más reciente de la zona de estudio (Plan Nacional de Ortofotografía Aérea, año 2012, resolución de 0.25 m © Instituto Geográfico Nacional de España). La fotointerpretación no permitió diferenciar entre estos dos últimos, ya que ambos se identifican con superficies de color verde que proyectan sombra, con lo que fue necesario diferenciarlos en el campo. El mapeado de individuos en las parcelas de estudio se completó identificando los individuos juveniles de sabina rastrera, enebro común y pino albar que no pudieron ser localizados en la ortofotografía por ser demasiado pequeños.

Por otra parte, también se utilizó el SIG para medir las superficies cubiertas por cada individuo de sabina rastrera de forma mucho más precisa que en campo, puesto que a veces cubren áreas de formas irregulares, lo que dificulta el cálculo de su superficie a partir de medidas de campo. Para asegurar la adecuada correspondencia de las medidas en campo y en el SIG, se midieron dos radios perpendiculares en una submuestra de individuos con forma más o menos circular, se estimó su superficie y se relacionó con la superficie medida en el SIG, obteniéndose un ajuste muy bueno ($R^2 = 0.937$). El empleo de esta metodología permitió por una parte cubrir áreas de estudio de varias hectáreas de superficie con relativamente poco esfuerzo, y por otra economizar los tiempos al reducir el número de jornadas de campo.

Dendrocronología con arbustos y herbáceas perennes

La aplicación de las técnicas dendrocronológicas a pequeños arbustos y herbáceas perennes requiere de una serie de procedimientos diferentes de los utilizados con árboles a la hora de obtener las muestras y prepararlas para su análisis. Estas técnicas se emplean en los capítulos 3, 4 y 5. En primer lugar, para obtener las muestras es necesario extraer el cuello de la raíz, ya que no es viable obtener testigos utilizando barrenas Pressler. En herbáceas perennes y pequeños arbustos, el cuello de la raíz es la parte de la planta que registra el número máximo de anillos de crecimiento y, por tanto, permite obtener la edad exacta de las plantas, así como medir el crecimiento secundario. Para extraerlo es necesario desenterrar los primeros cm de la planta hasta encontrar el punto donde comienzan a ramificarse y desarrollarse las raíces. Tras su obtención, se puede conservar o bien congelado para evitar la degradación de los carbohidratos no estructurales (capítulo 3) o bien en una solución de etanol, ácido acético y formaldehido en una proporción de 90:5:5 conocida como formalín (capítulos 4 y 5).

En segundo lugar, la preparación de las muestras para su análisis dendrocronológico requiere del empleo de microtomía, tinciones y fotografía, en contraste con las técnicas clásicas de montaje y pulido de testigos y rodajas de madera. El procedimiento habitual implica los siguientes pasos:

1. Realizar cortes transversales del cuello de la raíz de unos 10 µm de grosor utilizando por lo general un micrótomo de deslizamiento, como el desarrollado por H. Gärtner/F. H. Schweingruber (WSL, Birmensdorf, Suiza).

2. Colocar los cortes en un portaobjetos y teñirlos con algún colorante que ponga de manifiesto las células con lignina

(las que forman los anillos en el xilema) y algún colorante de contraste que tiña el resto de células. En este caso, los colorantes empleados son una solución de safranina al 1% en etanol y una solución de Alcian blue al 1% en ácido acético, de modo que las células no lignificadas se tiñen de azul y las lignificadas, de rojo.

3. Lavar el exceso de colorante de los cortes ya teñidos utilizando una serie de soluciones de etanol de concentración creciente que ayudan a desecar las muestras. Finalmente se lavan con xilol y se preservan de forma permanente utilizando algún fijador, por ejemplo Eukitt glue (Kindler GmbH, Freiburg, Alemania).

4. Fotografiar los cortes amplificados al microscopio óptico utilizando una cámara digital y un adaptador. En este caso se utilizó una cámara Nikon D90 acoplada a un microscopio óptico Nikon Eclipse 50i. Cuando un corte es demasiado grande como para ser fotografiado en una única imagen, se unen fotografías secuenciales, preferiblemente con algún software que evite distorsiones en los extremos de las imágenes, como PTGUI (ver. 8.3.10 pro, New House Internet Services B.V., Rotterdam, Holanda).

En los capítulos 4 y 5 se utiliza esta metodología, puesto que se trabaja con una herbácea perenne. En el capítulo 3, al tratarse de un arbusto, se pulen directamente las muestras con papel de lija de grano decreciente y se fotografían a la lupa. Una vez obtenidas las fotografías de los cortes o muestras de madera, se utiliza algún programa de análisis de imagen para medir la anchura de anillos. En esta tesis se emplea el software libre

ImageJ (v. 1.44; http://rsb.info.nih.gov/ij; W. Rasband, National Institutes of Health, Bethesda, MD, USA). Es necesario destacar que, a pesar de contar los anillos para obtener la edad de las plantas y medir su anchura para obtener una estimación del crecimiento secundario, en esta tesis no se aplica la dendrocronología sensu stricto, puesto que no se emplea la datación cruzada para determinar con exactitud la edad de cada anillo (Fritts 1976), ni se estandarizan los datos de crecimiento radial al no ser necesario para responder a las preguntas planteadas en cada uno de los capítulos.

Análisis estadísticos

➢ *Modelos de ecuaciones estructurales (SEM)*

Se trata de una técnica de análisis multivariado frecuentemente utilizada en el campo de la psicología cuya aplicación resulta de gran interés en el estudio de sistemas naturales. Los SEM permiten comprobar si un conjunto de datos es compatible con una determinada hipótesis o modelo causal, pero su característica más importante es que permiten abordar el estudio de procesos complejos en los que intervienen varios factores que interactúan entre sí (Grace 2006). Utilizando sistemas de ecuaciones se especifican modelos en los que se incluyen múltiples relaciones entre variables que implican influencias y respuestas simultáneas. Esto permite estudiar sistemas interactivos y evaluar muchas cuestiones que no pueden ser respondidas usando modelos simples con una sola variable respuesta, ya que éstos están diseñados para el estudio de procesos individuales o de efectos netos. En los SEM se combinan análisis de regresión, análisis factorial, modelización estadística y evaluación y selección de modelos.

A diferencia de otros modelos multivariados como el análisis de componentes principales (PCA), el análisis de correspondencias canónicas (CCA), el análisis de conglomerados (*cluster analysis*), las correlaciones canónicas (CA) o el análisis de la varianza multivariado (MANOVA), entre otros, los SEM examinan redes de interacciones que incluyen efectos directos e indirectos, no solo efectos netos. Además, son flexibles para representar el modelo que mejor se ajusta a una situación particular, es decir, tienen alta capacidad para especificar contenido teórico, mientras que en los otros casos se estiman modelos fijos genéricos (p. ej. las correlaciones canónicas). Por último, los SEM se pueden utilizar como análisis exploratorios, pero principalmente tienen carácter confirmatorio, es decir, se emplean para confirmar una serie de hipótesis establecidas a priori, mientras que los otros análisis multivariados son básicamente descriptivos y exploratorios.

En esta tesis se emplean los SEM en el capítulo 5 para evaluar efectos secuenciales, directos e indirectos entre diferentes variables relacionadas con el microambiente y el proceso reproductor de las plantas. Se emplea el software comercial AMOS 18.0 (AMOS Development Corp., Mount Pleasant, South Carolina, USA), que cuenta con capacidad de programación gráfica, es decir, permite especificar los modelos a través del diseño de un esquema de relaciones entre variables de forma visual sin necesidad de programar las ecuaciones una a una. Existen otros programas comerciales para ajustar modelos de ecuaciones estructurales (EQS, CALIS –un módulo de SAS–, Mplus), pero también es posible ajustarlos utilizando los paquetes sem (Fox et al. 2013) y lavaan (Rosseel 2012) en el entorno de programación R (R core team 2013). Se pueden encontrar excelentes explicaciones sobre el funcionamiento y uso de estos modelos en los libros de Grace (2006) y Shipley (2004).

➢ *Modelos lineales mixtos (LMM) y modelos lineales mixtos generalizados (GLMM)*

Los análisis estadísticos más frecuentemente utilizados en estudios ecológicos son los modelos lineales de regresión y análisis de la varianza (LM). Estos modelos se emplean para analizar el efecto de ciertas variables explicativas sobre una determinada variable respuesta. La forma de aplicarlos es bastante sencilla, si bien los datos deben cumplir una serie de asunciones para que el uso de estos modelos pueda considerarse correcto. Estas asunciones son: independencia de los datos, normalidad y homogeneidad de los residuos del modelo, y uso de variables explicativas (o efectos) fijas, es decir, cuyos niveles sean de interés para el experimentador. Sin embargo, con frecuencia los datos ecológicos violan estas asunciones, con lo que la aplicación de modelos lineales no es correcta y puede conducir a realizar interpretaciones y obtener conclusiones erróneas. Los LMM y GLMM ofrecen la posibilidad de resolver todos y cada uno de estos problemas sin necesidad de transformar los datos o utilizar modelos no paramétricos.

Los modelos mixtos reciben este nombre porque combinan los efectos fijos de los modelos lineales con diferentes efectos aleatorios (Zuur et al. 2009). Entre los efectos aleatorios se incluyen diferentes cuestiones. En primer lugar, es posible considerar factores cuyos niveles son sólo una realización de todos los posibles niveles procedentes de una población, es decir, son aleatorios. En estos casos, no interesa conocer el efecto concreto de cada uno de los niveles del factor sobre la variable respuesta, sino saber en qué medida la estimación de los valores medios de la variable respuesta puede variar en función de los niveles del factor que se seleccionen de modo aleatorio. Los modelos mixtos se utilizan cuando los datos presentan alguna estructura de no independencia, como los diseños de medidas repetidas, las series temporales o los diseños anidados. En otros casos, la no independencia de los datos se debe a que están relacionados entre sí desde un punto de vista espacial o temporal; la parte de efectos aleatorios de los modelos mixtos permite incorporar estructuras de autocorrelación espacial o temporal a la hora de realizar los análisis. En cuanto a las desviaciones de la normalidad, se incorporan del mismo modo que en los modelos lineales generalizados (GLM), es decir, utilizando distribuciones de errores diferentes a la normal, como pueden ser las distribuciones de Poisson, binomial o Gamma. En este caso se habla de GLMM. Por último, para hacer frente a la heterogeneidad (o heterocedasticidad) de los residuos existen dos vías: aplicar alguna transformación a los datos (generalmente logaritmos, potencias o raíces cuadradas) o incorporar estructuras de la varianza de los residuos que indiquen alguna relación entre éstos y los factores fijos. Por ejemplo, es posible incorporar en el modelo el hecho de que la varianza de los residuos aumente o disminuya a medida que aumentan los valores de un determinado efecto fijo continuo, o que la varianza de los residuos difiera entre los distintos niveles de un efecto fijo discreto. Cuando la parte de efectos aleatorios del modelo únicamente incorpora estas estructuras de la varianza de los residuos, pero no incluye ningún factor aleatorio, se habla de un modelo de mínimos cuadrados generalizados (GLS), o lo que es lo mismo, una regresión linear ponderada.

En esta tesis se emplean los modelos mixtos en los capítulos 3, 4 y 5 con el objetivo de incorporar estructuras de no independencia de los datos. En todos los casos, se trabaja con diseños de medidas repetidas, por lo que se incluye la identidad de cada individuo como factor aleatorio para considerar que los datos correspondientes a un mismo individuo no son independientes entre sí. Los modelos mixtos se ajustan utilizando los paquetes *nlme* (Pinheiro et al. 2013) y *lme4* (Bates et al. 2013) en R. El libro de Zuur et al. (2009) describe de forma sencilla y detallada el modo de aplicar los modelos mixtos y todas las posibilidades que ofrecen de cara al análisis de datos ecológicos.

➢ *Modelos aditivos*

En ocasiones la relación entre la variable respuesta y las variables explicativas no es lineal. Para solucionar este problema existen varias opciones, que pasan por incluir interacciones entre variables, utilizar funciones cuadráticas, añadir más variables explicativas o transformar los datos para linearizar las relaciones. Sin embargo, es posible que estas soluciones sólo funcionen para determinados niveles de un factor, que a pesar de aplicarlas la relación entre los residuos del modelo y las variables explicativas siga sin cumplir las asunciones de los modelos lineares, o que simplemente no tenga sentido utilizar estas opciones. Un ejemplo es la relación existente entre la edad de un árbol y su crecimiento secundario. Cuando el individuo es joven presenta anillos de crecimiento muy anchos, pero a medida que envejece los anillos van siendo cada vez más estrechos (Fritts 1976). Esto se debe a que la cantidad de madera acumulada año tras año hace que aumente el grosor y la altura del tronco. De este modo, la generación de un nuevo anillo de crecimiento pasa por añadir una nueva capa de madera alrededor de un volumen preexistente cada vez mayor, lo que se traduce en que las capas de madera van siendo cada vez más delgadas y por tanto los anillos de crecimiento se van estrechando con el tiempo. La relación entre estas dos variables (edad y crecimiento secundario) presenta claramente un patrón no lineal. En árboles esto se suele solucionar estandarizando los datos de crecimiento radial o utilizando como variable alternativa el incremento en área basal (Biondi y Qeadan 2008). En herbáceas perennes y en pequeños arbustos, sin embargo, esta relación no sigue patrones tan definidos, con lo que no suelen funcionar estas estandarizaciones. Una alternativa para solucionar este problema es aplicar modelos que permitan considerar la existencia de relaciones no lineares entre variables, como es el caso de los modelos aditivos.

Los GAM permiten modelizar la relación entre dos variables ajustando curvas que pueden presentar distintas formas. A su vez, permiten mantener relaciones lineares entre la variable respuesta y otras variables explicativas, con lo que es posible generar modelos híbridos. Un ejemplo de su aplicación para analizar datos de crecimiento radial en una herbácea es el trabajo de Olano et al. (2013a). En esta tesis se emplean los GAM en el capítulo 5 para modelizar la relación entre el crecimiento secundario de *H. foetidus* y su edad, incluyendo otros factores explicativos como el sitio o la altura de los individuos, que siguen una relación lineal con la variable respuesta. Se implementan utilizando el paquete *mgcv* (Wood 2006) en R, aunque también pueden ajustarse con el paquete *gam* (Hastie 2013). En los libros de Zuur et al. (2007, 2009) se describe el funcionamiento de estos modelos y se ofrecen distintas alternativas para su implementación.

➢ *Análisis espacial de patrones de puntos*

Un patrón de puntos consiste en un conjunto de objetos ecológicos que pueden ser caracterizados por su localización en el espacio. El interés en analizar estos patrones radica en la posibilidad de inferir los procesos ecológicos que los generaron o que se derivan de ellos. Por ejemplo, la localización de los individuos de distintas especies vegetales definen patrones de puntos que pueden ser analizados por separado (análisis univariados), dos a dos (análisis bivariados) o en conjunto (análisis multivariados) para tratar de inferir las relaciones existentes entre ellos. Por lo general, si la relación entre los individuos de dos especies es positiva (facilitación), los patrones de puntos que generan mostrarán atracción, es decir, los individuos de la especie protegida se agruparán en torno a individuos de la especie nodriza. En cambio, si la relación es negativa (competencia), los patrones de puntos mostrarán repulsión, esto es, los individuos de ambas especies tenderán a aparecer alejados entre sí. La tercera posibilidad es que la interacción entre ambas especies sea neutral y, por tanto, sus patrones de puntos no muestren ninguna relación concreta.

Un diseño apropiado del análisis puede revelar la conexión entre el patrón observable y los procesos ecológicos que lo producen o que se derivan de él. El análisis de patrones de puntos está enormemente condicionado por la estructura de los datos, pero en todos los casos se emplean técnicas similares. Las funciones y tests exploratorios permiten describir la estructura de los datos para después ajustar modelos que la incluyan. Se utilizan modelos nulos y procesos de puntos para detectar la presencia de estructuras espaciales significativas en el set de datos, resumir sus propiedades y comprobar hipótesis ecológicas acerca de los factores que pueden haberlas producido (Wiegand y Moloney 2014). El paso inicial suele ser comprobar si los datos contienen alguna señal espacial diferente de la producida por efectos estocásticos puros, para lo cual se contrastan con un modelo nulo de aleatoriedad espacial completa (*csr*). Si los datos se desvían de este modelo, se buscan mecanismos que puedan estar generando esta distribución, es decir, se buscan los procesos de puntos subyacentes para construir un modelo nulo que será contrastado con los datos. Para ello se ejecuta el modelo un número determinado de veces, se construyen envueltas con los resultados de las simulaciones y se calculan tests de bondad del ajuste para evaluar el ajuste entre los datos y el modelo nulo. Es importante destacar que las relaciones entre puntos varían en función de la escala, esto es, de la distancia para la cual se calcula la relación entre el punto focal y el resto de puntos.

El mundo de los análisis espaciales de patrones de puntos es muy complejo y existen multitud de posibilidades (ver algunos ejemplos en de la Cruz 2006, 2008). En esta tesis se emplean análisis univariados de patrones de puntos no marcados en el capítulo 3 para estudiar la estructura espacial de los individuos de *H. spinosa*. Los datos se contrastan frente a un modelo nulo de aleatoriedad espacial completa (*csr*) para comprobar si los individuos se distribuyen al azar, se agregan de alguna manera o se repelen entre ellos, y se describen los resultados con la función de correlación de par $g(r)$. En el capítulo 2 se estudian las interacciones entre plantas y los procesos de dispersión mediante análisis espacial de patrones de puntos bivariados. Para estudiar la dispersión anemócora de los pinos se emplean modelos nulos de Poisson heterogéneos y se obtienen la función de correlación de par bivariada $g_{12}(r)$, la función bivariada L de Ripley $L_{12}(r)$ y la distribución bivariada de distancias al cuarto vecino más próximo $D^4_{12}(r)$. Puesto que los individuos de sabina rastrera pueden cubrir áreas de hasta varios cientos de m^2, las relaciones espaciales entre las sabinas rastreras y los individuos de las otras dos especies (pinos y enebros) se analizan incorporando objetos con área definida en los análisis de patrones de puntos (Wiegand et al. 2006). Se contrastan los datos con modelos nulos *csr* y se describen los resultados con funciones de correlación de par bivariadas $g_{12}(r)$. También se calculan funciones de correlación de marcas r-mark m_2 y variogramas de marcas para analizar la autocorrelación espacial entre las edades de los individuos de sabina rastrera y los enebros rastreros. Estos análisis se realizan utilizando el software libre Programita (Wiegand and Moloney 2004), aunque también pueden realizarse con varios paquetes de R, e los cuales el más desarrollado es *spatstat* (Badeley y Turner 2005). El libro de Wiegand y Moloney (2014) presenta diferentes métodos de análisis espacial que pueden resultar útiles para extraer información de distintos patrones de puntos y describir los procesos subyacentes utilizando ejemplos ecológicos.

Principales resultados

Los datos obtenidos en el **capítulo 2** permitieron observar la importancia relativa de las interacciones bióticas y los procesos de dispersión en función de la especie, de las cohortes y del contexto ambiental. En primer lugar, se vio que la dispersión no estaba limitada en ningún caso para sabinas y enebros, ya que sus principales dispersores son aves (sobre todo zorzales) que pueden desplazarse fácilmente a distancias relativamente largas (Jordano 1993, Verdú y García-Fayos 2003). Por el contrario, en el caso de los pinos, que presentan semillas pequeñas dispersadas por el viento, los adultos juegan un papel muy importante para la recolonización de las parcelas. Esto hace que sabinas y enebros se establezcan en los cultivos abandonados antes que los pinos, que sólo comienzan a expandirse en los últimos 40 años. A su vez, las sabinas viejas de borde de cultivo actúan como puntos de nucleación para enebros y pinos al servir como protección frente a la herbivoría. Sin embargo, factores ambientales como la distancia al bosque, la extensión de las manchas de bosque circundante –que hacen que haya más o menos propágulos disponibles para la colonización de las parcelas, sobre todo pinos–

, o las variaciones históricas en la presión de herbivoría y en su naturaleza –ungulados salvajes frente a ganado doméstico–, pueden producir cambios en la velocidad de los procesos y en la dirección de las interacciones bióticas, que pueden pasar de positivas a neutrales o negativas.

En el **capítulo 3**, los transectos para el análisis espacial mostraron un resultado clásico: una distribución aleatoria en el valle y agregación de las plantas de *H. spinosa* bajo las superficies cubiertas por sabina en la ladera, lo que sugiere una interacción positiva cuando el estrés es mayor de acuerdo con la hipótesis del gradiente de estrés (Bertness y Callaway 1994). Al comparar las edades de las plantas de H. spinosa con las de las ramas de sabina más cercanas a cada individuo se vio que en la ladera todas las ramas de sabina analizadas eran más viejas que las plantas de *H. spinosa* que estaban junto a ellas, lo que corroboraría que los individuos de *H. spinosa* se establecieron bajo las superficies cubiertas ya por sabina, confirmando así la existencia de un proceso de facilitación. En cambio, en el valle ocurría lo contrario. Casi todas las plantas de *H. spinosa* eran más viejas que las ramas de sabina más próximas, lo que indicaría que el patrón espacial existente se originó por un proceso de "fagocitación" por el cual las plantas de *H. spinosa* se establecieron en áreas abiertas que fueron más tarde colonizadas por sabinas rastreras en expansión. Ambos procesos ocurrieron de modo simultáneo en el tiempo, como indica el hecho de que la estructura de edades de las plantas de *H. spinosa* era similar en ambos sitios y también entre individuos que crecían dentro y fuera de sabina. Además, el análisis de las estructuras de edades mostró que el reclutamiento no fue homogéneo en el tiempo, ya que la mayoría de los individuos se estableció entre los años 60 y los 80 del siglo pasado, lo que sugiere que las estructuras que se observan en la actualidad son el reflejo de procesos que ocurrieron durante una ventana temporal muy particular. Por otra parte, las variables relacionadas con la forma y tamaño de las plantas y el crecimiento secundario fueron denso-dependientes, ya que en ambos casos se vieron afectadas negativamente por la alta densidad de individuos en el valle (2.00 individuos m^{-2}) y favorecidas por las sabinas en la ladera, donde la densidad de individuos era tres veces menor (0.64 individuos m^{-2}). Los niveles de carbohidratos no estructurales fueron más elevados en el valle que en la ladera, pero el menor crecimiento secundario de las plantas que crecían en el valle sugiere una menor inversión en crecimiento y muestra que los mejores lugares a nivel poblacional no son necesariamente los mejores lugares a nivel individual.

En el **capítulo 4**, el análisis conjunto de los rasgos funcionales estudiados sugiere que las estrategias económicas de las plantas varían en relación con las condiciones ambientales, y que esta variación en general es independiente de la edad. En áreas abiertas las plantas siguen estrategias más conservativas en el uso de los recursos que aquéllas que crecen en las áreas cubiertas por sabina rastrera, y esto es especialmente notable cuando el estrés ambiental es mayor. Este resultado concuerda con la idea de que el uso conservativo de los recursos es ventajoso en ambientes que limitan las posibilidades para adquirir carbono de forma rápida (Westoby et al. 2002).

Las variaciones en el modo de adquirir y utilizar los recursos suelen tener consecuencias demográficas (Poorter et al. 2008, Easdale y Healey 2009), que quedan de manifiesto en el **capítulo 5**. En el caso del eléboro, la estrategia más adquisitiva de las plantas que crecen en las superficies cubiertas por sabina rastrera se traduce en una mayor probabilidad de reproducción y una fecundidad más elevada que la de las plantas que crecen en áreas abiertas, pero esto solo ocurre en el sitio más estresante –en el menos estresante no hay diferencias relacionadas con la presencia de la sabina–. Sin embargo, el crecimiento secundario es menor en las plantas que crecen bajo la sabina a pesar de que son más altas y tienen más hojas, lo que sugiere que la competencia por la luz con la planta nodriza modifica la arquitectura de la planta, primando el crecimiento primario frente al secundario. El análisis de la estructura de edades muestra que en el sitio más estresante los individuos que se establecen en áreas abiertas lo hacen mayoritariamente en un año concreto. Esta concentración en el establecimiento efectivo de individuos en un año particular podría estar reflejando una limitación severa para el establecimiento en el sitio más estresante, que se ve mitigada en las superficies cubiertas por sabina rastrera. Puesto que la sabina rastrera está relacionada con suelos más profundos y con mayor humedad edáfica (Verdú y García-Fayos 2003), este efecto paliativo podría deberse a la reducción del impacto negativo de la sequía estival en la emergencia y supervivencia de las plántulas (Garrido et al. 2002). En conjunto, la combinación de todos estos análisis muestra que en el sitio más estresante el efecto neto de la sabina rastrera es positivo y resulta de la suma de efectos positivos y negativos que actúan en distintos momentos del ciclo vital (Brooker y Callaghan 1998, Eckstein 2005).

Direcciones futuras

La anchura de anillos o la edad de las plantas no son las únicas variables que se pueden incorporar al estudio de la facilitación. En los anillos de crecimiento tanto de árboles como de arbustos y herbáceas perennes se puede analizar otra serie de parámetros que varían en función de las condiciones ambientales y cuya variación queda por tanto registrada en los anillos de crecimiento. Uno de estos parámetros es el contenido en isótopos estables, principalmente del carbono y el oxígeno, pero también otros elementos como nitrógeno o hidrógeno. Estos elementos proporcionan información de cuestiones funcionales, como la eficiencia en el uso del agua, el tipo de agua utilizada o el tipo de fuente de nitrógeno. En el caso de la eficiencia en el uso del agua, dado que las plantas nodriza modifican el grado de humedad del suelo (Verdú y García-Fayos 2003, Pugnaire et al. 2004), es esperable que reduzcan el grado de estrés hídrico a que están sometidas las plantas que crecen bajo sus copas (García-Cervigón et al. *in press*). También influirían en procesos relacionados con la adquisición de recursos, como la fotosíntesis, a través de la modificación de los rasgos funcionales de las hojas (Gross et al. 2009, Schöb et al. 2012, García-Cervigón et al. *in press*).

Del mismo modo que el papel de la facilitación como filtro ambiental relacionado con la variación de las estrategias económicas a nivel de hoja está siendo recientemente reconocido (McIntire y Fajardo 2014), también cabría esperar que la facilitación estuviese relacionada con variaciones en las estrategias económicas a nivel del tallo (*wood economics spectrum*, Chave et al. 2009). Existe una serie de variables anatómicas de la madera relacionadas con la eficiencia en el transporte de agua que se pueden cuantificar en los diferentes anillos de crecimiento y son indicadoras del compromiso existente entre presentar una mayor conductividad hidráulica o una mayor resistencia a la cavitación. La anatomía cuantitativa de la madera se está desarrollando sobre todo en relación con las variaciones climáticas y ambientales principalmente con árboles (Fonti et al. 2010), pero también es posible estudiarla con herbáceas (von Arx et al. 2012, Olano et al. 2013a) y pequeños arbustos (Eugenio et al. 2014) y en combinación con otras variables dendrocronológicas como la anchura de anillo o los isótopos del carbono (Olano et al. 2014). Algunas de las variables que se han relacionado con las condiciones ambientales son el tamaño medio de los vasos, la densidad y el área de vasos o el lumen del vaso más grande de cada anillo en el xilema de angiospermas, y la anchura de la pared y su relación con el lumen de las traqueidas o la abundancia y tamaño de los radios parenquimáticos en el xilema de coníferas (p. ej. Abrantes et al. 2013, Olano et al. 2013b). Puesto que la facilitación actúa como elemento que modifica las condiciones microambientales a las que se ven sometidas las plantas, es esperable que afecte a la variación de estos parámetros en la madera. Una variable anatómica recientemente incorporada es el grado de agrupamiento de los vasos conductores. El agrupamiento de los vasos puede mejorar la integración hidráulica y aumentar la resiliencia ante la cavitación a través de la redundancia de las rutas hidráulicas, pero a la vez puede facilitar la expansión de cavitaciones entre vasos vecinos (von Arx et al. 2013). Dado el efecto de las plantas nodriza en la humedad del suelo y en la atenuación de las temperaturas con respecto a las áreas abiertas, esta variable también podría verse afectada por la facilitación.

En esta tesis se muestran diversas formas de utilizar herramientas dendroecológicas como complemento a los estudios que abordan problemas ecológicos clásicos relacionados con las interacciones entre plantas para incluir la cuestión temporal. La edad y la anchura de anillo son las variables más inmediatas que se pueden extraer del análisis de los anillos de crecimiento, pero no son las únicas. La existencia de anillos de crecimiento en especies arbustivas y herbáceas perennes y su validez para ser analizados de forma similar a los de los árboles pone de manifiesto el potencial que esto supone de cara no solo al análisis de las interacciones entre plantas, sino también al estudio de otras cuestiones ecológicas.

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Chapter 2

Colonization in Mediterranean old fields: the role of dispersion and plant-plant interactions

The slow one now / will later be fast
As the present now / will later be past

Bob Dylan

Colonization in Mediterranean old fields: the role of dispersal and plant-plant interactions

Introduction

Land-use change is one of the main drivers of global change (Sala et al. 2000, Chazal and Rounsevell 2009). Forest clearing is the most relevant component of land use change, leading to biodiversity loss due to the conversion of native forests into cultivated or urban lands (Chazal and Rounsevell 2009). However, the opposite process (land abandonment) has also been recognized as a major driver of global change, particularly in Europe and high income countries (Palombo et al. 2013, Herrando et al. 2014). Industrialization and agricultural intensification promoted major changes in land-use throughout Southern Europe; both processes resulted in a strong migration of people from the countryside to cities after the Second World War, leading to drastic changes in land management. These changes triggered a massive abandonment of marginal lands and a sharp reduction in extensive livestock practices (Lasanta-Martínez et al. 2005). As a consequence the abandonment of marginal farmland promoted secondary succession in abandoned lands (Bonet and Pausas 2007), whereas reduction in livestock and wood harvesting favoured the encroachment of open woodlands and shrublands (Lasanta-Martínez et al. 2005, Olano et al. 2012, Herrando et al. 2014). This global change driver is reducing landscape heterogeneity, altering species composition and modifying ecosystems functioning (Zarovalli et al. 2007, Azcarate and Peco 2012, García-Tejero et al. 2013, Soliveres and Elridge 2014).

Colonization of abandoned lands depends on the so-called dispersal filter (Duncan 2006). Seed availability decreases as the distance to the plant source increases (Aide and Cavelier 1994), but being the critical distance and effectiveness for each species dependent on the dispersal syndrome. For instance wind dispersal range in anemochorous species depends on some functional traits of seeds and fruits, on the vegetation type, and on physical parameters such as wind speed (Heydel et al. 2014) whereas effectiveness of endozoochorous species will depend on the abundance and foraging behaviour of disperser animals (Verdú and García-Fayos 2003, Escribano-Ávila et al. 2014). After dispersal, effective colonization is modulated by other demographic processes such as germination, establishment, survival and growth of new plants (Rodríguez-Pérez and Traveset 2007). Recruitment success can be limited and exacerbated by a wealth of physical and biotic factors. For instance in semiarid climates, recruitment is restricted to years of high pluviosity (Castro et al. 2005, Olano et al. 2011); whereas high herbivory pressure can hamper recruitment for decades (Davis et al. 2011, Pinna et al. 2014). Positive plant-plant interactions may influence the early successional stages by increasing the establishment rate (Sthultz et al. 2007, Granda et al. 2014).

All these demographic processes leave an imprint in the spatial distribution of plants. The different dispersal mechanisms generate contrasting spatial distribution of recruits. This primary spatial template generated by dispersal may be modified by other processes acting at different stages of plant establishment. For instance the existence of negative-dependent mortality mediated by parasites and predators would reduce recruitment in the vicinity of mother plants (Janzen 1970, Connell 1971). Therefore, seedling survival decreases near adult conspecifics, generating gaps available for the recruitment of heterospecifics and in turn enhancing species coexistence. Similarly, positive plant-plant interactions usually generate clumped distributions of facilitated plants under the canopy of nurse plants, whereas competition between plants produces regular distributions related to an optimization of resource use (Tirado and Pugnaire 2005). Plant establishment would also leave clear spatial signals by responding to herbivory pressure, which would favour a higher concentration of individuals in areas or microsites with lower foraging pressure. Since similar patterns can be generated by different processes or combinations of them, the inference of processes from patterns needs the definition of a suitable set of hypothesis based on the previous knowledge existing about the system (McIntire and Fajardo 2009). In order to go forward for disentangling spatial patterns and mechanisms during colonization we do think that a two-fold approach should be followed. Since more of the methodological approaches to spatial patterns are static we could move on by considering time variation in the spatial patterns. This is especially critical in the case of colonization, which is a dynamic process and consequently presents variation over time. This could be done by explicitly considering the age of each tagged individual and more precisely by including dendrochronological information (Stefanini 2004). The second needs the inclusion of null models that consider some assembly mechanisms and filters (Muenkemueller et al. 2012).

In this paper we studied the colonization of abandoned crop fields in a high-altitude Mediterranean area in central-eastern Spain. Our aim was to assess the effects of dispersal syndromes, facilitation and herbivory in the dynamics of abandoned lands. We focused on the analysis of spatial patterns of the three dominant species (*Juniperus sabina* L., *J. communis* subsp. *hemisphaerica* (C. Presl) Nyman and *Pinus sylvestris* L.) to study the relative importance of dispersal and plant to plant interactions in the colonization dynamics. Seeds of both juniper species are mainly dispersed by birds, whereas pine seeds are dispersed by wind. We studied three sites that have undergone secondary succession under similar climatic conditions but show variations in the spatial context referred to the distance to closed forests (i.e. seed sources) and the browsing pressure and herbivore identity (sheep vs. red deer). We reconstructed the present and past spatial structure for the three focal species using growth rings. We wanted to describe their arrival in the old fields, to evaluate (1) the temporal pattern and rate of colonization of the three species along the studied period; (2) whether dispersal limitation had occurred for endozoochorous junipers, with recruitment occurring close to the plot boundaries or to old individuals; (3) whether dispersal limitation for wind-dispersed pines would have lead to seedling shadows around parent trees (Debain et al. 2007), (4) whether facilitation by *Juniperus sabina* individuals on the other two species had caused higher recruitment density within *J. sabina* canopy, as has been reported in other Mediterranean mountains (García-Cervigón et al. 2013, Verdú and García-Fayos 2003), and (5) whether the different browsing behaviours associated to herbivore identity may lead to different spatial patterns of recruits.

Material and methods

Study area and species

The study area is located in central-eastern Spain, at the Alto Tajo Natural Park. This is a high elevation area (around 1600 m a.s.l.) under continental Mediterranean climate with an annual mean temperature of 8.3 °C and a total amount of precipitation of 725 mm in the Sierra de Molina, located south of Checa village.

The seasonal distribution of precipitations (only 120 mm between June and August) and the increase of mean temperatures during summer (19.0 °C in July; data from the Digital Climatic Atlas of the Iberian Peninsula, Ninyerola et al. 2005) result in an extreme summer drought. Vegetation is dominated by low-density *Pinus sylvestris* woodlands with scattered areas of grasslands and cereal crops. Intense transhumant pastoralism and cereal cultivation have declined since the 60's, and many areas that were previously grazed and cultured have been abandoned and are being recolonized by surrounding vegetation.

We established three plots in previously cultured areas of the Sierra de Molina (**Fig. 1**). Plots 1 and 3 comprised 4.32 and 4.48 ha, respectively, and plot 2 has 13.80 ha. Surrounding vegetation was formed by very open pine (*P. sylvestris*) forests with *J. communis* and *J. sabina* in the understory, including other less abundant shrub species such as *Berberis vulgaris* L., *Crataegus monogyna* Jacq. and *Rosa* spp. These species were also found inside the plots. Substrate and surrounding vegetation were similar for the three plots, but pine forests were closer to plot 3 (Fig. 1). To determine the presence of sheep herbivory per plot we located the active and abandoned sheepfolds in the most updated aerial ortophotography of the study area and visually estimated distances to the plots. Based on this estimation and on field observations, we concluded that plots 1 and 2 are currently subjected to sheep grazing, whereas plot 3 is only grazed by red deer (*Cervus elaphus*).

We focused our study on the three dominant species present in the plots: the tree *Pinus sylvestris*, the erect shrub *Juniperus communis* subsp. *hemisphaerica* and the prostrate shrub *J. sabina*. These three conifer species are widely distributed in

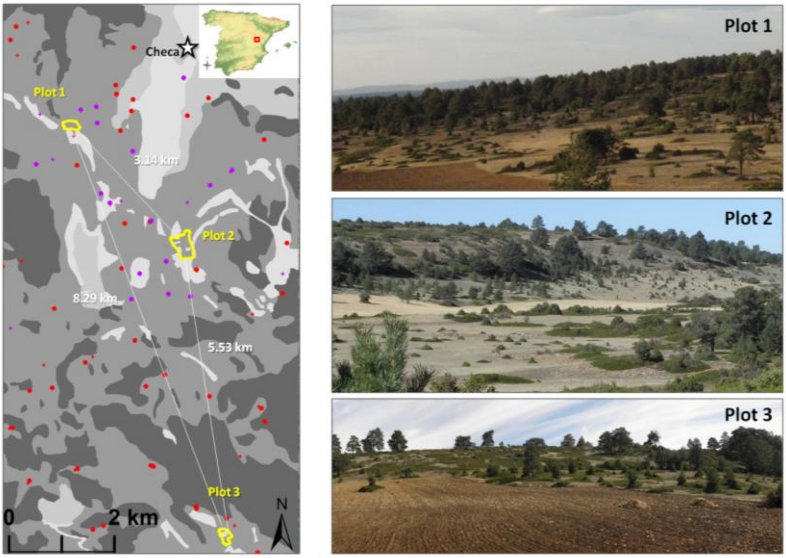

Figure 1. Location of study sites (left) and view of the three plots (right). Grey intensities from light to dark in the left bottom map represent respectively crops, open areas without junipers, open areas with J. sabina and some dispersed pine trees, and pine forests. Red dots and crosses represent bigger and smaller abandoned sheepfolds, respectively. Purple dots and crosses represent active farms.

Europe and Asia, with *J. communis* also present in North America, and share mountainous and cold continental habitats at the Iberian Peninsula (do Amaral Franco 1986). Dispersal mode and tolerance to herbivory differ between pine and junipers. Whereas the 3-5 mm-size winged seeds of *Pinus sylvestris* are dispersed by wind, those of both junipers are dispersed by the same guild of birds and carnivores (Jordano 1993, Verdú and García-Fayos 2003). As a result of land use changes red deer density has increased in the study area, exerting a strong herbivory pressure, especially affecting pine saplings. Individuals of the three species are usually spatially associated; juvenile individuals of both junipers appear at high concentrations under the old pines canopy, whereas *P. sylvestris* and *J. communis* individuals frequently grow in areas covered by *J. sabina*.

Data collection

Position of individuals

We followed a two-step process to build our maps and to spatially locate all individuals in the three plots. First, we used the most updated mosaic of ortophotographs of the study area (National Plan of Aerial Ortophotography PNOA, year 2012, pixel resolution 0.25 m, geodesic system of reference ETRS89, UTM projection, sheet 540 from the Topographic National Map at 1:50,000 –MTN50–, © Instituto Geográfico Nacional de España IGN) to draw junipers and pines in a Geographical Information System (GIS, ArcGIS 10.1). In a second step, we revised and completed the photointerpretation in the field. We distinguished between *J. communis* and pines, located individuals growing under pine and juniper canopies and included all individuals whose surfaces were under the image resolution and could not have been previously located. The coordinates of the central point of each individual were obtained in the GIS.

Age estimation and reproductive status

We estimated the age of all individuals from their size. First, we established linear relationships between size and age following a different procedure for each species. Twenty individuals of *J. sabina* per plot (60 in total) were randomly selected to estimate their ages. We chose a random branch, followed it from the edge until the central part of the individual and extracted a cross-section to count growth rings. Since *J. sabina* individuals tend to cover elliptical or circular areas, we measured two perpendicular diameters at the field and related the estimated age with the mean radius (radius = (area/π)-1/2) of each individual by means of a linear regression. To estimate age of *J. communis* individuals we randomly selected 30 plants out of the plots. We measured their height and two perpendicular diameters to calculate the plant volume and cut them at the soil level to obtain a cross-section from the bottom part of each individual, where we counted growth rings. We then related height, canopy area and volume to age through linear regressions. Finally, to estimate the age of *P. sylvestris* individuals we measured the height and counted the internode number of approximately 33% of plants inside the plots up to 3 m tall. Pine trees whose trunk was free of branches at the bottom were bored as closer as possible to the soil level to get their approximate age. Since individuals show a slower height growth during the suppressed (browsed) period, the relationship between height and age was established for two different groups, a height growth rate until a browsing limit of 1.80 m tall (Olano et al. 2008) and a different height growth rate after that height. After establishing the age-size relationships for the three species, we estimated ages of the rest of individuals of each species from their sizes measured in the field.

Males and females of both juniper species were identified by recording the presence of reproductive structures. In *J. communis*, due to the difficulty to find male cones, only females were recorded. Pine individuals with cones were recorded as reproductive individuals. We used logistic regressions to establish the minimum age at which half of the individuals become reproductive, assuming a sex ratio of 1:1 in junipers (pers. obs.).

In *J. communis*, since we only recorded reproductive females, we estimated the minimum age at which a quarter of the individuals were reproductive.

Data analysis

Age structure and individual density

To describe the age structure per plot and per species, individuals were grouped into age classes of 10 years, to minimize uncertainty derived from age estimation. We reconstructed the spatial patterns of the three focus species in each plot in 2000, 1980 and 1960, by considering only those individuals present in each moment according to their estimated age in 2014. We considered their size and reproductive status during the reconstructed period. The area of each plot available for recolonization (i.e former arable lands and pastures) at each moment was obtained from historical ortophotographs from2003 (© Centro Cartográfico de Castilla-La Mancha), 1984, 1977 and 1956 (© Instituto Geográfico Nacional de España), in which we visually delimited the former cultivated fields. We are aware that past pictures only consider the spatial pattern of surviving individuals, but not of those individuals that finally died. This was done for taking into consideration the role of such individuals as seed sources and perches for the current colonization.

Spatial patterns

We evaluated separately the role played by different mechanisms and processes in shaping the observed spatial distribution of individuals of the three species. Effects of dispersal and plant-plant interactions were evaluated by means of bivariate spatial point pattern analyses, using specific null models to describe each process. The role of herbivory was derived from the comparison of the consistence of observed processes between plots.

1. *Dispersal limitation in junipers*

To check dispersal limitation in junipers, we hypothesized that if seed limitation occurred in junipers, old females present in crop boundaries might have acted as seed source to colonize abandoned fields. If this was true, we would expect that age structure of individuals established in the abandoned fields after 1960 reflects this limitation, with older individuals growing closer to old females (the seed source). However, if seed dispersion was effective enough (Verdú and García-Fayos 2003) individuals would show similar ages all across the abandoned area.

To analyze the spatial correlation between ages of adult females already present in 1960 (pattern 1) and the rest of junipers currently present (pattern 2), we estimated mark correlation functions considering age as a quantitative mark. Analyses were run separately per species, but the same procedure was followed in both cases. The r-mark correlation function m_2 (Stoyan and Stoyan 1994) allowed to determine how the proximity of a focal point of type 1 (i.e., the old reproductive females) influenced the marks of nearby points of type 2 (i.e., the age of juvenile junipers). This function returns the mean age of plants of pattern 2 at distance r of individuals of pattern 1. If individuals of pattern 2 closer to individuals of pattern 1 were older than those located further, our observed function would be beyond the upper limit of the confidence envelopes. To calculate mark correlation functions we randomized marks (i.e., ages) of pattern 2 individuals and kept fixed marks of pattern 1 individuals in the null models. We considered junipers as points located at the centroid of each individual and used a bin width of 3 m, a ring width of 3 m and a maximum radius of 50 m. To construct confidence envelopes we run 199 Monte Carlo simulations and performed goodness of fit tests (Wiegand and Moloney 2014).

2. *Pines dispersal*

To search for dispersal processes underlying the spatial pattern of pines in the study plots, we hypothesized that the presence of adult pines within the study plots and in the surrounding areas in 2014 (pattern 1) would generate an aggregated pattern of seedlings and juveniles (pattern 2) around them, according to the wind dispersal (Debain et al. 2007). To test this hypothesis, we simulated a Cox process, which causes an aggregated point pattern. We used a null model of heterogeneous Poisson with double clustering, by which the distribution of a point pattern (pine recruits) is defined by an intensity function generated by a given pattern of parent points (adult pines) following a kernel function (Wiegand and Moloney 2014). In our case, recruits were distributed following a Gaussian function around adult pines at two scales: a smaller one ($\sigma = 2$ for plots 1 and 3, $\sigma = 5$ for plot 2) and a larger one ($\sigma = 16$ for plots 1 and 3, $\sigma = 22$ for plot 2). Both scales had similar weights (1:1) to obtain the intensity function. Sigma values were selected for being those adjusting better to our data and based on the dispersal kernel estimated by Debain et al. (2007) for *P. sylvestris*. The contribution of older adult pines was higher than that of younger pines (Mukassabi et al. 2012) to generate the intensity function in a proportion of 1:2:4:8 for pines between 21-30, 31-40, 41-50 and more than 50 years old, respectively.

We combined three summary functions to describe spatial patterns at multiple scales. The more commonly used summary function is the bivariate pair-correlation function $g_{12}(r)$, which gives the expected number of points of pattern 2 (juveniles) at distance r from an arbitrary point of pattern 1 (adults). We also used the bivariate Ripley's L-function $L_{12}(r)$ and the bivariate distribution function of distances r to the fourth nearest neighbour $D^4_{12}(r)$. $L_{12}(r)$ is a cumulative function that gives the expected number of points of pattern 2 within a given distance r of an arbitrary point of pattern 1 (in contrast with $g_{12}(r)$, Wiegand and Moloney 2004). The function $D^4_{12}(r)$ assesses the probability of founding the fourth nearest neighbour of type 2 within a distance r of an arbitrary point of pattern 1. Values of this function range from zero (scales at or below which no nearest neighbours have been encountered in the pattern) to one (scales at or above which all points have a nearest neighbour, Wiegand and Moloney 2014). For all cases we chose a ring width of 5 m ($r = 5$). To check the adjustment degree of models to the observed data, we obtained simulation envelopes from 199 Monte-Carlo simulations of the model and calculated goodness of fit tests. If the parameters selected for constructing the null models were compatible with the underlying mechanism generating the observed spatial distribution, the observed function should fit inside the simulation envelopes. Departures from the simulation envelopes would indicate a poor fit of the model to describe the underlying processes of the observed spatial pattern.

3. *The role of biotic interactions*

Based on existing evidence about the role of *J. sabina* as a nurse plant in Mediterranean mountains (Verdú and García-Fayos 2003, García-Cervigón et al. 2013), we hypothesized that *J. sabina* may facilitate the establishment and survival of pines and *J. communis* individuals. To check this, we combined spatial point pattern analysis for objects of finite size (Wiegand et al. 2006) with χ^2 tests. Chi-square tests were used to check whether the observed number of pine and *J. communis* individuals growing within areas covered by *J. sabina* was similar to that expected by chance according to the surface of plots occupied by the prostrate shrubs. Despite the limitations of the χ^2 tests (they ignore the important question of scale, as well as additional information that may exist for each point), they were considered as a good complement because they allow evaluating if pines and *J. communis* individuals are growing exactly within areas covered by *J. sabina*, an issue that spatial point pattern analyses do not fully cover (but see Pescador et al. 2014).

As an alternative we considered individuals of *J. sabina* as objects of finite size (and not as points) in the spatial analyses, because one single individual may cover areas of several hundred m^2. In these analyses we would expect significant attraction between the pattern of prostrate junipers and the pattern of the other two species up to the scale corresponding to *J. sabina* surfaces (see **Table 1** for mean radius of *J. sabina* individuals). To check this hypothesis we used a null model of complete spatial randomness (csr) keeping fixed the positions of *J. sabina* areas and randomizing positions of pines and *J. communis*, running separate analyses for each species. To construct confidence envelopes we run 199 Monte-Carlo simulations and performed goodness of fit tests. If pines and *J. communis* individuals were

Table 1. Mean ± SE and median radius (m) of *J. sabina* individuals at the study plots and in the four temporal moments.

	Plot 1		Plot 2		Plot 3	
	Mean ± SE	Median	Mean ± SE	Median	Mean ± SE	Median
2014	2.02 ± 0.09	1.74	2.12 ± 0.06	1.86	1.27 ± 0.08	1.27
2000	2.37 ± 0.09	1.97	2.66 ± 0.06	2.39	2.69 ± 0.14	2.01
1980	3.34 ± 0.14	2.75	3.45 ± 0.07	3.1	3.99 ± 0.21	3.16
1960	4.98 ± 0.20	4.87	4.45 ± 0.09	4.03	5.53 ± 0.31	4.52

positively associated to *J. sabina* plants, we would expect a positive departure from csr, with the observed function outside the uppermost confidence envelope. Conversely, if the observed function were below the lowermost confidence envelopes, it would indicate repulsion between both patterns, that is, a negative association between them. If there were a random association, the observed function would fit inside the confidence envelopes. Data were analysed altogether and also separated by cohorts. Three temporal windows were considered for *J. communis* (1960-1980, 1980-2000, 2000-2014), and two for *P. sylvestris* (1980-2000, 2000-2014) given the low number of individuals present in the study plots before 1980. To evaluate the effect of *J. sabina* on the effective recruitment of both species, only new recruits in each study period were included in the analyses. It is worth noting that the number of new recruits between 2000 and 2014 was much higher than for the other periods because mortality processes may be currently acting. The total area of the study plots included in the spatial analyses was reduced at each temporal window, according to the reduction in the surface of abandoned fields available for recolonization. All spatial point pattern analyses, including those for objects of finite size, were run with the software Programita (Wiegand and Moloney 2004).

Results

Linear regressions between age and size showed a good fit for the three species. Age of *J. sabina* individuals was estimated as the mean radius divided by 0.0585 (R^2 = 0.5419). The best fit for *J. communis* plants was obtained between age and height (R^2 = 0.6793), and allowed to estimate age as the height divided by 0.0244. Age of pines up to 1.80 m tall was estimated as the height divided by 0.06 (R^2 = 0.7182), whereas for pines taller than 1.80 m age was estimated dividing height by 0.1468 (R^2 = 0.5641). Reproductive age was established at 26 years for *J. sabina*, 37 years for *J. communis* and 21 years for pines.

Colonization dynamics differed between species. Older individuals of *J. communis* and *J. sabina* were already present in the study plots at the beginning of the study period (1960), mainly occupying crop boundaries. The density of both juniper species steadily increased from 1960 to 2014, especially in plot 3. On the contrary, *Pinus sylvestris* colonized the plots more recently, between 30 and 40 years ago (**Fig. 2**). The density of pines increased from 1980 to the present much faster than that of junipers. Pines younger than 10 years old represented 36 % of the individuals in plot 1, 56 % in plot 2 and 83 % in plot 3.

Old junipers located in crop boundaries of the study plots were not important as seed source for recolonization. The observed values of the r-mark correlation functions fitting within the simulation envelopes (**Fig. S1**) indicated that ages of junipers were randomly distributed across the study plots. Older individuals were thus not closer to other old individuals and to plot boundaries, meaning that there was no dispersal limitation for the two juniper species. In the case of pines, new recruits were clearly aggregated around adults in plots 1 and 2, but not in plot 3

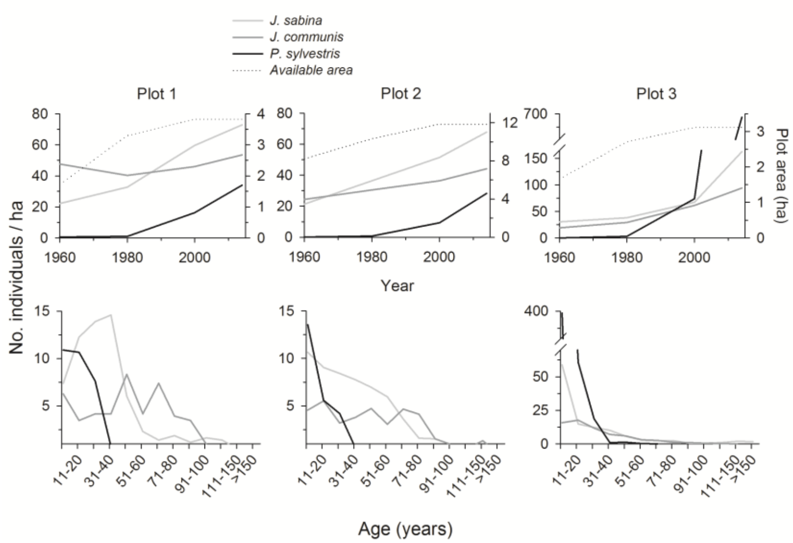

Figure 2. Density of survival individuals over time respect to the area available to be colonized (i.e., not cultivated at each temporal moment, upper graphs) and age structure of individuals present in 2014 (lower graphs).

(**Fig. 3**). In plot 1 the heterogeneous Poisson null model with double clustering at σ = 2 and 16 described adequately the observed pattern except for distances of about 20 m from the focal point. At this distance the observed function indicated that the number of new recruits around adult trees was lower than expected by the null model. In plot 2 the observed pattern adjusted to the model in the $g_{12}(r)$ and $L_{12}(r)$ functions until 25-30 m from the focal point. At larger distances, the observed number of recruits was lower than expected by the model. Observed values of the $D^4{}_{12}(r)$ function were lower than the simulation envelopes from 10 m onwards, indicating that the probability of finding the fourth nearest neighbor was lower than expected. In plot 3 none of the three summary functions adjusted to the observed data, indicating that the proposed model of

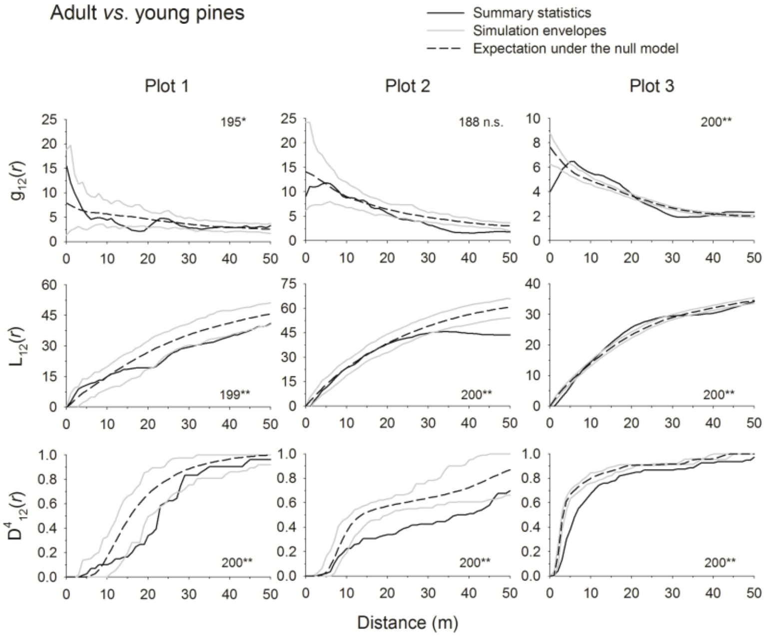

Figure 3. Values of the bivariate pair correlation function $g_{12}(r)$, Ripley's L $L_{12}(r)$ and fourth nearest neighbor $D^4_{12}(r)$ between adult pines (older than 21 yr) and new recruits (younger than 21 yr) in 2014 as a function of distance (r). Simulation envelopes result from 199 simulations of a heterogeneous Poisson null model with double clustering (σ = 2 and 16 for plot 1; σ = 5 and 22 for plot 3; σ = 2 and 16 for plot 3) and represent 5th and 95th percentiles. Values of summary statistics above the upper simulation envelope indicate higher attraction than expected by the model between adult pines and new recruits, whereas values under the lower simulation envelopes indicate higher repulsion between both patterns than expected by the model. Values and significance of the GoF tests are indicated within each plot (n.s. = non significant, * = $P < 0.05$, ** = $P < 0.01$).

heterogeneous Poisson with double clustering could not explain the distribution of new recruits. In this plot there were probably some processes other than dispersion shaping the spatial pattern of individuals.

Patterns of pines and *J. sabina* were independent at any scale in plot 1. In fact, *Juniperus sabina* only acted as nurse plant for *P. sylvestris* in plot 2, where the number of pines growing under juniper canopies was higher than expected by chance at scales below 20 m (**Table 2, Fig. 4**). The mean radius of *J. sabina* individuals was 1.86 m (**Table 1**), which combined with the significant chi-square test indicate that pines were growing within surfaces covered by *J. sabina* in a significantly higher proportion than expected by chance. However, the positive relation between both species was only detected for pines established between 1980 and 2000, whereas the distribution of pines established between 2000 and 2014 was independent from the presence of *J. sabina*. When taking data altogether (from 1980 to 2014) the

Table 2. Results of the Chi-square tests contrasting the number of *J. communis* and *P. sylvestris* individuals within and outside *J. sabina* canopies. Analyses for *J. communis* consider only *J. sabina* females, whereas analyses for *P. sylvestris* consider all *J. sabina* individuals. Superscripts indicate that there are more (+) or less (-) individuals within *J. sabina* canopies than expected by chance.

	Time period	Plot 1		Plot 2		Plot 3	
		χ^2	*P*	χ^2	*P*	χ^2	*P*
Juniperus communis	1960-1980	5.358	**0.021**$^{+}$	161.562	**<0.001**$^{+}$	0.172	0.678
	1980-2000	0.266	0.606	188.870	**<0.001**$^{+}$	4.117	**0.042**$^{-}$
	2000-2014	0.881	0.348	7.866	**0.005**$^{+}$	8.951	**0.003**$^{-}$
	1960-2000	15.430	**<0.001**$^{+}$	376.448	**<0.001**$^{+}$	2.971	0.085
	1960-2014	1.628	0.202	267.506	**<0.001**$^{+}$	13.873	**<0.001**$^{-}$
Pinus sylvestris	1980-2000	3.238	0.072	28.808	**<0.001**$^{+}$	0.622	0.430
	2000-2014	3.644	0.056	1.795	0.180	818.051	**<0.001**$^{-}$
	1980-2014	3.210	0.073	70.782	**<0.001**$^{+}$	241.212	**<0.001**$^{-}$

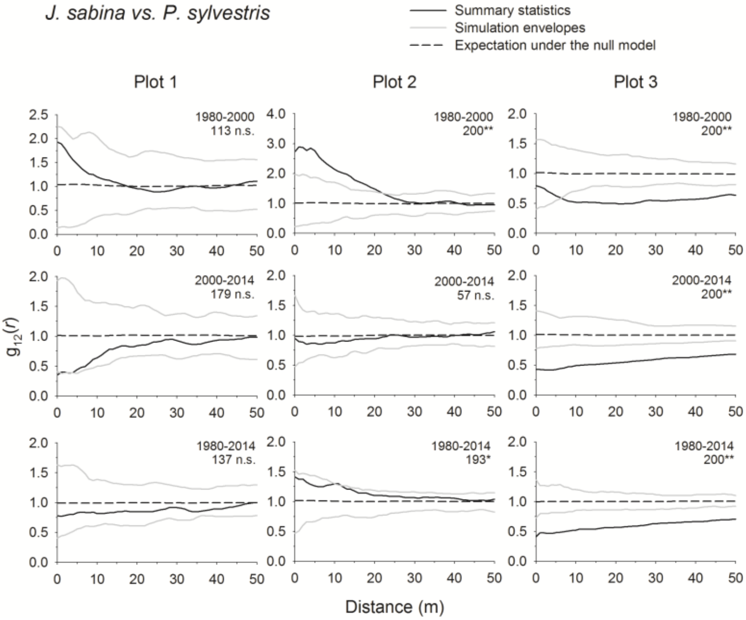

Figure 4. Values of the bivariate pair correlation function $g_{12}(r)$ between *J. sabina* areas and *P. sylvestris* points as a function of distance (r) analyzed by cohorts and altogether. Simulation envelopes result from 199 simulations and represent 5th and 95th percentiles. Values of $g_{12}(r)$ above 1 and the upper simulation envelope indicate attraction between junipers and pines, whereas values under 1 and the lower simulation envelops indicate repulsion between both patterns. Values and significance of the GoF tests are indicated within each plot (n.s. = non significant, * = $P < 0.05$, ** = $P < 0.01$).

positive relation between both species remained at small scale (around 10 m). In plot 3, both patterns showed repulsion at any scale when considering data altogether (from 1980 to 2014) and when considering new recruits between 2000 and 2014. The pattern of pines established between 1980 and 2000 was independent of the pattern of *J. sabina* at small scales (up to 7 m), but also showed repulsion at larger scales. The chi-square tests confirmed these results (**Table 2**).

Analyses of spatial patterns of *J. communis* in relation with *J. sabina* showed very similar results than those for pines. In plot 1 both patterns were independent when considering individuals established by periods or altogether (**Table 2, Fig. 5**). Individuals of *J. communis* established between 1960 and 2000 showed attraction for *J. sabina* at small scale (3-4 m). The mean radius of *J. sabina* (1.74 m, under the scale of attraction) combined with the significant chi-square test might indicate that the number of *J. communis* growing in areas covered by *J. sabina* was higher than expected by chance at plot 1 between 1960 and 2000. In plot 2 the spatial patterns of *J. communis* and *J. sabina* showed attraction at scales of up to 30 m independent of the period considered, and the chi-square test confirmed that the number of *J. communis* growing in areas covered by *J. sabina* was higher than expected by chance. The chi-square test for new recruits established between 2000 and 2014 was significant, but the spatial analysis showed no significant attraction between both patterns. In plot 3 results were similar to those obtained for pines, with independent patterns at small scales that varied between 10 and 30 m depending on the period, and repulsion at larger scales. *Juniperus communis* established between 1960 and 1980 were independent of *J. sabina*.

Discussion

Colonization patterns of abandoned crops in our Mediterranean system were determined by seed dispersal syndromes. Endozoochorous species (*Juniperus sabina* and *J. communis*) were the first to colonize abandoned fields, with this process being related to the presence of individuals in small crop boundaries. The initial dispersal in the anemochorous *Pinus*

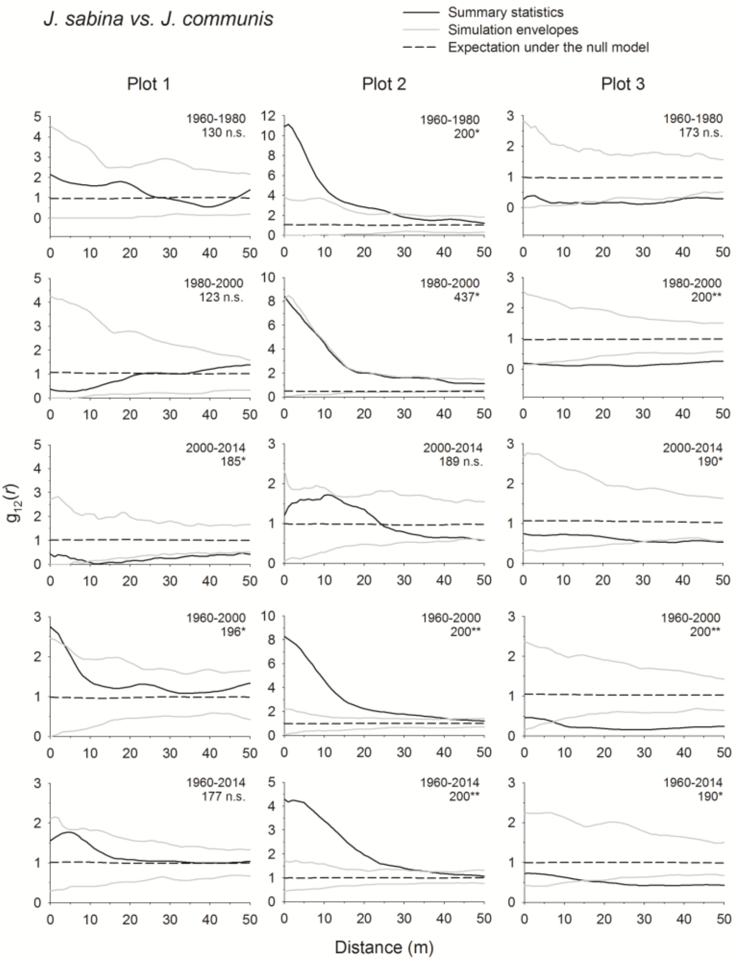

Figure 5. Values of the bivariate pair correlation function $g_{12}(r)$ between *J. sabina* areas and *J. communis* points as a function of distance (r) analyzed by cohorts and altogether. Simulation envelopes result from 199 simulations and represent 5th and 95th percentiles. Values of $g_{12}(r)$ above 1 and the upper simulation envelope indicate attraction between *J. sabina* and *J. communis*, whereas values under 1 and the lower simulation envelops indicate repulsion between both patterns. Values and significance of the GoF tests are indicated within each plot (n.s. = non significant, * = $P < 0.05$, ** = $P < 0.01$).

sylvestris was less effective and individuals arrived later to the plots. However, once adult pines were established, they expanded much faster than junipers, favoring a dense seedling shadow. The importance of *J. sabina* as nurse plant was site-dependent. Whereas in one plot *J. sabina* favored survival of *J. communis* and *P. sylvestris* individuals, this effect was very marginal in the second plot and changed to negative in the third plot.

Differences in colonization dynamics of the three species can be explained by a combination of processes related to seed production and dispersal efficiency. Endozoochorous dispersal in junipers is carried out by a diverse assemblage of birds and carnivore mammals (Escribano-Ávila et al. 2012), which makes this process highly effective. Differences in feeding and movement behavior between both groups of animals generate functional divergences between them: whereas birds are the main dispersers in woodlands, mammals are more important in open areas (Escribano-Ávila et al. 2014). The complementary role played by both groups of dispersers increases the effectiveness of dispersal and promotes the colonization of abandoned fields, explaining the earlier arrival of junipers to the study plots compared with pines. In contrast, *P. sylvestris* have a relatively limited dispersal capacity, since their seeds transported by wind may arrive at shorter distances (Camarero et al. 2005, Débain et al. 2007). However, once established, pine recruitment is much faster than in junipers due to a higher seed productivity (two to three orders of magnitude higher; García et al. 2000, Mukkasabi et al. 2012) that is even more enhanced in isolated trees (Débain et al. 2003). This pattern was reflected by the exponential increase in pine density observed in the study plots, especially in plot 3. Here, spatial patterns of pine recruits did not adjust at all to the proposed dispersal model, which might indicate the existence of additional processes modulating their spatial distribution. The much higher individual density compared to plots 1 and 2 might have carried the emergence of negative density-dependent effects acting on seed and seedling survival. In fact, the lower number of recruits close to the parent trees, combined with a higher number at intermediate distances than expected by the dispersal model, might point to Janzen-Connell processes (Janzen 1970, Connell 1971). According to them, the lower number of recruits closer to parent canopies might be explained by the presence of seed and seedling enemies that would act more strongly in the vicinity of adults and in denser patches.

The role of *J. sabina* as a nurse plant in Mediterranean ecosystems has been proved for herbaceous and shrubby beneficiary species (Verdú and García-Fayos 2003, García-Cervigón et al. 2013, Castellanos et al. 2014). However, in our study design spatial relationships between *J. sabina* and the other two species were site dependent. Positive associations between *J. sabina* and both *J. communis* and *P. sylvestris* spatial patterns suggesting the existence of facilitative processes were only evident in plot 2 and marginally in plot 1, although this weak association in plot 1 may be a consequence of the lower number of individuals compared to plot 2, which may have decreased the power of spatial analyses. Among the battery of described facilitative mechanisms (Stachowicz 2001, McIntire and Fajardo 2014), three of them could have acted in our study system. First, seed rain in *J. communis* is associated with bird perching sites; since birds are expected to feed on *J. communis* and *J. sabina* in environments where both species coexist, *J. sabina* females could act as perching sites for birds that have eaten *J. communis* fruits, favoring the deposition of *J. communis* seeds under *J. sabina* female canopies (García 2001, Verdú and García-Fayos 2003). However, this would result on higher *J. communis* recruitment under *J. sabina* females, but both sexes were not differentially associated with *J. communis* (data not shown). Second, the higher individual density under *J. sabina* canopies may be associated to the amelioration of abiotic conditions under juniper canopies. Increases on soil nutrient content and reduction of summer drought stress levels (Verdú and García-Fayos 2003, García-Cervigón et al. in press) may reduce seedling mortality and increase effective recruitment of *J. communis* and *P. sylvestris* (García 2001, Gómez-Aparicio et al. 2008, Otto et al. 2010). In this case, similar results should have been observed in the three study plots, since all are subject to the same climatic constraints. Moreover, since the amelioration of abiotic conditions would act on seedlings from their first years, the positive effect of *J. sabina* should have been appreciated especially in juvenile plants. Instead, the analysis of spatial patterns separately by cohorts showed positive associations with *J. communis* and *P. sylvestris* individuals established before 2000, but not with individuals established between 2000 and 2014. This may suggest that *J. sabina* plants acted on survival of established plants rather than on effective recruitment, pointing to a different facilitation mechanism. Protection against herbivory may explain this pattern. Junipers show a great tolerance to herbivory (Olano et al. 2008), and low palatable nurse plants as *J. sabina* act as shelter for seedlings and adults of other more palatable species. The large surfaces of *J. sabina* individuals of up to 1 m height may discourage grazers such as sheep from feeding on plants growing under *J. sabina* canopies.

Spatial analyses in plot 3, conversely, indicated no facilitation, but interference, between *J. sabina* and individuals of the other two species. This inconsistency may be related to a shift in the dominant herbivore (from sheep to deer) compared to plots 1 and 2. Sheep are grazers, feeding mainly on grasses and eating seedlings of woody plants when present in open areas, whereas red deer are browsers and woody plants are a basic part of their diet (Ferreira et al. 2013, Zweifel-Schielly et al. 2012). Both herbivores also differ in their spatio-temporal use patterns. Whereas sheep avoid grazing under *J. sabina* canopies, red deer feed within *J. sabina* individuals and even use them as resting places (pers. obs.), thus increasing their use of this microhabitat. Moreover, whereas sheep are moved to milder areas or remain stabled during winter, red deer have to keep on the meager alimentary resources present during winter. Under winter harsh conditions, with snow cover and almost no grass, perennial woody plants become the main food supply for deer (Bee et al. 2010, Häsler and Senn 2012). Accordingly, deer pressure might center on *J. sabina* plants and, consequently, on juvenile individuals of *J. communis* and *P. sylvestris* growing beneath *J. sabina* canopies. Thus, when sheep stopped grazing in plot 3, a much higher number of pine and *J. communis* seedlings could have survived in open areas, highly increasing effective recruitment rates compared to those under *J. sabina* canopies and changing the colonization dynamics.

The analysis of spatial distribution of individuals combined with age estimations allowed describe temporal variations in the colonization patterns of abandoned crops, identifying interactions between plants at different temporal moments. The importance of including a temporal perspective in the analysis of point patterns was highlighted by the emergence of positive relations between spatial patterns of *J. sabina* and *P. sylvestris* when considering individuals by cohorts, relations that were not observed when analyzing data altogether. Spatial analyses by cohorts in *J. communis* allowed detect the lack of relationship with *J. sabina* for juvenile individuals, highlighting the effect of *J. sabina* on survival rather on establishment. Our results suggest that colonization patterns are driven by species-specific dispersal mechanisms, but that colonization dynamics under a priori similar plots depend on the identity of biotic actors. Substitution of a domestic grazer by a wild browser determined sharply different spatial patterns. Projections of the effects of the current land-use change should consider the replacement of extensive livestock by wild herbivores on successional dynamics of abandoned areas.

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Supplementary material

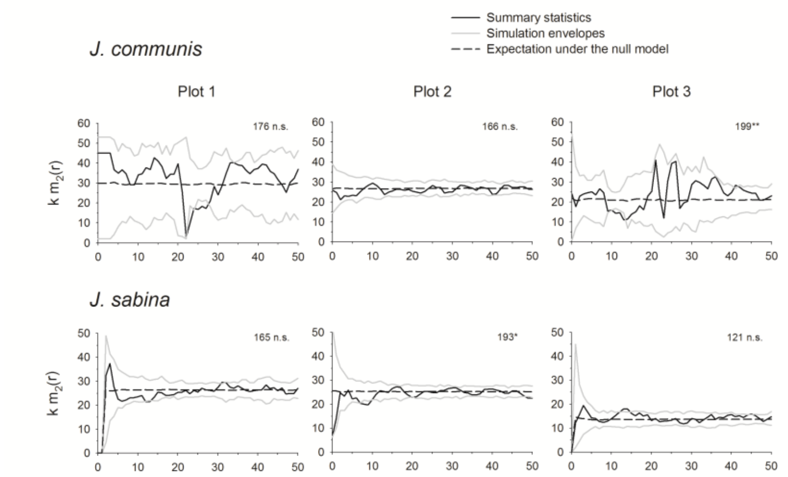

Figure S1. Results of the r-mark correlation functions m_2 to check for dispersal limitation in both juniper species. Values and significance of the GoF tests are indicated within each plot (n.s. = non significant, * = $P < 0.05$, ** = $P < 0.01$).

Chapter 3

Intraspecific competition replaces interspecific facilitation as abiotic stress decreases: the shifting nature of plant-plant interactions

These mist covered mountains / are a home now for me
But my home is the lowlands / and always will be

Dire Straits

Intraspecific competition replaces interspecific facilitation as abiotic stress decreases: the shifting nature of plant-plant interactions

Introduction

Together with competition, facilitation is one of the major forces driving community structure (Connell and Slatyer 1977, Bertness and Callaway 1994, Callaway and Walker 1997). Positive interactions between plants can affect local species richness and promote changes in the interaction networks among the facilitated species (Soliveres et al. 2011), and their consequences go beyond community organization. Facilitation may have evolutionary consequences in terms of group selection (McIntire and Fajardo 2011) or the phylogenetic structure of communities (Valiente-Banuet and Verdú 2007). In fact, the inclusion of positive interactions into evolutionary theory has already been proposed by Kikvidze and Callaway (2009), who suggest that they are the underlying mechanism driving major evolutionary transitions such as that explained by the serial endosymbiotic theory (Margulis et al. 2000).

Understanding the balance of positive and negative plant-plant interactions (i.e., facilitation and competition) on species coexistence has been a major focus in plant ecology over the last decade (Brooker et al. 2008, Maestre et al. 2009). Under mild environmental conditions competition is considered to be the leading interaction, because increased resource availability enhances biomass production and reduces the presence of suitable sites for establishment (Connell and Slatyer 1977). Conversely, under harsh environmental conditions nurse plants can facilitate seedling establishment by ameliorating abiotic conditions (Kitzberger et al. 2000, Núñez et al. 2009), by increasing soil nutrient levels (Anthelme et al. 2012) or by protecting from herbivory (Rousset and Lepart 2000). Thus, the net balance between positive and negative interactions may well depend on the environmental context. This is the core idea of the Stress Gradient Hypothesis (SGH) (Bertness and Callaway 1994), which postulates that facilitative interactions would be dominant in harsh environments, shifting to competitive ones as abiotic conditions ameliorate (Maestre et al. 2009, Soliveres et al. 2010). However, identifying the changes in biotic interactions is usually not so straightforward. Facilitation and competition act simultaneously on a given set of individuals (Holmgren et al. 1997, Callaway and Walker 1997), and the net observable outcome is a result of the combination of both positive and negative interactions (Brooker and Callaghan 1998, Holzapfel and Mahall 1999). Nurse and facilitated species may have also reciprocal effects that can be quantified separately (Holzapfel and Mahall 1999) and the effect of facilitated species on their nurse plants can often be negative (Holmgren et al. 1997). Moreover, a given level of environmental stress does not have the same effect on all the species of a community, and this depends on the level of stress tolerance of each species (Holmgren et al. 1997, Choler et al. 2001, Soliveres et al. 2011). When stress levels are critical for the less tolerant species, facilitation would allow them to endure the stress and thus helping to expand their distribution range. Nevertheless, the environmental conditions are sometimes so extreme that biotic interactions cannot overcome their effect (Choler et al. 2001, Soliveres et al. 2011). There is empirical evidence supporting the SGH in different plant communities subjected to stressful conditions such as semiarid steppes (Pugnaire and Luque 2001) and alpine and arctic tundra (Choler et al. 2001, Callaway et al. 2002, Pellissier et al. 2010), but other studies have shown this hypothesis to be inconsistent (Maestre and Cortina 2004, Maestre et al. 2005, Gross et al. 2010, Soliveres et al. 2011). As a consequence, the debate on the suitability and general applicability of the SGH is still open, and further field studies are still necessary in order for it to be better understood (Callaway 2007, Sthultz et al. 2007, Maestre et al. 2009).

A major challenge when testing the SGH is the dynamic behaviour of plant performance in response to the changing levels of stress that result from at least two major sources of temporal variability, namely climate variation (Wang et al. 2008) and plant ontogeny (Callaway and Walker 1997, Miriti 2006). Climatic conditions at a regional scale change through time, thereby modifying the stress experienced by plants and thus influencing the balance between competition and facilitation (Kitzberger et al. 2000, Sthultz et al. 2007). In relation to ontogeny, adult plants may initially enhance the establishment of other individuals of the same or different species, but as individuals grow this relationship may become negative (Callaway and Walker 1997, Rousset and Lepart 2000, Núñez et al. 2009). Since temporal changes in the levels of stress may induce transient changes in the strength and direction of biotic interactions at different scales (from monthly during a whole growth season to annually during several consecutive years), it is therefore necessary to include a temporal perspective in the SGH framework (Callaway and Walker 1997, Brooker and Callaghan 1998, Holzapfel and Mahall 1999). Although this could be achieved by monitoring plants through their entire life-cycle to estimate, for instance, their survival rates, it would require long periods of study prior to reaching any conclusive results, particularly in the case of long-lived species. The retrospective study of radial growth using ring-width series is a valid, albeit incomplete, alternative to this method. It can provide a temporal framework for evaluating the

SGH in perennial plants (Soliveres et al. 2010), since it can be used to quantify past growth changes in species with long lifespans (Fritts 1976).

The initial formulation of the SGH was done at the interspecific level, thereby highlighting its importance on driving community structure (Bertness and Callaway 1994, Callaway and Walker 1997). However, intraspecific interactions should also be considered in the framework of the SGH, since conspecific competition is a major force driving population dynamics (Connell and Slatyer 1977) that could ultimately affect the net outcome of interspecific interactions at the community level. Intraspecific facilitation occurs between plants at different stages of their life cycle (e.g. Callaway 1995, Fajardo et al. 2006), but it can also occur among individuals of the same cohort (Goldenheim et al. 2008, Fajardo and McIntire 2011, McIntire and Fajardo 2011). Nevertheless, the impact of intraspecific interactions within SGH has remained almost unexplored (but see Tielbörger and Kadmon 2000, Madrigal-González et al. 2012).

The aim of our study was to evaluate how abiotic conditions modulate intraspecific interactions in the cushion plant *Hormathophylla spinosa* (L.) P. Küpfer, at the same time as considering its interspecific interactions with a local dominant co-occurring species, the shrubby Savin juniper (*Juniperus sabina* L.). We assessed whether these interactions varied locally by comparing not a gradient *sensu stricto*, but two sites with contrasting levels of abiotic stress (valley bottom *vs*. slope) that are representative of mild and harsh conditions in a Mediterranean mountain area. Studies on the magnitude and shift of biotic interactions are particularly relevant in Mediterranean plant communities (Maestre et al. 2005, Matesanz et al. 2009) due to the critical role of drought stress in plant establishment (Olano et al. 2011) and the positive effects of nurse plants on water availability (Padilla and Pugnaire 2006). Moreover, since trends of increasing aridity due to rising temperatures and more frequent severe droughts are expected for Mediterranean areas under different global warming scenarios (Gao and Giorgi 2008), most research has focused on plant-plant interactions in water-constrained communities such as semiarid steppes (Escudero et al. 2000, 2005, Maestre et al. 2001). However, less effort has been directed towards understanding this process in Mediterranean high mountains (e.g. Gómez-Aparicio et al. 2004, Cavieres et al. 2006), despite the fact that in contrast with alpine regions they are high-altitude areas that are constrained by both low temperatures and water deficit (Giménez-Benavides et al. 2007, García-Cervigón et al. 2012).

To test the SGH simultaneously at intra and interspecific levels, we developed an observational approach combining several techniques of analysis. We carried out spatial analyses that are commonly used to infer patterns related to positive or negative associations between plants (e.g. Schenk et al. 2003, Fajardo et al. 2008), allowing inferences regarding patterns and processes (Tirado and Pugnaire 2005). To evaluate the status of the target plants, we used complementary measures of plant performance (Lortie and Callaway 2006) including canopy form, non-structural carbohydrate concentrations in wood and radial growth. We also included a temporal perspective by reconstructing the historical patterns of the two species' association. Specifically, we aimed to evaluate if (i) interspecific interactions shift from positive to neutral or even negative when comparing a stressful vs. a mild site, and (ii) increased intraspecific competition outweighs the benefits of improved abiotic conditions in the site with lower abiotic stress level (see **Table 1** for a more detailed explanation).

Material and methods

Study site and target species

The study site was located near Javalambre peak (40º 07’ N, 1º 01’ W), in the Iberian mountain range, Teruel, eastern Spain (**Fig. 1a**). The site is above the tree line (1,940-1,965 m a.s.l.), and vegetation is dominated by large prostrate Savin junipers (*Juniperus sabina*). The substrate is mainly composed of shallow, stony soils developed on limestone bedrock. Climate is continental and Mediterranean with a marked summer water stress period (García-Cervigón et al. 2012). Mean summer and winter temperatures are 17.1 ºC and -0.2 ºC, respectively, and estimated total precipitation is 520 mm, with a summer drought period lasting from July to August (Ninyerola et al. 2005).

Hormathophylla spinosa is a cushion plant from the western Mediterranean Basin that grows at altitudes of up to 3400 m a.s.l. It is a species inhabiting sites with stony and rocky soils (Küpfer 1993). *Juniperus sabina* is the dominant shrub above the tree line and up to 2750 m a.s.l in calcareous Mediterranean high mountains, being widely distributed along central and southern Europe, northern Africa and western Asia (López González 2004). A single individual of this prostrate juniper species can cover areas of up to 0.1 ha, being a keystone species of mountainous ecosystems with a large number of woody and herbaceous species establishing within its canopy (García-Cervigón pers. obs.). This may be due to protection from herbivory but also to the better microenvironmental conditions provided by soils developed beneath *J. sabina* plants, which have higher organic matter, N and P contents, higher soil moisture and lower maximal temperatures (Verdú and García-Fayos 2003).

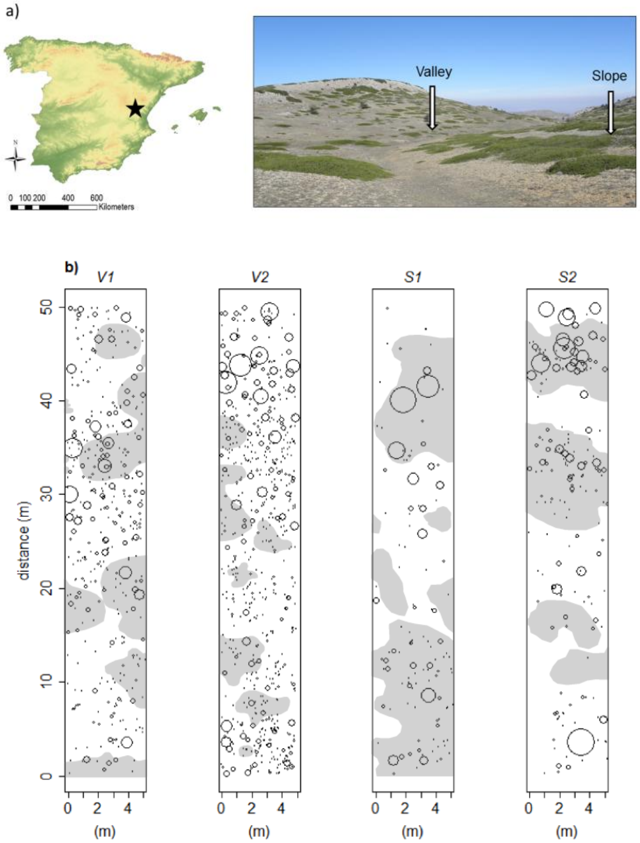

Figure 1. View and location of the study area in Javalambre, Teruel, eastern Spain. a) Location of transects. b) Location of *H. spinosa* plants inside the sampled rectangular transects (V1, valley transect 1; V2, valley transect 2; S1, slope transect 1; S2, slope transect 2). The size of symbols is proportional to the canopy diameter of plants. Grey areas represent surfaces covered by *J. sabina*.

Hormathophylla spinosa and *J. sabina* coexist and are widely distributed in Iberian Mediterranean mountains.

Table 1. Specific objectives, related hypotheses and expected patterns, and the methods and analyses used to test them. Abbreviations: GLM, General Linear Models; LMM, Linear Mixed Models; SGH, Stress Gradient Hypothesis.

Objective	Hypotheses and expected patterns	Methods and analyses
Testing the SGH at the interspecific level.	In the more stressful site (slope) *H. spinosa* would appear aggregated and associated with juniper canopies due to nurse effects on establishment. In the mild site, the spatial pattern of *H. spinosa* would be either random or negatively associated with juniper canopies.	• Univariate point patterns analysis based on the pair-correlation function (*g*(*t*)) to compare spatial patterns in slope vs. valley sites. • χ^2 test to assess and compare *H. spinosa*–juniper potential associations between contrasting sites (slope *vs*. valley) and microsites (within *vs*. outside juniper canopy).
Checking if the inclusion of intraspecific interactions improves the vision of the study system provided by the analysis of interspecific ones.	In the mild site (valley) *H. spinosa* density would be higher than in the more stressful site (slope) leading to higher intraspecific competition. This effect may outweigh the amelioration of abiotic conditions in the valley, with individual plant performance being higher on the slope than in the valley.	• ANOVA to compare intraspecific interactions between sites and microsites. • GLM to study the combined effect of intraspecific interactions, site and microsite on plant canopy form and on non-structural carbohydrate concentrations in wood. • LMM to quantify how radial growth is related to site and microsite.

Field and laboratory work

We selected two contrasting sites: a valley bottom (hereafter referred to as valley) with relatively favourable conditions (deep soils, increased moisture and wind protection) and the nearest west-facing slope (hereafter referred to as slope) where abiotic conditions were harsher, due to shallower soils and higher run-off. This sampling scheme does not comprise a true abiotic gradient, but conditions are contrasting enough to enable comparison between a favourable and a less favourable site for the target species. Moreover, both sites are close enough to ensure that other possible confounding factors such as climate or historical human management have been similar. As a proxy of the abiotic stress degree we measured soil depth, considered as the distance between the soil surface and the bedrock, by performing 80 random measures at each site, 40 of them beneath *J. sabina* and the other 40 on the open ground, using a metal stick and a centimetre ruler. Two rectangular transects (5 m x 50 m) were randomly placed at each site (valley transects V1 and V2; slope transects S1 and S2, these two located in the direction of the maximum slope). This transect size is particularly appropriate for the species under study since it tends to encompass several juniper individuals and a large number of *H. spinosa* plants. For each *H. spinosa* individual we recorded its position, microsite (i.e., within or outside the area occupied by a juniper canopy) and size (plant height and two perpendicular canopy diameters). Additionally, we mapped the area covered by juniper individuals in each transect (**Fig. 1b**). *Hormathophylla spinosa* size data were used to calculate two variables reflecting the canopy form and size: slenderness (height divided by mean canopy diameter) and area (considering the horizontal canopy projection as an ellipse). These measures were taken for all individuals in each transect. However, to estimate their age, we collected all plants in the two transects located on the slope, but only half of them (from the first 25 m of the transect) in both transects in the valley site, due to the elevated plant density there. Age was estimated by taking a transversal section from the root collar, sanding it carefully with sandpapers of progressively finer grains until rings were clearly visible, and counting growth rings under a binocular lens (Nikon SMZ800).

In order to know whether *H. spinosa* established after or before the nearest juniper, at each site we randomly sampled 20 additional *H. spinosa* plants that were growing within the area of a juniper canopy, as well as the largest juniper branch contiguous to each *H. spinosa* individual. Ages of *H. spinosa* individuals and juniper branches were again estimated by counting annual rings in basal sections.

To obtain an estimate of *H. spinosa* individual reserve levels, we collected 40 additional plants from each site, 20 per microsite (within and outside juniper canopy areas), and measured non-structural carbohydrate (NSC) concentrations in the wood. NSC levels have been used as a proxy for carbon balance in plants under different scenarios as response to disturbances (Clarke et al. 2013), successional dynamics (Olano et al. 2006) or environmental gradients (Körner 2003a). Since secondary growth is under stronger environmental constraints than photosynthesis (James et al. 1994), high NSC levels at high altitudes would be due to lower growth (sink) activity (Körner 2003a, Fajardo et al. 2012), and we would therefore expect a negative relationship between growth and NSC levels. NSCs were measured using the anthrone method (Morris 1948, see Olano et al. 2006 for a detailed description of the methodology). We collected wood samples from the main stem 1 cm below root collars, which were frozen immediately after being collected to avoid the degradation of NSCs. Before the chemical procedure, samples were debarked, oven-dried at 80ºC for 48 h and finely ground.

Finally, to estimate how radial growth varies through time we measured the annual rings of 80 *H. spinosa* individuals, 40 per site and 20 per microsite. To measure annual rings we took photographs of the sanded samples under a binocular lens at 10-63 magnifications depending on the samples' size and analyzed them with the ImageJ v.1.44 software (available at http://rsb.info.nih.gov/ij; developed by W. Rasband, NIH, Bethesda, MD). Photographs were converted into greyscale images and we traced a radius from pith to bark, visually delimited each annual ring and measured the ring widths along two radii per plant. Since some stems were partially rotten, we used a final sample size of 20 and 15 plants located within juniper canopy areas and 18 and 17 plants outside juniper canopy areas at the valley and slope sites, respectively.

Statistical analyses

Hypothesis 1. Testing the SGH at the interspecific level

In order to test whether the juniper presence and site affected soil characteristics we compared soil depth between sites and microsites through an Analysis of Variance (ANOVA).

We explored whether the spatial patterns of *H. spinosa* plants varied between the two sites by using univariate point pattern analyses. We preferred univariate over bivariate analyses to study the relationship between *H. spinosa* and juniper because each juniper individual covered large areas of the transects (see

Fig. 1b): the representation of each juniper plant with a single point would mean analysing the interspecific effect only as related to the centre of each juniper individual, not to all its surface. To describe the small-scale spatial pattern in each transect, and to visually compare the patterns in the two studied sites, we used the pair-correlation function g(t), which is a useful second-order statistic to characterize patterns at small spatial scales (Diggle 2003). Values of g(t) higher and lower than one indicate clustered or regular spatial patterns, respectively. We considered the null hypothesis of a homogeneous Poisson process for complete spatial randomness of *H. spinosa* plants. To test if the represented spatial pattern departs significantly from random we used Monte Carlo test with 999 unrestricted permutations. The analyzed distance range was from 0.25 m to 2.5 m, establishing a maximum limit equal to half the minor axis of each transect (i.e. 2.5 m). We used a numerical approach which uses an underlying grid of cells (0.25 m side in our case) and does not require edge-effect correction (Wiegand and Moloney 2004). Finally, we calculated a goodness of fit test and its associated probability level to summarize the deviation between the observed pattern and the theoretical results across the distances tested in each analysis (Diggle 2003). The spatial analyses were done using the software Programita (Wiegand and Moloney 2004).

To determine whether juniper affects the spatial distribution of *H. spinosa*, we compared plant density per microsite in both sites. We performed χ^2 tests for comparing the expected and observed number of individuals per microsite according to the surface of each transect covered by juniper. We checked the ages of *H. spinosa* individuals and contiguous juniper branches and analyzed the age structure at each microsite in the four transects. We used 5-year age classes to take into account the uncertainty in age estimation associated with possible missing or false rings, since samples were not cross-dated (Fritts 1976).

Hypothesis 2. Including intraspecific competition in the SGH predictions

Intraspecific competition in *H. spinosa* was estimated by using the modified influence index of Woods (2000) in a radius of 50 cm around each focal plant:

$$\text{Influence index} = \Sigma_{\text{dist}\leq 50\text{cm}} Area_{\text{n}}/dist_{\text{n,f}}$$

where Area_n is the canopy area of an individual neighbouring plant, and distn,f the distance between the neighbouring plant (n) and the focal plant (f), considering only plants in the same microsite. Since the belowground influence area of a plant is proportional to its volume (Casper et al. 2003) and the volume for *H. spinosa* plants ranged from 2.80 to 89.34 dm^3, we selected a radius of 50 cm as a compromise between considering the maximum lateral root spread area (assuming the general equation obtained by Casper et al. 2003, **Fig. S1**) and avoiding larger distances that could respond to ecological factors other than intraspecific competition. High values of the influence index indicate the presence of large neighbouring individuals located at short distances, whereas low values indicate the presence of few small neighbouring conespecifics. The resulting parameter was log-transformed to fit normality of residuals due to their asymmetric distribution biased to low values. We compared the influence index values in the different site x microsite combinations through an ANOVA and a Tukey post-hoc test. These analyses were performed with the spatstat package (Baddeley and Turner 2005) in R environment (R Development Core Team 2011).

We used General Linear Models (GLM) to test the effect of intra- and interspecific interactions on several estimates for H. spinosa performance: plant form (slenderness and canopy area) and reserve levels (NSC concentrations). Canopy form was related to the nominal factors site and microsite, and to quantitative variables plant age and the log-transformed influence index, a proxy of intraspecific interaction. Influence index was log-transformed since a preliminary analysis suggested a logarithmic relationship between influence index and response variables. We used the gamma distribution to normalize and homogenize residuals. We related NSC concentrations to the same nominal factors and to plant age as a covariate (intraspecific interaction was not considered because the samples for NSC analysis were not collected within transects), using the Gaussian distribution. Finally, we used Linear Mixed Models (LMM, McCullagh and Nelder, 1989) to assess the combined effect of site and microsite and their interaction on *H. spinosa* radial-growth trends during the last 21 years. This time interval was selected to include most *H. spinosa* individuals while minimizing the effect of age trends in young plants. We considered site, microsite and year as fixed effects (year as covariable), and the identity of *H. spinosa* individuals as random effect. Since we only considered one random effect, we checked if its inclusion improved the beyond optimal model for fixed effects (i.e., that with the more complex structure), in which we did not include the triple interaction. We estimated both models (with and without random effect) with the restricted maximum likelihood method (REML) and selected the optimal one by comparing them with the Akaike Information Criterion (AIC, see Bolker et al. 2009 for a summary of model selection methods and their advantages and main concerns). In a second step, we searched for the best structure of the fixed component by fitting all potential models that included the selected random effect structure and estimating them with the Maximum Likelihood (ML) method. We compared all possible models with AIC values and the final model was then refitted using REML to obtain estimates of factor effects. Since the residuals of the final model showed heterogeneity in the variances between groups by site, we incorporated an identity variance structure (varIdent) to satisfy model assumptions (Zuur et al. 2009). LMM analyses were performed with the nlme package (Pinheiro et al. 2011) in R environment (R Development Core Team 2011).

Results

Testing the SGH at the interspecific level

We compared soil depth within and outside the juniper canopy to evaluate how abiotic factors depend on microsite conditions and found that soils were deeper within the juniper canopy (mean depth ± SE = 11.8 ± 0.6 cm) than outside it (8.7 ± 0.7 cm; F = 10.389; *P* = 0.002). The interaction between site and microsite on soil depth was significant (F = 7.669; *P* = 0.006); at the slope site, soils were deeper within (12.6 ± 0.9 cm) juniper canopies than outside them (6.8 ± 1.0 cm), whereas at the valley site, there were no significant differences in soil depth between microsites (11.0 ± 1.0 cm within vs. 10.6 ± 0.8 cm outside juniper canopies).

Hormathophylla spinosa individuals at the slope site displayed an aggregated pattern with significant clustering at specific distances of 0.25 and 1 m from the focal plant (transect

S1) or from 0.25 up to 2.5 m (transect S2) (**Fig. 2**). At the valley site, H. spinosa individuals either did not show significant aggregation (transect V1) or presented only short-distance clustering up to 0.5 m (transect V2). Although spatial patterns on transects S1 and V2 may seem similar, the detected clustering on transects S1 and S2 were positively related to the presence of juniper canopies (S1: $\chi^2 = 23.934$, $P < 0.001$; S2: $\chi^2 = 32.595$, $P < 0.001$), whereas clustering on transect V2 was negatively associated with juniper canopies ($\chi^2 = 21.052$, $P < 0.001$). Plants on transect V1 were independent of juniper canopies ($\chi^2 = 1.553$; $P = 0.213$).

The age structure of *H. spinosa* populations was similar between sites and microsites (**Fig. 3**), with the oldest individual being 76 years old. Most individuals had established between the 1960s and 1980s, with low establishment rates since then. *H. spinosa* density was three times larger in the valley (2.00 individuals m^{-2}), than in the slope (0.64 individuals m^{-2}). In the valley, juniper branches were older than neighbouring *H. spinosa* individuals only in 17% of all analyzed cases (**Fig. 4a**), whereas on the slope all juniper branches were older than neighbouring *H. spinosa* individuals (**Fig. 4b**).

Including intraspecific competition in the SGH predictions

Influence index differed between sites, being significantly higher in the valley (mean ± SE = 40.12 ± 10.67 cm) than on the slope (15.52 ± 2.81 cm; n = 1286; F = 28.609; $P < 0.001$). The interaction term between site and microsite also had a significant effect on the influence index (F = 55.780; $P < 0.001$). In the valley the influence index was larger outside (46.22 ± 12.75 cm) than within juniper canopies (8.84 ± 1.29 cm), whereas the inverse

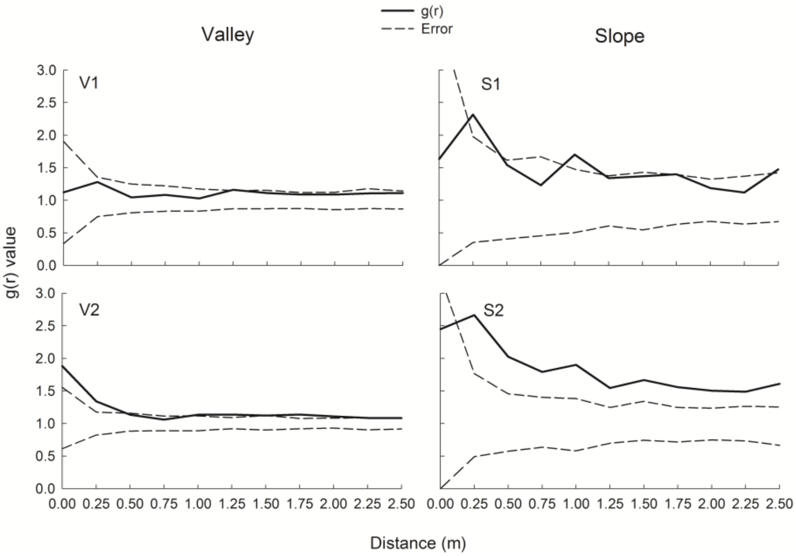

Figure 2. Values of the univariate pair-correlation function (g(r), continuous lines) calculated as a function of distance (r) and 5th and 95th percentiles based on 999 permutations of the original data (dashed lines) of the four transects in the two sites (valley, transects V1 and V2; slope, transects S1 and S2). Values of the g(r) functions above 1 and the upper percentile envelope indicate significantly aggregated patterns, whereas g(r) values above 1 but inside the envelopes indicate aggregated patterns which do not differ from spatial randomness.

pattern occurred on the slope, with higher influence indices within junipers (19.93 ± 3.91 cm) than outside them (4.85 ± 1.10 cm).

H. spinosa plants slenderness was affected by intra- and interspecific interactions, (residual deviance = 34.80 %), whereas canopy area responded to intraspecific interactions (residual deviance = 28.69 %). Irrespective of the site (i.e. valley vs. slope), plants were more slender within juniper canopies (mean ± SE =

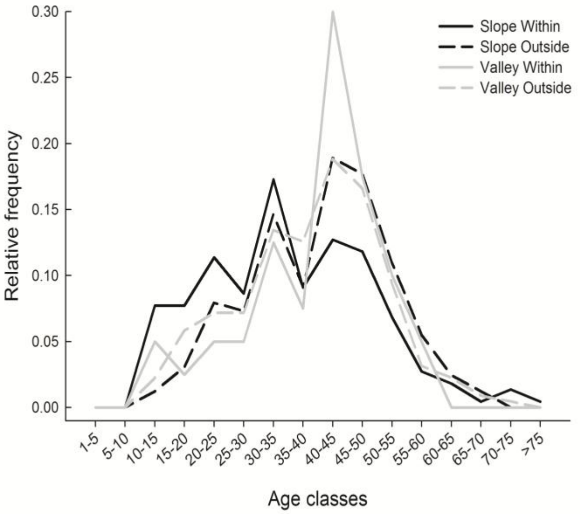

Figure 3. Age structure (5-year classes) of *H. spinosa* at slope and valley sites and considering each microsite (within or outside juniper canopy).

0.98 ± 0.03) than outside them (0.65 ± 0.02; **Table 2**). The interaction between site and microsite was also significant. *Hormathophylla spinosa* individuals outside junipers were more slender in the valley (0.70 ± 0.02) than on the slope (0.51 ± 0.03), whereas within juniper canopies the pattern was reversed, with plants being more slender on the slope (1.00 ± 0.03) than in the valley (0.91 ± 0.06). Age decreased plant slenderness, whereas conspecific density increased it. Canopy area differed marginally between the slope (373.9 ± 26.73 cm^2) and the valley (183.6 ± 11.62 cm^2), with no significant microsite effect. Intraspecific competition and age exerted negative and positive effects on canopy area, respectively.

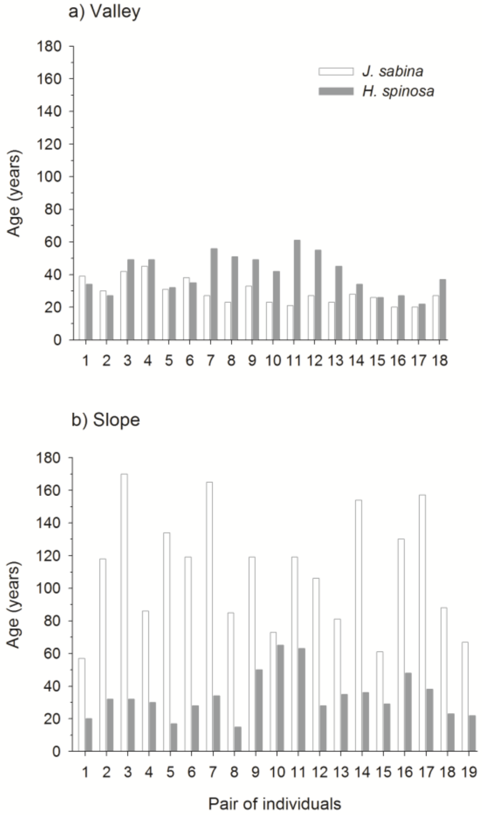

Figure 4. Paired ages of H. spinosa individuals and the nearest juniper (*J. sabina*) branch at valley and slope sites. a) At the valley site *H. spinosa* individuals were mostly (15 out of 18) older than junipers, indicating that they established in bare areas that were later colonized by junipers. b) At the slope site, by contrast, *H. spinosa* plants were always younger than junipers, and thus had established within areas previously occupied by junipers.

NSC concentrations in wood were affected by site and microsite (Table 2, $R^2_{adj} = 0.365$; $P < 0.001$) but not by plant age. NSC concentrations (mean ± SE) were higher in plants growing in the valley (10.06 ± 0.53 %) than on the slope (6.62 ± 0.39 %); they were also higher outside (8.79 ± 0.42 %) than within juniper

Table 2. Statistics of the General Linear Models used to evaluate differences between sites (valley vs. slope) and microsites (within vs. outside neighbouring juniper canopies) for slenderness, canopy area and non-structural carbohydrate (NSC) concentrations in wood of *H. spinosa* plants. An influence index was calculated in a radius of 50 cm around each focal plant. Bold values correspond to significant effects (P<0.05)

		Slenderness (n=561)		Area (n=561)		Total NSC (n=82)	
		F	*P*	F	*P*	F	*P*
Factors	Site	0.016	0.899	3.637	0.057	**27.762**	**<0.001**
	Microsite	**83.704**	**<0.001**	3.122	0.078	**6.111**	**0.016**
	Site* Microsite	**13.366**	**<0.001**	0.142	0.706	**6.245**	**0.015**
Covariates	Age	**47.334**	**<0.001**	**25.050**	**<0.001**	1.946	0.167
	Influence index	**32.160**	**<0.001**	**28.998**	**<0.001**	-	-

canopies (7.89 ± 0.63 %). The interaction between site and microsite significantly affected NSC concentrations. At the slope site, plants had higher NSC concentrations outside (8.11 ± 0.43 %) than within juniper canopies (5.21 ± 0.45 %), whereas in the valley there was no significant difference between them (10.72 ± 0.79 % within and 9.44 ± 0.70 % outside the canopy).

The optimal structure in GLMM fitted to radial growth included the site × position and site × year interactions as fixed factors, individual as random factor and an identity variance structure based on site and individual (**Table 3, Fig. S2**). All fixed factors and interactions significantly influenced radial growth (**Table 4**). Ring width was much lower in the valley (mean ± SE = 0.091 ± 0.008 mm) than on the slope (0.168 ± 0.017 mm) and this difference was stronger for plants located within juniper canopy areas (0.085 ± 0.010 mm vs. 0.196 ± 0.027 mm for valley and slope sites, respectively) than outside them (0.097 ± 0.013 mm vs. 0.144 ± 0.020 mm). Ring width decreased from 1990 to 2010 in all site × microsite combinations, but it was more pronounced at the slope than at the valley site (**Fig. 5a**) and also outside juniper canopies than within them (**Fig. 5b**).

Discussion

Our results support the hypothesis that both intra- and interspecific interactions among neighbouring plants are affected by environmental conditions: under lower stress conditions the net effect of intraspecific interactions shifted from neutral to negative, while interspecific interactions changed from exerting a net positive to a neutral or negative effect (Holzapfel and Mahall 1999, Sthultz et al. 2007, Soliveres et al. 2010, Xu et al. 2010). The range of analyses performed allowed us to detect the combined effects of intra- and interspecific interactions, abiotic conditions and historical processes on *H. spinosa* spatial patterns and performance. Overall, our findings point to the importance of considering both intra- and interspecific interactions when testing the SGH, despite its initial formulation at an interspecific level (Bertness and Callaway 1994, Callaway and Walker 1997). In this case it allowed us to develop a more complete picture of the system than that provided by the analysis of interspecific interactions alone. Although our study presents some limitations, since we only consider interactions between two species and in two contrasting environmental situations rather than across a whole gradient, it provides evidence of the need to consider the intraspecific component when studying plant-plant interactions in relation to stress at the community level.

Table 3. Linear mixed model construction (Zuur et al. 2009) and selection following the Akaike Information Criterion (AIC). Fixed effects are represented by site (valley or slope) and microsite (within or outside juniper canopies) as factors and year as covariable. Random effects comprise the identity of H. spinosa individuals (indicated as 1|Individual). BOM: beyond optimal model including the more complex structure of fixed effects without considering the triple interaction = Site x Microsite + Site x Year + Microsite x Year. RANDOM: represents the selected structure of random component = 1|Individual, varIdent (Individual|Site). ΔAIC: increment on AIC values respect to that of the model with lowest AIC. ML: maximum likelihood. REML: restricted maximum likelihood. NPar: number of estimated parameters.

Model	AIC	ΔAIC	NPar
Random component selection, adjusted with REML			
Growth ~ BOM + (1|Individual), varIdent(Individual|Site)	9175.735	0.000	10
Growth ~ BOM + (1|Individual), varIdent(Site)	9443.834	268.099	9
Growth ~ BOM, varIdent(Individual|Site)	9665.735	490.000	9
Growth ~ BOM, varIdent(Site)	9905.453	729.718	8
Fixed component selection, adjusted with ML			
Growth ~ Site*Position + Site*Year + RANDOM	9171.651	0.000	9
Growth ~ Site*Position + Site*Year + Position*Year + RANDOM	9175.735	4.084	10
Growth ~ Position+Site*Year + RANDOM	9181.058	9.407	8
Growth ~ Site*Position+Year + RANDOM	9181.502	9.851	8
Growth ~ Site*Year + RANDOM	9182.820	11.169	7
Growth ~ Site*Position + Position*Year + RANDOM	9185.052	13.401	9
Growth ~ Site+Position+Year + RANDOM	9190.912	19.261	7
Growth ~ Site+Year + RANDOM	9192.665	21.014	6
Growth ~ Site+Position*Year + RANDOM	9194.459	22.808	8
Growth ~ Site*Position + RANDOM	9197.262	25.611	7
Growth ~ Site+Position + RANDOM	9206.678	35.027	6
Growth ~ Site + RANDOM	9208.418	36.767	5
Growth ~ Position+Year + RANDOM	9228.113	56.462	6
Growth ~ Year + RANDOM	9229.215	57.564	5
Growth ~ Position*Year + RANDOM	9231.654	60.003	7
Growth ~ Position + RANDOM	9243.906	72.255	5
Final model, adjusted with REML			
Growth ~ Site*Position + Site*Year + (1|Individual), varIdent (Individual|Site)			9

Spatial patterns reflected the influence of interspecific interactions. Juniper plants modify the small-scale soil conditions, creating fertility islands by increasing soil depth, improving infiltration, increasing nutrient and organic matter contents and diminishing compaction (Verdú and García-Fayos 2003, Escudero et al. 2004). At the slope site, improved soil conditions may have enhanced the establishment of *H. spinosa* individuals within juniper canopies, a process already observed for this and other juniper species (Verdú et al. 2004, Montesinos et al. 2007, DeSoto et al. 2010), and thus leading to a clumped pattern of H. spinosa plants within juniper canopies. Contrastingly, on the deep soils found in the valley, where it is likely that the juniper canopy did not lead to deeper soils, *H. spinosa* establishment occurred preferentially in open areas. In the valley site, the age comparison between *H. spinosa* plants and the nearest juniper branch indicated that most of the *H. spinosa* individuals appearing within juniper plants had in fact also originally established in open areas, which were subsequently colonized by spreading junipers. Consequently, these results supported the SGH predictions (Bertness and Callaway 1994, Schenk et al. 2003), since under harsh environmental conditions *H. spinosa* was facilitated by juniper through the amelioration of soil conditions, but under mild conditions the interaction between both species was mainly neutral or even negative.

Combining different measurements of plant performance provided a more accurate view about the net response of individuals to intra- and interspecific interactions under contrasting environmental conditions. The more slender habit of *H. spinosa* plants within juniper canopies than outside them was probably due to altered light transmittance through the juniper canopy (Xu et al. 2010). At the same time, the more slender habit of plants growing in the valley than on the slope could be related to negative density-dependent effects resulting from higher intraspecific competition (Goldenheim et al. 2008). This could be

Table 4. Main statistics of the fixed factors for the Generalized Linear Mixed Models fitted to evaluate differences in annual radial growth (last 21 years) of *H. spinosa* plants as a function of site (valley *vs*. slope), microsite (within vs. outside neighbouring juniper canopies) and time (year, covariable). The identity of *H. spinosa* individuals was included as random factor. All fixed effects are significant (P<0.05).

Model: Growth ~ Site*Position + Site*Year + (1|Individual), varIdent (Individual|Site)

Fixed effects	Estimate	SE	*t*	*P*
Intercept	551.479	92.349	5.972	<0.001
Site (valley)	-422.175	102.609	-4.114	<0.001
Position (outside juniper)	-4.891	1.577	-3.101	0.003
Year	-0.266	0.046	-5.763	<0.001
Site (valley) * Position (outside juniper)	6.103	2.095	2.913	0.005
Site (valley) * Year	0.206	0.051	4.009	<0.001

Random effect	SD	SE
Individual	4.162	0.109

Variance function	Multiplication factor	Residual SE
Site = valley	0.528	0.100
Site = slope	1.000	0.189

the same reason that secondary growth was depressed in the valley, whereas the higher secondary growth within juniper on the slope was possibly due to interspecific facilitation. As expected, NSC levels showed an inverse pattern to secondary growth, and this concurs with previous work showing sink activity (i.e., investment on secondary growth) to be the major factor driving NSC levels in alpine environments (Körner, 2003a, Fajardo et al. 2012). Secondary growth shows higher sensitivity to environmental conditions than photosynthesis (Körner 2003b), and when secondary growth is limited plants can continue

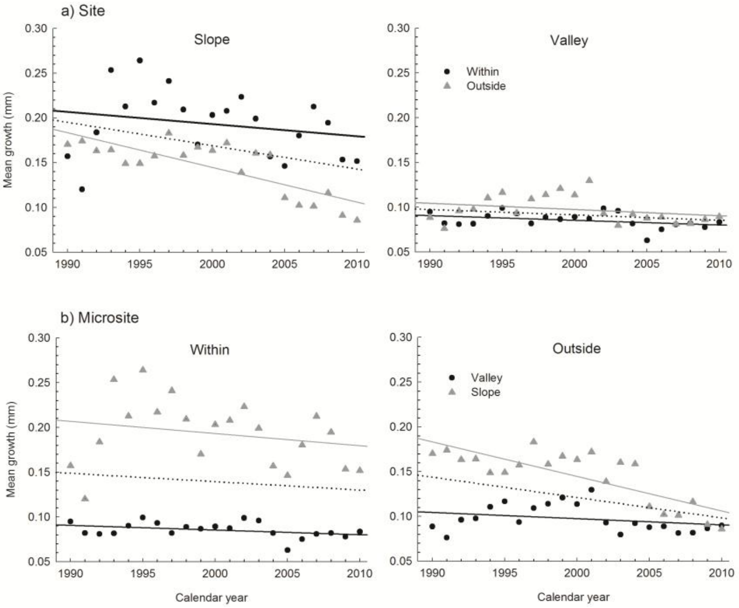

Figure 5. Trends in annual radial growth (shrub-ring width) of H. spinosa individuals as a function of site (slope vs. valley) (a) and microsite (within vs. outside juniper canopies) (b). Means were obtained from 15 individuals within juniper canopies and 17 outside them at the slope, and 20 individuals within and 18 outside juniper canopies at the valley. Continuous lines indicate linear growth trends for each site/microsite combination while dotted lines correspond to mean trends for each graph.

incorporating carbon up to a certain threshold and thus leading to an accumulation of NSC. Secondary growth limitation in alpine environments is mainly driven by low temperatures (Körner 2003b), but summer water deficit poses an additional constraint in Mediterranean high mountains (Giménez-Benavides et al. 2007, García-Cervigón et al. 2012). In fact, soil conditions have been considered more important than radiation as a limiting factor for plant development in Mediterranean mountains (Gómez-Aparicio et al. 2004). Our results are consistent with this hypothesis, through the impact on *H. spinosa* secondary growth and NSC levels of the amelioration of the harsh environmental conditions by the juniper on the slope (Maestre et al. 2002, Michalet et al. 2006). Moreover, negative effects of intraspecific interactions in the valley can be interpreted as the result of the enhancement of summer drought stress, due to increased root competition between conspecifics (Robberetch et al. 1983, Deng et al. 2006), resulting in lower growth rates and higher NSC accumulation for individual plants.

Conclusions

Our study highlights the temporal and spatial complexity of facilitative-competitive processes in plant communities from Mediterranean high mountains. At the population level, the more favourable abiotic conditions in the valley, compared to the slope, enabled greater *H. spinosa* establishment there. At the individual level, however, the stronger biotic constraints exerted by neighbouring conspecifics in the valley led to reduced individual growth rates and high NSC accumulation despite the more favourable abiotic conditions. The study of annual rings made possible the interpretation of the origins of plant-plant interactions within a temporal context.

Intra- and interspecific effects should be considered in order to evaluate the outcome of plant-plant interactions as a function of abiotic conditions. In our study case, under mild environmental conditions intraspecific competition was the leading force, whereas under harsher conditions interspecific facilitation became the dominant interaction. The direction, intensity and nature of biotic interactions changed as a consequence of the amelioration in abiotic conditions. The simultaneous analysis of intra- and interspecific interactions allowed us to improve our understanding of a two-species study system, and this should be considered when studying plant-plant interactions at the community level.

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Supplementary material

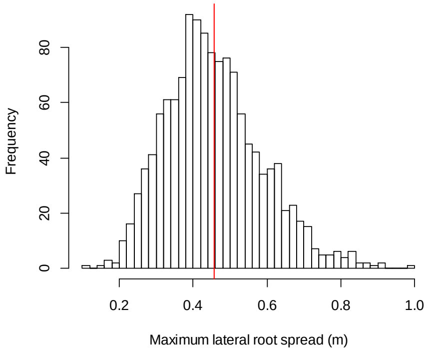

Figure S1. Histogram of the maximum lateral root spread of *H. spinosa* individuals, indicated as the distance from the stem. Red line represents the mean value. Maximum lateral root spread was calculated following the general equation obtained by Casper et al. (2003).

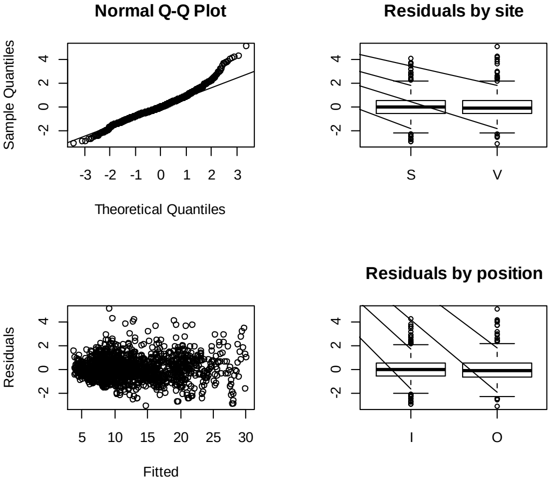

Figure S2. Residuals analysis of the LMM. Normal Q-Q plot, predicted vs. residuals plot and two boxplots of residuals by site (S, slope; V, valley) and by position (I, within juniper; O, outside juniper), the two assessed fixed factors, are shown.

Chapter 4

Facilitation promotes changes on leaf economics traits of a perennial forb

You can climb a ladder up to the sun
Or write a song nobody has sung
Or do something that's never been done

Coldplay

Facilitation promotes changes on leaf economics traits of a perennial forb

Introduction

Plants share a common strategy to convert sunlight into carbon-based chemical energy. However, they show strong differences in the way this energy is allocated into different tissues. Optimal allocation of resources is crucial in order to maximize plant success in an ecological and evolutionary context. The rationale behind these processes of investment and re-investment has a clear economic parallel, and has thus been termed ‘plant economics’ (Bloom et al. 1985). Different evidences suggest plant economics is driven by a general trade-off between rapid resource acquisition and resource conservation, leading to contrasting resource-acquisitive versus resource-conservative strategies (Díaz et al. 2004, Wright et al. 2004). The outcome of different strategies is expected to be reflected in demographic rates, in which variation is mediated by shifts in functional traits (Violle et al. 2007, Poorter et al. 2008, Martínez-Vilalta et al. 2010, Rüger et al. 2012). The trade-off between short-term carbon gain and long-term leaf persistence is linked to the balance between plant growth and survival (Poorter and Bongers 2006). High growth rates, for example, are promoted by cheaper, low-construction cost leaves and related to a resource-acquisitive strategy, whereas high survival is enhanced by the formation of long-lived and well protected leaves, following a more resource-conservative strategy (Poorter and Bongers 2006).

Functional traits vary in response to shifts in environmental conditions both at the inter- and intra-specific level (Ackerly et al. 2000, Westoby et al. 2002, Cornwell and Ackerly 2009, Albert et al. 2010b, Thuiller et al. 2010, Kichenin et al. 2013). As a result, distribution of trait values in a given community is mainly defined by abiotic and biotic filters that modify the wider and local environments (Cornwell et al. 2006). Negative interactions (predation and competition) have been considered as the main biotic filters, although the relevance of positive plant-plant interactions is being increasingly recognized (Callaway 1995, Brooker et al. 2008) and integrated into ecological theories (Stachowicz 2001, Bruno et al. 2003, Lortie et al. 2004, McIntire and Fajardo 2014). Nurse plants modify abiotic and biotic conditions and lead to net positive effects through different mechanisms, both direct (such as stress amelioration or novel habitat creation) and indirect (such as promoting the spread of negative competitive effects that cause a net positive outcome; Soliveres et al. 2011, Roy et al. 2013, McIntire and Fajardo 2014), and these processes may ultimately filter the trait distribution of facilitated species (McIntire and Fajardo 2014). So far it has been shown that facilitation increases the variability of trait values observed in the local community (Gross et al. 2009, 2013, Butterfield and Briggs 2011, Schöb et al. 2012).

Most research on trait variability has considered individual species as homogeneous entities by using mean trait values per species, despite great within-community variation in trait values being typical. Intraspecific trait variation appears to be an additional source increasing the variability of trait values that can allow individuals to adjust their performance along large environmental gradients, reducing or increasing the overlap in interspecific trait values. Although interspecific variability is usually higher than intraspecific (Hulshof and Swenson 2010), in some cases both are comparable (Messier et al. 2010) and, indeed, intraspecific trait variation plays a strong role in community assembly, promoting species coexistence by enabling species to pass through both abiotic and biotic filters (Jung et al. 2010). Since dispersion patterns of trait values within communities have been widely studied, the focus should now turn to mechanistic models focused on plant functional tradeoffs and their relationship with environment, as proposed by Adler et al. (2013). Considering intraspecific trait variation is a first key step towards this, since its omission may mask the detection of complex patterns that can even change the direction of the relationship between traits and environment (Albert et al. 2010b). Studies at the population level accounting for intraspecific trait variation and its relationship with environmental heterogeneity driven by both abiotic conditions and biotic interactions are still scarce (e.g. Gross et al. 2009, Schöb et al. 2012), and the simultaneous evaluation of several interrelated functional traits in this context is still missing.

Identifying filters that limit plant performance within a particular environment is necessary in order to detect the relevant traits that may be responding to those filters (Butterfield and Callaway 2013). In Mediterranean high mountains plants are subjected to two periods of climatic stress over the year: low winter temperatures delay the start of the growing season, while summer drought poses severe limits to growth during the optimal thermal period (García-Cervigón et al. 2012, Olano et al. 2013). Nurse plants, including cushions and prostrate shrubs, play an important role in these ecosystems, since they are associated with deeper and richer soils that alleviate summer drought stress (Verdú and García-Fayos 2003, Schöb et al. 2012, García-Cervigón et al. 2013). In addition, nurse canopies may create a more shaded environment that limits light availability for plants growing underneath (Holmgren et al. 1997). Disentangling the relevance of these potentially co-occurring filters requires the selection of an adequate battery of functional traits. In these ecosystems, the study of plant functional responses in a facilitative context would therefore require consideration not only of the main traits used to describe leaf strategies such as specific leaf area and foliar nutrient content (Westoby et al. 2002, Roche et al. 2004, Wright et al. 2004, 2005), but also other traits indicative of plant water balance and light interception.

In this paper we evaluate intraspecific shifts in leaf functional traits of a highly plastic perennial forb (*Helleborus foetidus* L.) in relation to the presence of a nurse plant (*Juniperus sabina* L.) in two geographically close sites with contrasting abiotic stress levels within a Mediterranean high mountain. Since the net outcome of plant-plant interactions may vary according to plant ontogeny and size (Callaway and Walker 1997, Miriti 2006, Núñez et al. 2009), we include plant age and height in our evaluation. We hypothesized that individuals growing under nurse canopies are subjected to lower abiotic stress and therefore show a more resource-acquisitive strategy than individuals growing in open areas. Consequently, plants under nurse canopies would be expected to show higher leaf nutrient contents and lower leaf construction costs related to lower investments on dry mass per area (that is, higher specific leaf area values, Westoby et al. 2002, Wright et al. 2004). Effects of habitat amelioration by nurse plants on the establishment, development or maintenance of a given species have been widely tested in the framework of the stress gradient hypothesis (Bertness and Callaway 1994, Maestre et al. 2009), whereby facilitative effects are expected to increase as stress does (He et al. 2013). In our study area, *J. sabina* causes a net positive effect on *H. foetidus* by increasing its fitness when stress is higher (García-Cervigón et al., unpublished data); we expected this positive effect to be reflected by variation in leaf functional traits of the beneficiary species (Poorter and Bongers

2006). We aimed to answer two specific questions: (1) are individual values of leaf functional traits different under nurse canopies and in open areas? And (2) how do intraspecific leaf economics traits shift according to stress reduction?

Material and methods

Study sites

Fieldwork was carried out in the Sierra de las Nieves Natural Park, Málaga province, southern Spain (36° 35’ N, 4° 59’ W, **Fig. 1a**). Soil parent material is limestone and climate is characterized by low winter temperatures and summer drought, a typical characteristic of Mediterranean high mountains that leads to a short vegetative period (García-Cervigón et al. 2012, Olano et al. 2013). Summer drought lasts approximately four months, from June to September (**Fig. 2**). In the study area, vegetation was dominated by *Juniperus sabina* (Savin juniper) with several species of spiny cushion plants (e.g. *Hormathophylla spinosa* (L.) P. Küpfer, *Astragalus granatensis* Lam., *Bupleurum fruticescens* subsp. *spinosum* (Gouan) O. Bolòs & Vigo).

We selected two sites above the tree-line elevation, which is located about 1700 m a.s.l., with contrasting water stress levels. The first site was located at 1850 m a.s.l. on the northwest-facing slope of the Torrecilla peak (Torrecilla hereafter). This site is characterized by stony and extremely shallow soils (**Fig. 1b**), with an average temperature of 9.3 °C and mean summer and winter temperatures of 17.4 and 2.7 °C, respectively (Ninyerola et al. 2005, **Fig. 2**). The second site was situated at 1700 m a.s.l. in a north-facing area near the Pilar de Tolox (Pilar hereafter), with deeper soils and higher soil moisture caused by oozing rock walls

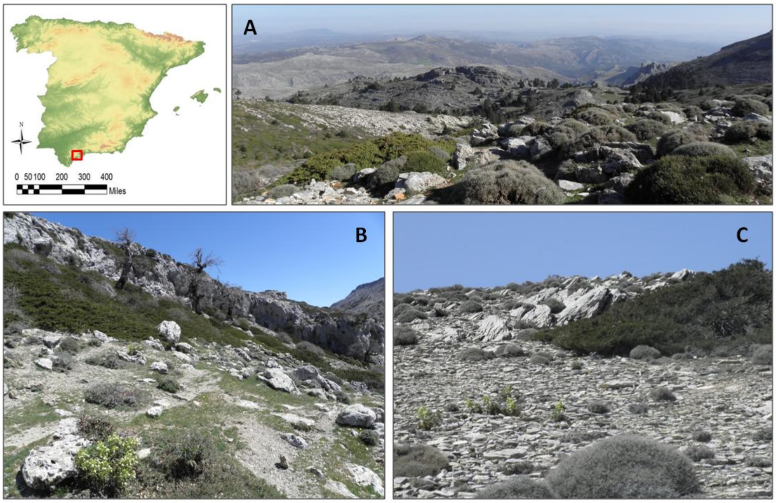

Figure 1. View and location of the study area. A. General view of the Sierra de las Nieves. B. Pilar de Tolox. C. Torrecilla peak. Note the differences in substrate texture.

(**Fig. 1c**). Average temperatures are only slightly higher than at Torrecilla (annual, summer and winter temperatures are 9.8, 17.9 and 3 °C, respectively, Ninyerola et al. 2005). Although mean annual precipitation is similar at both sites (1220 mm with a high inter-annual variation, 540-2600 mm), there are differences related to lower water availability at Torrecilla due to shallower soils and steeper slope than at Pilar.

Target species

Helleborus foetidus (Ranunculaceae) is a winter-flowering perennial forb that inhabits stony soils, hedgerows, scrublands and forest fringes. It prefers calcareous substrata in

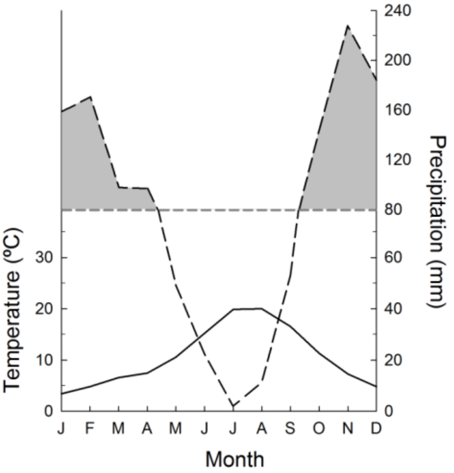

Figure 2. Climatic diagram of the study site constructed for the period 1965-2004. Data were provided by Quejigales meteorological station (36º 41' N, 5º 2' W, 1290 m a.s.l.).

humid and shady areas from 200 up to 2000 m a.s.l., and is widely distributed in western and southern Europe, also occurring in northern Africa (Nieto 1986). All plant parts accumulate glycosids that are highly toxic (Font Quer 1993) and as a result, foliage herbivory is virtually nonexistent (Herrera et al. 2002). *Juniperus sabina* (Cupressaceae) is a prostrate juniper species dominating landscapes above the tree line up to 2750 m a.s.l. in calcareous Mediterranean mountains. It is widely distributed across central and southern Europe, northern Africa and western Asia (López González 2004). *Juniperus sabina* acts as nurse plant in other Mediterranean high-mountains for different species (Verdú and García-Fayos 2003, García-Cervigón et al. 2013). A single individual can cover areas up to 0.1 ha, allowing a large number of woody and herbaceous species to establish within its canopy

(García-Cervigón pers. obs.). The juniper canopy provides protection from herbivory and ameliorates micro-environmental soil conditions by lowering maximal temperatures and increasing soil moisture and nutrient contents, although at the same time light availability is decreased (Verdú and García-Fayos 2003). Since *H. foetidus* is self-protected from herbivory, improvement of soil conditions seems the most plausible mechanism for *J. sabina* facilitation on *H. foetidus*. Juniper enhances fitness of *H. foetidus* in our study area (534 ± 87 seeds per plant under juniper canopies, 416 ± 63 seeds in open areas). The positive effect of juniper on fitness is especially pronounced at Torrecilla (460 ± 53 seeds under juniper canopies, 239 ±41 seeds in open areas), where juniper also leads to higher individual density (0.21 individuals m^{-2} under juniper canopies, 0.04 individuals m^{-2} in open areas; χ^2 = 70.555, $P < 0.001$; García-Cervigón et al. unpublished data).

Field sampling

Fieldwork was performed during spring 2011. In order to describe soil characteristics in both sites and how they were modified by juniper presence, we took 20 randomly selected soil samples at the surface (to a depth of 5 cm) at each site, 10 of them under juniper canopies and the other 10 in open areas (microsites hereafter). We took 10 additional soil samples from the whole study area in July, during the summer drought period, to compare soil water content under juniper canopies and in open areas (5 samples by microsite). Soil water content was also measured at each study site by time domain reflectometry (TDR) in the upper 50 cm of the soil profile. We used four permanent probes (ECH2O, Decagon Devices, Inc., Pullman, WA, USA) per site buried at 50 cm depth to take hourly measurements from February 2009 to

December 2014. We performed a calibration curve to convert soil dielectric measurements to estimates of volumetric water content (see Linares et al. 2012 for a detailed description of these measurements).

We randomly selected 120 *H. foetidus* individuals at each site, 60 located under the canopies of different juniper individuals and 60 in open areas. For each plant we recorded height, the number of conspecifics in an area of 1 m^2 and soil depth based on four measures within that area. Soil depth was considered as the distance between the soil surface and the bedrock, and was measured with a metal stick and a centimetre ruler. Each plant was sampled including aerial parts and the upper part of the root. The root collar was cut and preserved in formalin (ethanol, acetic acid and formaldehyde at 90:5:5). Aerial parts were sent to the laboratory and frozen until processed.

Laboratory processing

Soil analysis

As a prerequisite to confirm that the effect of the nurse plant promoted changes in environmental conditions that might reduce abiotic stress levels, we analyzed several soil parameters under juniper canopies and in open areas. Organic matter content, assimilable P and total N were determined following the Walkley, Olsen and Kjeldahl methods respectively (Olsen et al. 1954, Page 1982). Soil exchangeable K and Mg were determined by using atomic absorption spectrophotometer and extraction with ammonium acetate (Porta et al. 1986). pH was measured with a Crison MicropH 2001 pH-meter. Fresh samples to determine relative soil water content were weighed and then oven dried at 80°C until constant weight.

Trait data

Five leaves per individual were randomly selected among those showing no external damage (i.e., with no missing leaflets or insect wounds). In the case of individuals with less than five leaves, we processed all available leaves (only 13 individuals). Leaves were scanned with an Epson Perfection V750 PRO scan (Seiko Epson Corp., Japan) at 300 dpi resolution. Lamina and petiole lengths and areas were measured by using the ImageJ v.1.44 software (available at http://rsb.info.nih.gov/ij; developed by W. Rasband, NIH, Bethesda, MD). After scanning, samples were oven-dried at 80°C for 48 h and weighted with a 0.0001 g resolution. Three parameters were obtained for each individual leaf: leaf area (LA, cm^2), lamina/petiole length ratio (LPR) and specific leaf area (SLA, cm^2 g^{-1}), calculated as LA divided by the dry weight. Higher SLA means that leaf thickness, leaf density or both are lower. Among the several strongly interrelated traits that define the leaf economics spectrum (Wright et al. 2004, 2005), SLA has proved fundamental to describe leaf strategies (Westoby et al. 2002, Roche et al. 2004). SLA is positively related to maximum photosynthetic rates, stomatal conductance and relative growth rate, and negatively to leaf construction cost and leaf lifespan (Wright et al. 2004, Butterfield and Briggs 2011). However, the combined exploration with other traits can show a more accurate view of plant functioning. In our case, LA and LPR were selected as complementary to SLA for being related to light interception (Poorter 2009).

Dry leaves belonging to the same individual were pooled and finely ground. Leaf organic nitrogen and ortho-phosphate contents were estimated following the Kjeldahl method in a SKALAR San++ Analyzer (Skalar, Breda, The Netherlands) and expressed on a dry mass basis (mg g^{-1}). The ratio between them (N:P) was calculated as an indicator of nutrient limitation: ratios higher than 16 indicate P-limitation, whereas ratios lower than 14 indicate N-limitation (Koerselman and Meuleman 1996). Nitrogen and phosphorous contents in leaves are directly related to photosynthetic capacity. The proteins of Calvin cycle and the thylakoids represent most of the leaf nitrogen (Evans 1989), and leaf phosphorous is required for Rubisco regeneration (Jacob and Lawlor 1992) being thus positively correlated to the maximum carbon assimilation rate (Reich et al. 1995). Moreover, leaf phosphorous can influence the relation between photosynthesis and nitrogen (Reich et al. 2009). At the inter-specific level, a higher photosynthetic capacity is a result of higher leaf N and higher SLA (Reich et al. 1995), and therefore species with high SLA are expected to have higher leaf nutrient concentrations and photosynthetic rates (Wright et al. 2004).

Estimation of plant water use efficiency

Intrinsic water use efficiency (iWUE) is an indicator of the relationship between photosynthetic rates and stomatal conductance (McCarroll and Loader 2004). It has been widely used as an indicator of plant water relations and is potentially related to the leaf economics spectrum (Fortunel et al. 2012). To assess intrinsic water-use efficiency (iWUE) in the *H. foetidus* individuals, we measured their $^{13}C/^{12}C$ isotope ratios ($\delta^{13}C$) in leaves. The leaf powder previously obtained was weighed into tin cups and analyzed for $\delta^{13}C$ using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (UC Davis Stable Isotope Facility - University of California, Davis). Results were expressed as relative differences in $\delta^{13}C$ of leaves in parts per thousand (‰) relative to the standard V-PDB ($\delta^{13}C$ plant). $\delta^{13}C$ plant was used to calculate isotopic discrimination (Δ; Farquhar and Richards 1984); we then calculated the intrinsic water-use efficiency (iWUE; expressed in μmol of CO_2 per mol of H2O), using available data for $\delta^{13}C$ in atmospheric CO_2 and atmospheric CO_2 concentrations (McCarroll and Loader 2004) following the formula:

$$\mathrm{iWUE} = \ C_a(b' - \Delta)/[1.6\ (b' - a)]$$

where a is the fractionation during CO_2 diffusion through the stomata (4.4‰; Farquhar et al. 1981), b' is the fractionation associated with reactions by Rubisco and PEP carboxylase (27‰; Farquhar and Richards 1984) and Ca is the atmospheric CO_2 mole fraction.

Age estimation

Plant age was estimated from root collars. We obtained cross-sections about 10-15 μm-thick from root collars with a sledge microtome (H. Gärtner/F. H. Schweingruber, WSL, Birmensdorf, Switzerland), placed them on a slide and stained them with safranin (safranin 1% solution in ethanol) and Alcian blue (Alcian blue 1% solution in acetic acid). Following this procedure, unlignified cells appeared blue and lignified cells (i.e., those constituting growth rings) red; annual rings were then visually counted using the whole root collar surface.

Data analysis

Environmental variables

Differences in soil parameters (depth, pH and organic matter, P, N, Mg and K contents) between sites (Torrecilla and Pilar) and microsites (under juniper canopies and in open areas) were tested by two-factor Analysis of Variance (ANOVA) with the interaction and a Tukey post-hoc test. Given the low number of soil samples (n=40), we used the Benjamini and Hochberg (1995) method to adjust P values in post-hoc differences between sites and microsites. This method controls the false discovery rate and increases the power of the analysis. Soil water content under juniper canopies and in open areas was tested by one-factor ANOVA. To compare soil water content between sites we performed a one-factor ANOVA considering data for July.

Functional traits

To describe the level of intraspecific variation on our selected functional traits we calculated coefficients of variation (CV) for each one of the seven dependent variables (LA, LPR, SLA, N, P, N:P, iWUE) for all individuals and for each site × microsite combination. CV were computed as standard deviation divided by the mean value of each trait. For those parameters where multiple leaves per individual were measured (LA, LPR and SLA) averaged individual values had previously been calculated.

Linear Mixed Models (LMM) were used to test differences by site and microsite for those variables with five measurements per individual, namely LA, LPR and SLA. LA and LPR were previously log-transformed to fit normality and variance homogeneity of residuals. We constructed models including the fixed factors site and microsite and their interaction, as well as several covariables directly related to individuals to control for possible sources of intrinsic individual variation: plant age, plant height and the number of conspecifics. Microhabitat variables were not included because they were representative of sites and microsites (Fig. 3) and thus redundant. Individuals of *H. foetidus* were included as random effect to account for the non-independence structure of our data (five measurements of the same variable per plant). Since residuals of all models were normally distributed and had homogeneous variance, it was not necessary to include variance structures to satisfy model assumptions. We used *nlme* (Pinheiro et al. 2011) package in R environment (R Core Team 2013) for LMM construction. To calculate post-hoc differences between sites and microsites we used the Tukey test included in the *lsmeans* package (Lenth and Hervé 2014), and the *vioplot* package (Adler 2005) to create violin plots.

We constructed Linear Models (LM) for variables with one measurement per individual: leaf nitrogen, leaf phosphorous, N:P and iWUE. We used the same structure of explanatory variables and covariables as for LMM excluding the random part, and calculated post-hoc differences using the Tukey test in the *lsmeans* R package (Lenth and Hervé 2014).

To have a synthetic view of the relationships among all the dependent variables, we performed a principal component analysis (PCA) on an individual x leaf parameters matrix and plotted the results to show how leaf traits were distributed in each of the four site × microsite combinations. We previously analysed bivariate Pearson’s correlations between the seven dependent variables and excluded N:P from the PCA since it is a function of N and P contents. Moreover, to evaluate the effect of site and microsite on leaf traits configuration we performed a redundancy analysis (RDA, Legendre and Legendre 2012), including the environmental parameters per plant matrix (site × microsite) as a constraining matrix. The significance of all terms was evaluated using a Monte Carlo test with 999 permutations. These analyses were performed with the *vegan* package (Oksanen et al. 2012).

Results

Effects of juniper on environmental characteristics

Soil parameters showed a strong response to site and microsite effects. Soils were deeper at Pilar than at Torrecilla ($F_{1,185} = 52.1$, $P < 0.001$; **Fig. 3, Table S1**), and also deeper under juniper canopies than in open areas at both sites ($F_{1,185} = 35.1$, $P < 0.001$; model $R^2_{adj} = 0.32$, model $F_{3,185} = 29.7$, model $P < 0.001$). Organic matter content was higher under juniper canopies than in open areas ($F_{1,36} = 6.8$, $P = 0.013$). This difference was significant at Torrecilla, whereas at Pilar there were no differences between microsites ($F_{1,36} = 9.8$, $P = 0.004$; model $R^2_{adj} = 0.26$, model $F_{3,36} = 5.5$, model $P = 0.003$). Soil pH was higher at Torrecilla ($F_{1,36} = 5.8$, $P = 0.021$) and in open areas ($F_{1,36} = 6.8$, $P = 0.013$; model $R^2_{adj} = 0.21$, model $F_{3,36} = 4.05$, model $P = 0.009$). Soils had more N under juniper canopies than in open areas ($F_{1,36} = 14.6$, $P < 0.001$; model $R^2_{adj} = 0.31$, model $F_{3,36} = 6.7$, model $P = 0.001$); this difference was stronger at Torrecilla, whereas at Pilar the difference between microsites was not significant ($F_{1,36} = 5.0$, $P = 0.032$). Soil P was also higher at Pilar ($F_{1,36} = 14.9$, $P = 0.001$; model $R^2_{adj} = 0.244$, model $F_{3,36} = 5.2$, model $P = 0.004$). Soil K showed significant effects of both site ($F_{1,36} = 27.4$, $P < 0.001$) and

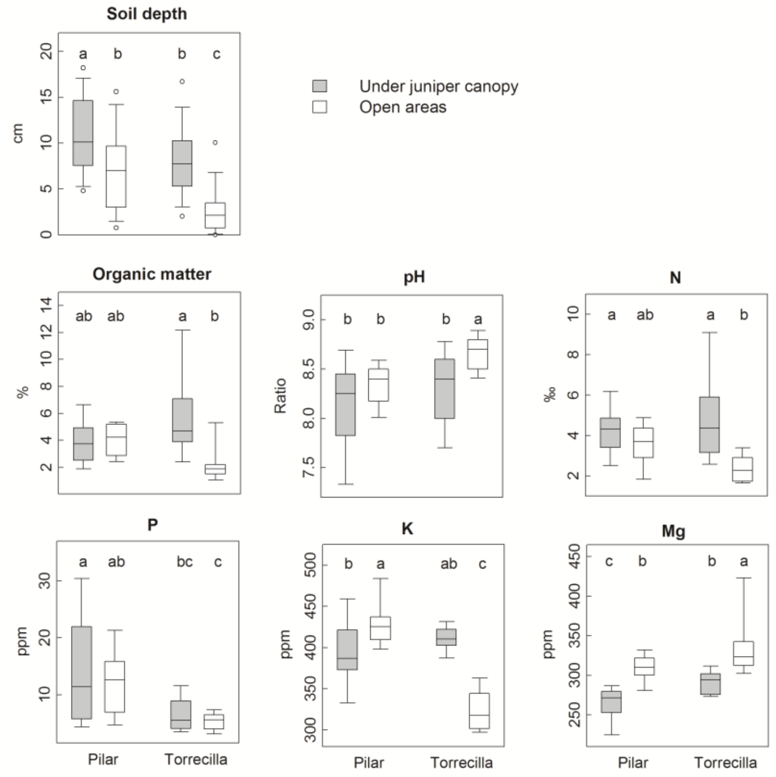

Figure 3. Boxplots of the seven soil variables. Boxes represent median, 25th and 75th percentiles, lines indicate 10th and 90th percentiles, and dots mark 5th and 95th percentiles. Significant differences are indicated by different letters.

microsite ($F_{1,36} = 10.0$, $P = 0.003$) with a highly significant interaction ($F_{1,36} = 56.6$, $P < 0.001$); K concentrations were higher under juniper canopies at Torrecilla whereas this pattern was reversed at Pilar (model $R^2_{adj} = 0.70$, model $F_{3,36} = 31.4$, model $P < 0.001$). Mg was higher at Torrecilla ($F_{1,36} = 11.57$, $P = 0.002$) and in open areas ($F_{1,36} = 33.7$, $P < 0.001$; model $R^2_{adj} = 0.52$, model $F_{3,36} = 15.1$, model $P < 0.001$). Soil water content was higher under juniper canopies (13.27 ± 2.95 %, mean ± SE) than in open areas (2.47 ± 0.76 %; $F_{1,8}$ = 12.6, P = 0.008; model R^2_{adj} = 0.56, model $F_{1,8}$ = 12.6, model P = 0.008), and was also higher at the low-stress site (21.56 ± 0.19 %) than at the high-stress site (17.13 ± 0.23 %; $F_{1,370}$ = 216.5, $P < 0.001$; model R^2_{adj} = 0.37, model $F_{1,370}$ = 216.5, model $P < 0.001$).

Effects of site and juniper on functional traits of *H. foetidus*

Functional traits showed high intraspecific variability (**Table S2**). Highest coefficients of variation were found for leaf area (52.3), followed by SLA (37.8) and N:P (30.5). LPR (24.5) and N (21.7) showed lower values, and the lowest coefficients of variation were for leaf phosphorous (16.35) and iWUE (12.21).

Plants had larger leaves with lower N:P ratios (model R^2_{adj} = 0.07, model $F_{6,182}$ = 3.5, model P = 0.002) and higher P content (model R^2_{adj} = 0.22, model $F_{6,182}$ = 10.0, model $P < 0.001$) at Pilar than at Torrecilla (**Table 1, Fig. 4, Table S1**). Plants also showed larger leaves with longer petioles in relation to laminas, higher SLA and lower iWUE (model R^2_{adj} = 0.18, model $F_{6,182}$ = 7.6, model $P < 0.001$) under juniper canopies than in open areas, but differences between microsites for all these variables were only significant at Torrecilla. When the effect of site and microsite was controlled, leaves were larger in taller plants and leaf N content was lower in older individuals with more conspecifics (model R^2_{adj} = 0.07, model $F_{6,182}$ = 3.0, model P = 0.004).

First, second and third PCA axes explained 38.40 %, 20.79 % and 14.15 % of the variance, respectively (**Fig. 5**). Leaf area and SLA showed similar positions and opposite directions to LPR and iWUE contributing to the largest part of first axis of variance. Leaf

Table 1. Main statistics of the linear models (N, P, N:P, iWUE) and linear mixed models (LA, SLA, LPR) fitted for the seven functional variables. LA: leaf area, SLA: specific leaf area, LPR: lamina/petiole ratio. $F_{1,182}$ for all explanatory variables in linear models; $F_{1,201}$ for all explanatory variables in linear mixed models. Significant *P*-values ($P < 0.05$) are highlighted in bold. Positive and negative significant estimates for the three covariables (plant age, plant height and number of conspecifics) are indicated by + and – signs in parentheses.

	Site		Microsite		Site × Microsite		Plant age		Plant height		Conspecifics	
	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
N	2.0	0.162	2.2	0.136	2.9	0.091	6.6	**0.011 (–)**	0.4	0.512	4.0	**0.046 (–)**
P	51.0	**<0.001**	0.1	0.807	4.3	**0.039**	0.2	0.649	2.8	0.094	1.9	0.173
N:P	4.4	**0.038**	1.5	0.221	5.1	**0.025**	4.7	**0.032 (–)**	1.1	0.315	4.6	**0.034 (–)**
iWUE	1.9	0.171	37.1	**<0.001**	4.2	**0.042**	0.1	0.781	2.3	0.130	0.0	0.960
LA	0.4	0.510	65.5	**<0.001**	9.2	**0.003**	1.3	0.259	17.7	**<0.001 (+)**	3.7	0.057
		Random effect SD		16.235								
SLA	34.9	**<0.001**	50.4	**<0.001**	14.8	**<0.001**	1.0	0.321	0.1	0.818	3.3	0.073
		Random effect SD		23.083								
LPR	1.7	0.199	38.1	**<0.001**	5.8	**0.017**	0.5	0.496	0.3	0.584	0.7	0.401
		Random effect SD		0.235								

nitrogen and phosphorous were orthogonal to the other four variables, with phosphorous increasing when nitrogen decreased. SLA was not correlated to leaf nitrogen ($r = 0.122$; $P = 0.091$) and showed a negative correlation with leaf phosphorous ($r = -0.260$; $P < 0.001$). Plants under juniper canopies showed larger leaves with higher SLA, greater petiole length to lamina ratios and lower iWUE. RDA showed that 34.35 % of the variance of the data could be explained by site, microsite and their interaction. Monte Carlo permutation tests showed that the effect of site, microsite and their interaction was significantly related to plant trait combinations (site $P < 0.001$; microsite $P < 0.001$; interaction $P < 0.001$).

Discussion

The change in leaf functional traits of our study species, H. foetidus, in response to the presence of the nurse plant, differed between sites. At the more stressful site (Torrecilla), four out of seven functional traits varied between plants growing in open areas and under nurse canopies, whereas at the less stressful site (Pilar) only one out of seven traits varied with juniper presence. Our results thus supported the hypothesis that alleviation of stress by junipers in more stressful environments (as Torrecilla site) may modify individual leaf functional traits promoting a more resource-acquisitive strategy (Westoby et al. 2002; Díaz et al. 2004). Leaf nutrient levels were not related to SLA or other traits indicative of light interception and water use efficiency, suggesting the existence of a different pattern of trait covariation at the intraspecific level (Boucher et al. 2013; Laforest-Lapointe et al. 2014) than generally observed at an interspecific level (Wright et al. 2004, 2005).

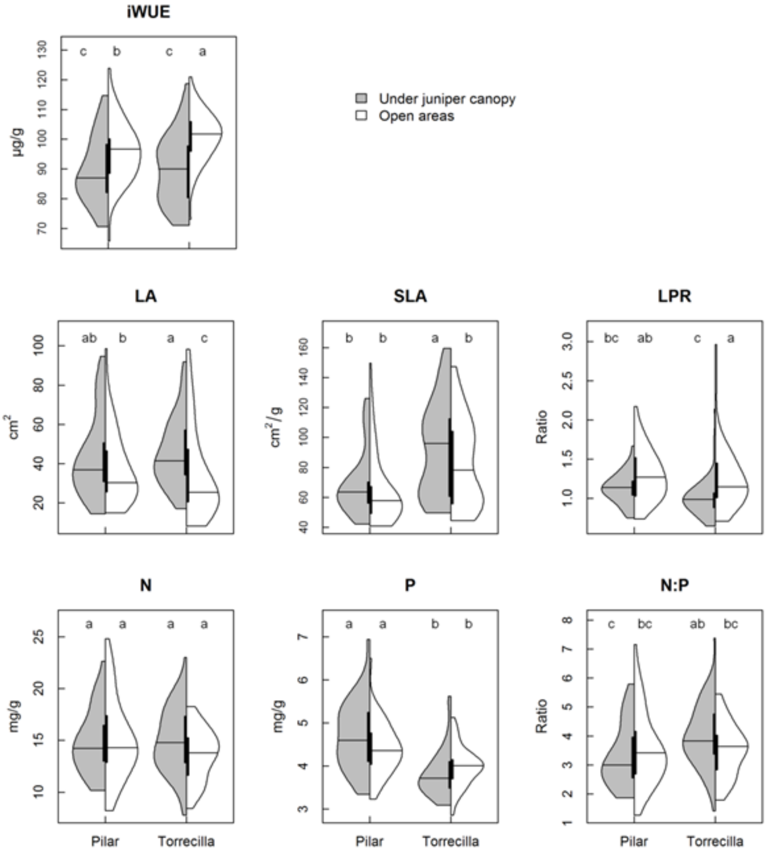

Figure 4. Split violin plots of the seven dependent variables: leaf area (LA), specific leaf area (SLA), limbo/petiole ratio (LPR), leaf nitrogen per mass (N), leaf phosphorus per mass (P), nitrogen/phosphorus ratio (N:P) and water use efficiency (iWUE). Boxes represent median, 25th and 75th percentiles, horizontal line indicates median. Vertical curves correspond to a rotated kernel estimate of probability density function of each parameter. Significant differences are indicated by different letters and can be consulted in Table 1.

Functional traits showed a considerable degree of intraspecific variation. Rates of variation found in a small area (two sites separated by a distance of 700 m) were comparable to those detected in other studies at both intra- and interspecific levels across much broader geographical gradients (see Fajardo and Piper 2011). However, Mediterranean species have scarcely been considered when accounting for interspecific trait variability (see Wright et al. 2004, 2005) and our study species has not previously been included in the literature about trait variability at all. Among all the traits we measured, leaf area and SLA showed the highest coefficients of variation (52.3 and 37.8, respectively).

This is consistent with some other studies on trait variation at the intraspecific level showing that SLA (or its inverse leaf mass per area) is one of the most variable leaf functional traits both at broad and at small scales (Albert et al. 2010a, Fajardo and Piper 2011, Boucher et al. 2013, Auger and Shipley 2013). The existence of this variability at small scales can be attributed to factors that modulate the micro-environmental context to which plants are subjected. Among these factors, biotic interactions play a key role. Competitive interactions between plants have been widely related to niche partitioning processes affecting functional traits even at the intraspecific level (Boucher et al. 2013). However, the role of positive interactions between plants in driving functional responses has been scarcely recognized (Butterfield and Callaway 2013; McIntire and Fajardo 2014). The proportion of the variation observed in our data that could be attributed to facilitative processes under contrasting abiotic environments highlights the importance of facilitation as a relevant source of intraspecific trait variation.

The effect of nurse plants on leaf functional traits at the more stressful site (Torrecilla) resulted in a positive effect on fitness (García-Cervigón et al. unpublished data) and was probably related to the alleviation of drought stress. At this site, plants growing under juniper canopies had larger leaves with a

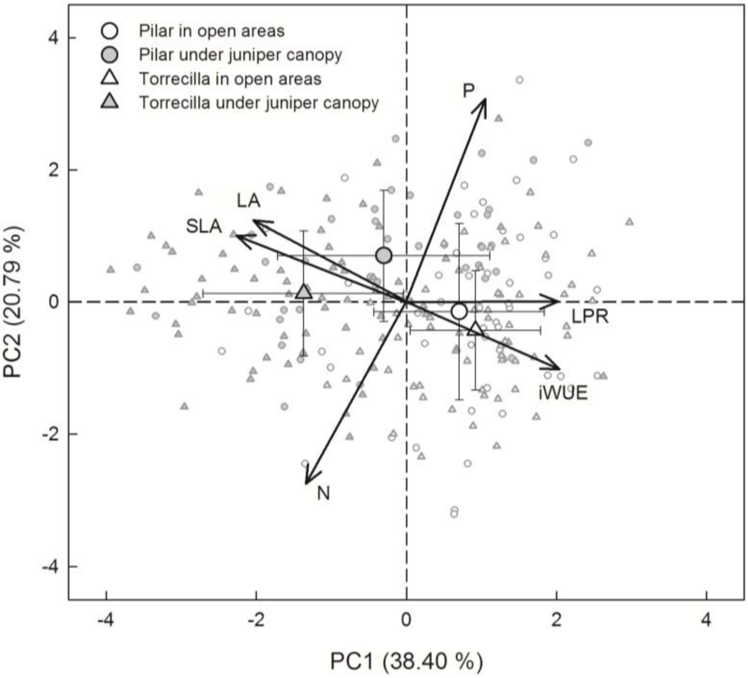

Figure 5. Principal component analysis plot showing the positions of individuals and the seven functional variables along the first two axes. LA: leaf area, SLA: specific leaf area, PRL: lamina/petiole ratio. Bigger symbols represent the mean value of all individuals on each of the four site × microsite combinations. Error bars represent one standard deviation.

higher SLA, longer petioles in relation to laminas and a lower iWUE. This may reflect the alleviation of drought stress under juniper canopies due to deeper soils, improved soil moisture (Verdú and García-Fayos 2003) and increased shading leading to a reduced transpirational demand. SLA increases with precipitation (Lamont et al. 2002) and water availability in soils (Cornwell and Ackerly 2009), but also may respond to additional environmental factors, increasing with higher temperatures (Roche et al. 2004; Poorter et al. 2009) or lower light levels

(Carlucci et al. 2015). In our case, the lower iWUE values and larger leaf areas combined with higher SLA support the predominant role of water stress in directing the observed shifts in these functional traits. Water availability is the main factor driving iWUE in drought-constrained Mediterranean environments (Moreno-Gutiérrez et al. 2012; Olano et al. 2014). Observed increases in the iWUE of plants growing in open areas were thus likely to be caused more by reductions in stomatal conductance, due to a more efficient stomatal regulation under dry conditions, than by higher photosynthetic rates (Ferrio and Voltas 2005; Linares and Camarero 2011). The lower SLA of plants growing in open areas supports this interpretation, given the usually observed positive correlation between SLA and photosynthetic rates (Shipley et al. 2005). Although water relations might play a more relevant role than the light environment in determining leaf structure (Koch et al. 2004), reduced light levels under juniper canopies could also promote leaves with larger surfaces and longer petioles in relation to laminas, exacerbating the differences in SLA between microsites. Shade may modify plant habit (García-Cervigón et al. 2013), promoting leaves with increased light interception surfaces to compensate for the decreased light levels (Carlucci et al. 2015). The increased self-shading effect caused by larger leaves could be mitigated by elongating petioles to achieve an optimal leaf spatial distribution under a context of higher competition for light with the nurse plant (Holmgren et al. 1997; Valladares and Niinemets 2008).

Leaf nutrient levels were not related to nurse plant presence at any of the two study sites. This was particularly remarkable in the case of nitrogen because the low N:P ratios found in all site × microsite combinations indicated a severe N-limitation (Koerselman and Meuleman 1996), and nitrogen content in the soil was significantly increased under juniper canopies at the high-stress site. The higher density of facilitated plants under nurse canopies at this site suggest that increased intraspecific competition for resources under favourable environmental conditions (Fajardo and McIntire 2011) may constrain leaf nitrogen. However, nurse plants might also be strong competitors for the soil N resources, constraining leaf nitrogen of facilitated plants. The positive effect of nurse plants observed on fitness at the high-stress site (García-Cervigón et al. unpublished data) does not invalidate this possibility, since the overall impact of plant-plant interactions is usually a combination of positive and negative effects acting simultaneously (Brooker and Callaghan 1998). Otherwise, while the total nitrogen content in the soil was higher under nurse canopies, the fraction available to the forbs might still be low due to the poor quality of organic matter generated by nurse plants, which would lead to low mineralisation rates (Murphy et al. 1998, Montane et al. 2010). The effect of site on leaf phosphorous content probably reflects site-specific differences in soil properties, since leaf phosphorous responds to soil phosphorous availability (Aerts and Chapin 2000) which is driven by geological processes (Delgado-Baquerizo et al. 2013).

Leaf nutrient content was orthogonal to the axis defined by the other leaf traits. At the interspecific level, leaf nitrogen and phosphorous show a positive correlation with SLA and LA, and a negative correlation with iWUE (Wright et al. 2004, Fortunel et al. 2012), but at the intraspecific level these relations are not so well established (Jackson et al. 2013). In our case, leaf nitrogen did not follow variations in SLA, and leaf phosphorous even showed a negative correlation with SLA. Similar results have been found in studies for single species (Boucher et al. 2013, Laforest-Lapointe et al. 2014) or when considering intraspecific trait covariation in studies with multiple species (Albert et al. 2010a). Albert et al. (2010b) proposed an explanation for the different patterns of covariation at inter– and intraspecific levels based on gradient lengths and differences in the species' niche. However, the contrasting responses of the two components of SLA (leaf density and thickness) to variations in nutrient availability at the intraspecific level pose an alternative explanation for this lack of correlation (Lamont et al. 2002). Small scale abiotic and biotic factors could have conditioned individual leaf nutrient levels, independently of other factors affecting SLA, LA and iWUE. In fact, when environmental conditions are fixed for all individuals, the intraspecific relation between leaf nitrogen and SLA remains positive (Vasseur et al. 2012). Although we only investigated a single species, our data support the findings of Jackson et al. (2013) identifying another species for which the pattern of intraspecific trait covariation does not reflect the generalised patterns observed in regional and global data sets (Díaz et al. 2004, Wright et al. 2004).

Plants living in open areas followed a more resource-conservative strategy than those growing under juniper canopies at the more stressful site (Torrecilla), where facilitation increased fitness and individual density (García-Cervigón et al. unpublished data). This result is in agreement with the idea that resource-conservative strategies are advantageous in environments that limit the possibilities for rapid carbon gain (Westoby et al. 2002). However, previous studies on the impact of facilitation on plant economic strategies at the interspecific level have shown differing outcomes. Butterfield and Briggs (2011) found that facilitated species show more resource-conservative strategies in the deserts of southwestern North America, whereas in another Mediterranean mountain site Schöb et al. (2012) found more resource-acquisitive strategies of facilitated plants under high abiotic stress levels. This discrepancy may be related to the degree of environmental stability, which may be critical in determining the functional strategy of facilitated plants (Butterfield and Callaway 2013). In the desert environments studied by Butterfield and Briggs (2011) favourable conditions in open areas are related to sporadic rains. This favours opportunistic strategies in which plants can exploit short water pulses, whereas more stable conditions under nurse plants promote more conservative functional strategies (Reich et al. 1995, Butterfield and Callaway 2013). In contrast, in Mediterranean mountains where stress is persistent during the growing season (Olano et al. 2013), nurse plants increase the long term resource levels, which favours less efficient resource-acquisitive strategies. Our results agree with those found in a similar Mediterranean mountain system at the interspecific level (Schöb et al. 2012). However, although the definition of the main ecological strategies (acquisitive vs. exploitative) may be robust to intraspecific variability (Albert et al. 2010a), it is worth noting that the opposite has also been documented. The variation in economics strategies at the intraspecific level is species-specific (Jackson et al. 2013) and the main interspecific functional trade-offs might not be reflected at the intraspecific level, as they differ even within and among populations of the same species (Boucher et al. 2013).

With this study we demonstrate that facilitation may be as important as abiotic conditions in driving intraspecific functional responses and leaf economics strategies. Alleviation of drought stress under nurse canopies was probably the main driver of shifts on SLA, leaf area, lamina/petiole ratio and iWUE at the more stressful site, leading to more resource-acquisitive strategies than in open areas. The relation between leaf nutrient content and the other traits was orthogonal, supporting the possible existence of an intraspecific pattern of trait covariation which is different from that observed at regional and global scales (Díaz et al. 2004, Wright et al. 2004, Jackson et al. 2013). We therefore conclude that negative and positive biotic interactions, as well as intraspecific trait variability, should be considered in future mechanistic models of plant functional responses to the environment; their synthesis would further increase the general understanding of community processes that trait-based approaches can bring.

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Supplementary material

Table S1. Mean ± standard error values for the seven environmental and the seven functional variables by site, microsite and their interaction. LA: leaf area; SLA: specific leaf area; LPR: lamina/petiole ratio; iWUE: intrinsic water use efficiency; W: within juniper canopies; O: open areas. Means and standard errors for LA, SLA and LPR were calculated by using the mean value of five leaves per individual.

	Site		Microsite		Site × microsite			
	Pilar	Torrecilla	Within	Open areas	Pilar W	Pilar O	Torrecilla W	Torrecilla O
Soil depth (cm)	9.19 ± 0.41	4.89 ± 0.39	8.57 ± 0.46	5.38 ± 0.39	10.70 ± 0.59	7.99 ± 0.53	6.92 ± 0.60	2.90 ± 0.35
Organic matter (%)	3.99 ± 0.31	3.88 ± 0.64	4.72 ± 0.56	3.15 ± 0.35	3.85 ± 0.52	4.13 ± 0.36	5.60 ± 0.94	2.16 ± 0.41
pH	8.25 ± 0.08	8.49 ± 0.05	8.24 ± 0.09	8.50 ± 0.05	8.16 ± 0.14	8.33 ± 0.06	8.31 ± 0.12	8.66 ± 0.05
N (‰)	3.88 ± 0.24	3.57 ± 0.43	4.51 ± 0.36	2.94 ± 0.23	4.21 ± 0.34	3.56 ± 0.31	4.80 ± 0.64	2.33 ± 0.21
P (ppm)	12.96 ± 1.72	5.89 ± 0.51	10.17 ± 0.77	8.69 ± 1.17	13.86 ± 3.06	12.06 ± 1.74	6.47 ± 0.90	5.31 ± 0.46
K (ppm)	410.0 ± 8.1	366.6 ± 11.0	401.4 ± 6.4	375.2 ± 13.3	391.9 ± 11.6	428.1 ± 8.3	410.9 ± 4.3	322.2 ± 7.4
Mg (ppm)	287.6 ± 6.3	312.8 ± 7.8	278.7 ± 4.7	321.7 ± 6.8	265.9 ± 6.2	309.3 ± 4.9	291.5 ± 4.3	334.1 ± 11.8
LA (cm^2)	40.9 ± 2.21	38.39 ± 2.03	50.25 ± 2.10	28.94 ± 1.51	46.56 ± 3.64	36.28 ± 2.52	52.47 ± 2.52	22.99 ± 1.37
SLA (cm^2/g)	65.6 ± 2.68	85.53 ± 2.85	90.97 ± 3.14	64.07 ± 2.16	71.16 ± 3.83	61.08 ± 3.62	102.93 ±	66.49 ± 2.56
LPR	1.15 ± 0.02	1.1 ± 0.03	1.01 ± 0.02	1.23 ± 0.03	1.07 ± 0.03	1.21 ± 0.03	0.97 ± 0.03	1.25 ± 0.04
N (mg/g)	15.01 ± 0.4	14.37 ± 0.27	14.95 ± 0.32	14.32 ± 0.33	14.86 ± 0.52	15.12 ± 0.59	15.00 ± 0.41	13.68 ± 0.34
P (mg/g)	4.54 ± 0.08	3.91 ± 0.05	4.16 ± 0.08	4.18 ± 0.06	4.7 ± 0.13	4.42 ± 0.09	3.83 ± 0.07	3.99 ± 0.06
N:P	3.45 ± 0.13	3.77 ± 0.10	3.75 ± 0.12	3.53 ± 0.11	3.3 ± 0.18	3.56 ± 0.19	4.01 ± 0.14	3.51 ± 0.12
iWUE (µmol CO_2/mol H_2O)	92.96 ± 1.27	95.08 ± 1.11	89.58 ± 1.19	98.67 ± 0.99	89.41 ± 1.91	95.84 ± 1.59	89.69 ± 1.53	100.97 ± 1.16

Table S2. Coefficients of variation for the nine functional variables in general and by site, microsite and site × microsite. LA: leaf area, SLA: specific leaf area, LPR: lamina/petiole ratio, W: within juniper canopies, O: open areas.

	Total	Site		Microsite		Site x Microsite			
		Pilar	Torrecilla	Within	Open areas	Pilar W	Pilar O	Torre W	Torre O
LA	52.28	47.70	55.69	40.24	51.16	46.30	45.53	36.63	43.37
SLA	37.83	36.08	35.15	33.24	32.99	31.87	38.88	27.17	28.03
LPR	24.50	18.36	28.25	22.49	22.27	16.79	17.93	25.18	25.11
N	21.70	23.46	20.16	20.74	22.57	20.71	25.62	20.92	17.95
P	16.35	15.68	13.35	18.74	13.78	16.99	13.92	14.58	11.74
N:P	30.50	34.55	27.38	30.29	30.59	32.79	35.73	27.12	25.88
iWUE	12.21	12.09	12.26	12.79	9.80	12.64	10.91	12.99	8.33

Chapter 5

Deconstructing facilitation along the life cycle: impacts of plant-plant interactions at vegetative and reproductive stages in a Mediterranean forb

Many times I've lied / Many times I've listened
Many times I've wondered how much there is to know

Led Zeppelin

Deconstructing facilitation along the life cycle: impacts of plant-plant interactions at vegetative and reproductive stages in a Mediterranean forb

Introduction

Positive interactions between plants are one of the major forces shaping community structure and diversity (Brooker et al. 2008, McIntire and Fajardo 2014). Nurse plants facilitate the presence and persistence of protégées through different mechanisms including the amelioration of various stresses, whether physical (e.g., direct effects of wind), physiological (e.g., freezing by low temperatures or desiccation by drought) or biotic (e.g., competition or predation; Stachowicz 2001). Through these effects facilitative interactions may modify community structure by influencing population dynamics as well as inter- and intraspecific relationships among individuals of the facilitated species (Eckstein 2005, Soliveres et al. 2011). As a consequence, facilitation may enable the expansion of distribution ranges of species by enlarging their tolerance limits, thus increasing local species richness (Hacker and Gaines 1997), maintaining plant diversity and allowing the persistence of communities in highly stressful environments (Le Bagousse-Pinguet et al. 2014, Soliveres and Maestre 2014).

The effect of an overall facilitative interaction on the population dynamics of protégées may vary depending on the vital rate under consideration, since the nurse-protégées interaction does not impact all vital rates simultaneously and in the same direction (Holzapfel and Mahall 1999, Eckstein 2005).

For example, the different microenvironmental conditions created under canopies of the nurse plant with respect to those existing in open areas can enhance seedling survival due to the alleviation of water stress, but at the same time the effect on reproduction and growth may be negative due to competition for light (Soliveres et al. 2010). The importance of these effects may shift depending on local moisture availability; that is, they may vary depending on the environmental context (Eckstein 2005, He et al. 2013). Moreover, the absence of a net observable effect of facilitation in the observed values of a given vital rate does not mean the absence of contrasting effects of plant-plant interactions. For instance, higher flowering probability in open areas due to lower competition for light than under nurse canopies (Eckstein 2005) combined with higher fruit set under nurse canopies due to shared pollinators between nurse and facilitated plants (Moeller 2004) might produce a similar net reproductive output in open areas and under nurse canopies. In that case, open areas and nurse canopies would be equally suitable for a given species (balanced selection, Barton and Keightley 2002) despite the contrasting effects of plant-plant interactions. Thus, the final outcome of plant-plant interactions on population dynamics is the net result of the simultaneous action of positive and negative effects on vital rates and on different parts of the life cycle.

Mediterranean high-mountains are harsh environ-ments for plants due to the combination of a long cold period in winter and drought during part of the short growing season (Giménez-Benavides et al. 2007, García-Cervigón et al. 2012, Olano et al. 2013). Facilitation is a decisive force in these singular conditions, enabling the maintenance of a diverse plant community with a high degree of endemicity (Väre et al. 2003, McIntire and Fajardo 2014). Understanding the effects of plant-plant positive interactions on different stages of the life cycle of protégées is critical to predict high-mountain response to ongoing climate change scenarios, especially since Mediterranean high mountains are particularly vulnerable to climate warming (Nogues-Bravo et al. 2008). In order to shed light on this topic, we studied the effects of a dominant shrub (*Juniperus sabina* L.) on several vegetative and reproductive variables of a perennial forb (*Helleborus foetidus* L.) comparing two geographically close sites with contrasting abiotic conditions (García-Cervigón et al. in press). On the one hand, we wanted to test if these plant-plant interactions affected the different stages of the life cycle of *Helleborus foetidus* simultaneously and in the same direction (positive or negative). Secondly, we studied in detail the impact of the dominant shrub on fecundity, examining sequential effects on different stages of the reproductive process, from flowering to seed production. We expected that variations in the direction and intensity of the nurse plant effect would depend on the abiotic conditions, the vital rate under consideration and the step of the reproductive process taken into account. Due to the combination of direct and indirect effects included in our hypotheses, we adopted a statistical framework of structural equation modeling (Grace 2006) combined with linear and additive models to answer the following questions: (1) Are plant density, age structure, growth, reproduction and fecundity of *Helleborus foetidus* modified by the presence of the nurse plant? (2) Are the effects of the nurse plant on these vital traits similar under contrasting abiotic conditions? And (3) which phases of the reproductive process are affected by the nurse plant?

Material and methods

Study area and target species

The study area was located in the Sierra de las Nieves Natural Park, Málaga province, southern Spain (36º 35’ N, 4º 59’ W), in the oromediterranean climatic belt (Rivas-Martínez and Loidi 1997). Average annual temperature is 10.6 °C with mean summer and winter temperatures of 18.8 and 4.4 °C, respectively. Mean annual precipitation (1220 mm) shows a high inter-annual variation (540-2600 mm) and is not uniformly distributed throughout the year; rainfall has its minimum in summer, leading to a drought period that lasts from June to August. Vegetation is dominated by the Savin juniper (*Juniperus sabina*) and a rich assemblage of spiny cushion plants including *Hormathophylla spinosa* (L.) P. Küpfer, *Astragalus granatensis* Lam. and *Bupleurum fruticescens* subsp. *spinosum* (Gouan) O. Bolòs & Vigo.

Helleborus foetidus (Ranunculaceae) is a perennial forb that inhabits stony soils, hedgerows, scrublands and forest fringes preferably in calcareous substrata of humid and shady areas. It is widely distributed in Western and Southern Europe, reaching northern Africa (Nieto 1986) with an altitudinal range from 200 up to 2000 m a.s.l. Each plant consists of one to several stems that develop a terminal inflorescence in early-mid winter. Flowers typically contain five nectaries and are mostly pollinated by bumblebees (Canto et al. 2008, Vesprini et al. 2008), although *H. foetidus* may be an autonomous self-pollinated plant (Herrera et al. 2001). Flowers are apocarpous, with one to five carpels (usually two or three) each containing eight to fifteen elaiosome-bearing seeds (Herrera et al. 2002) that are dispersed by ants (Garrido et al. 2002). The main fruit and seed predator is the wood mouse (*Apodemus sylvaticus* L.; Fedriani 2005). All plant parts accumulate glycosids that are highly toxic (Font Quer 1993) and as a result, foliage herbivory is virtually nonexistent (Herrera et al. 2002).

The prostrate Savin juniper (*Juniperus sabina*) is a dominant species above the treeline in calcareous Mediterranean mountains. It is widely distributed along central and southern Europe, northern Africa and western Asia and can appear up to 2750 m a.s.l. (López González 2004). *Juniperus sabina* acts as nurse plant for different species mainly through the amelioration of soil abiotic conditions (Verdú and García-Fayos 2003, García-Cervigón et al. 2013). A single individual can cover areas up to 0.1 ha, allowing a large number of woody and herbaceous species to establish under its canopy (García-Cervigón, pers. obs.). In the study area, *J. sabina* modifies the leaf functional traits and economics strategies of *H. foetidus* (García-Cervigón et al. in press).

Field sampling

We selected two sites above the treeline, at 1700 and 1850 m a.s.l. respectively, that provide contrasting environmental conditions. The higher altitude site is characterized by stony and extremely shallow soils, is highly exposed to prevalent winds and contains less nitrogen, phosphorous and potassium than the lower altitude one, which has deeper soils and is sheltered from dominant winds by rock walls. Abiotic stress is thus higher at the high altitude site (high-stress site hereafter) than at the lower altitude one (low-stress site). Soil depth increases and magnesium levels decrease under juniper canopies at both sites, and at the high-stress site organic matter, nitrogen and potassium levels also increase under juniper canopies. Soil characteristics in both sites and how they differ in relation to juniper presence are analyzed in detail in García-Cervigón et al. (in press).

Dataset 1: In spring 2012 we randomly sampled 120 *H. foetidus* individuals at each site, 60 under juniper canopies and 60 in open areas (microsites hereafter). For each individual we recorded its height, number of leaves and number of flowers, and collected the root collar. Root collars were cut and preserved into formalin (ethanol, acetic acid and formaldehyde at 90:5:5) until processed in the lab.

Dataset 2: In summer 2013 we identified two transects per site. Transects were located outside the previously sampled areas and perpendicular to the maximum slope. Sampling involved walking along the transect and searching within a 10 m wide band until we counted 60 individuals. At the low-stress site this required two transects of 10 x 30 m and 10 x 40 m; at the high-stress site plant density was lower, and transects of 10 x 30 m and 10 x 80 m were needed. For each *Helleborus* plant that was found along the transects, we measured the height and recorded the microsite (whether they lay under juniper canopies or in open areas), reproductive status (vegetative or reproductive individuals) and number of conspecifics in a radius of 1 m. In order to assess the effect of site and juniper on fecundity, we collected flowering stems of 40 individuals per site (20 per microsite), selecting the first 10 individuals of each microsite type in each transect. When not enough flowering individuals were found in a particular microsite in one transect, we randomly selected individuals outside transects until the required number was achieved.

Laboratory processing

Age and secondary growth

Plant age and secondary growth were estimated using cross-sections of approximately 10-15 µm from root collars collected in dataset 1. Cross-sections were obtained with a sledge microtome (H. Gärtner/F. H. Schweingruber, WSL, Birmensdorf, Switzerland), placed on a slide and stained with safranin (safranin 1% solution in ethanol) and Alcian blue (Alcian blue 1% solution in acetic acid), so that unlignified cells appear blue and lignified cells (i.e. those constituting growth rings) red. Thin-sections were dehydrated using a series of solutions of increasing ethanol concentration, washed with xylol and then permanently preserved by embedding them into Eukitt glue (Kindler GmbH, Freiburg, Germany). Images of the whole sections were captured with a Nikon D90 digital camera mounted on a Nikon Eclipse 50i optical microscope with different levels of magnification (from x 20 to x 200). When a whole section could not be captured in a single picture, sequential images were merged (PTGUI, ver. 8.3.10 pro, New House Internet Services B.V., Rotterdam, the Netherlands). We converted photographs into grayscale images and traced a radius from pith to bark, visually delimited each annual ring and measured them. Since some stems were partially rotten, we used a final sample size of 48 individuals under juniper canopies at the low-stress site and 60 in all other cases. Image analysis was performed with ImageJ (v. 1.44; http://rsb.info.nih.gov/ij; W. Rasband, National Institutes of Health, Bethesda, MD, USA).

Reproductive variables

We counted the number of developed and aborted flowers per plant and the number of developed and aborted carpels per flower on individuals from dataset 2. We also counted the number of developed and aborted seeds per carpel on 15 flowers per plant (or all of them when plants had less than 15 flowers). We calculated fruit set (percentage of fructifying flowers), mean number of developed carpels per flower and mean number of developed seeds per carpel. We defined individual fecundity as the total number of developed seeds per plant, and this was estimated by multiplying the mean number of developed seeds per carpel by the number of developed carpels per flower and the number of developed flowers per plant.

Statistical analyses

Age structure and spatial distribution

We used data on individual plant age from dataset 1 to evaluate if site by microsite combination affected *Helleborus* age structure. Confidence intervals for mean age were calculated by bootstrapping with 999 replications. We used *H. foetidus* transect data from dataset 2 to determine whether juniper affected the spatial distribution of the protégée plant. We compared plant density per microsite in both sites and performed χ^2 tests to compare the expected and observed number of individuals per microsite according to the surface of each transect covered by juniper (**Table 1**).

Table 1. List of vegetative, reproductive and environmental variables used in the study and analyses in which they were included.

Variable	**Obtention**	**Analyses in which it is included**
Vegetative		
Secondary growth	Ring width (μm)	GAMM
Age	Number of rings	Mean age comparison, age structure, LM and GAMM (covariate)
Number of leaves	Total count	LM
Plant height	From soil surface to the top (cm)	LM; LM and GLM (covariate)
Reproductive		
Probability of reproduction	Reproductive *vs*. vegetative plants in transects	GLM
Number of flowers	Total count	LM, SEM
Fruit set	Percentage of fructifying flowers	LM, SEM
Carpels per flower	Average number from all flowers per plant	LM, SEM
Seeds per carpel	Average number from 15 flowers per plant	LM, SEM
Fecundity	Total seed number (seeds per carpel x carpels per flower x flowers per plant)	LM, SEM
Environmental		
Neighbours	Number of conspecifics in 1 m^2	LM (covariate)
Spatial distribution	Position in transects	χ^2 for plant density

Vegetative and reproductive variables

To determine which factors affected number of leaves we built a linear model including site (high-stress versus low-stress), microsite (under juniper canopies versus in open areas), their interaction, age and height as explanatory variables using dataset 1. Factors determining plant height were evaluated by means of a linear model including site, microsite, their interaction and age as explanatory variables. Potential factors affecting secondary growth were adjusted to a generalized additive mixed model (GAMM) including site, microsite, their interaction, plant height and age of each ring as fixed factors. Age was modeled with a spline curve due to the non-linear relationship between age and secondary growth in forbs (Olano et al. 2013) and individual identity was included as random factor, since we had repeated measures of secondary growth per plant, corresponding to the different annual rings (**Table 1**).

We used data from dataset 2 to analyse which factors affected the reproductive output. The probability of reproduction (flowering *versus* non-flowering plants) was adjusted to a logistic regression (generalized linear model with binomial family of error distribution) including site, microsite and their interaction, and plant height as explanatory variables. We adjusted linear models for reproductive variables (total number of flowers, fruit set, mean number of carpels per flower, mean number of developed seeds per carpel and fecundity) considering the same explanatory variables (site, microsite, their interaction and plant height) and the number of conspecifics within a radius of 1 m^2. Total number of flowers and fecundity were previously log-transformed to achieve normality. We calculated post-hoc differences between sites and microsites using the pairwise t-test and the Benjamini and Hochberg (1995) method to adjust *P* values (see explanation at the end of this section). These analyses were performed in R environment (R core team 2013) using the mgcv package (Wood 2006, 2011) for GAMM analysis and lsmeans package (Lenth and Hervé 2014) for post-hoc comparisons.

Reproductive process

We used a structural equation model (SEM) to evaluate the effects of microsite on fecundity at several sequential steps of the reproductive process from flower production to seed maturation (**Fig. 2a**). SEM assess how well data support a set of hypothesized causal relationships between different variables by including both direct and indirect effects (Grace 2006) that cannot be identified with either linear models or GAMM. We built a hypothesized set of relationships that was tested separately for each site because we expected that different stress levels may lead to variations in the proposed relationships between variables. We included direct effects of microsite on number of carpels per flower, fruit set and number of seeds per carpel, since microsites differ in resource availability (García-Cervigón et al. in press), and this may influence plant investment in reproduction. In the case of fruit set microsites may also differ in pollinator service due to differential attraction to bumblebees under nurse canopies vs. open areas or to differences in flowering phenology (Herrera et al. 2001). Fecundity was considered the result of the additive effect of number of flowers, fruit set, number of carpels per flower and number of seeds per carpel.

Model parameters were estimated with maximum likelihood. Global model fit was assessed using the likelihood chi-square value complemented by the goodness of fit index (GFI), the normed-fit index (NFI) and the root mean square error of approximation (RMSEA). Non-significant P-values corresponding to the chi-square test indicate a good fit. GFI and NFI range between 0 and 1, with values above 0.90 indicating a good fit. Finally, RMSEA is less than 0.05 for very good models (those with a close fit), less than 0.1 for models that fit adequately, and

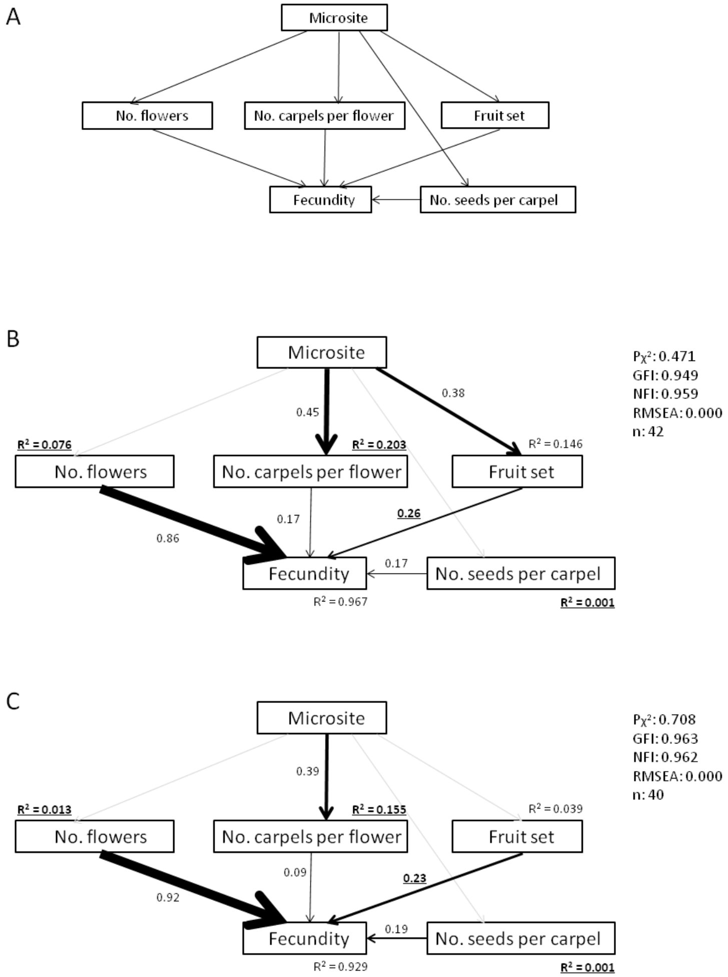

Figure 2. Hypothetical (A) and adjusted structural equation models at the high-stress (B) and low-stress (C) site for the effects of facilitation on all the steps of the reproductive process from flower number to seed production. Only significant path coefficients are shown in adjusted models, while non-significant paths appear as light grey arrows. Arrow width is proportional to path coefficients. Path coefficients that were significantly different between sites are highlighted in bold. Model fit statistics: $P\chi^2$, probability value associated to χ^2 statistic; GFI, goodness of fit index; NFI, normed fit index; RMSEA, root mean square error of approximation.

greater than 0.1 for poorly fitted models. Significance of path coefficients was evaluated by a multivariate Wald test.

Adjusted models were then statistically compared between sites using multigroup SEM to determine which paths differed in their behavior depending on site. A constrained model in which all free parameters were forced to be equal across the two sites was built, developing then a series of nested models where equality constraints were removed one at a time to detect which one would significantly improve the model (Shipley 2002). Differences in $\chi 2$ statistics between the fully constrained model and models with a particular free constraint indicated differences in that parameter value between the two sites. Since the number of models to compare was relatively high, we used the classical one-stage method of correction based on false discovery rates of Benjamini and Hochberg (1995) to evaluate their significance. Multiple comparison procedures based on false discovery rates are less conservative than the most commonly used Bonferroni correction, which increases the number of wrong rejections of true hypotheses as the number of hypotheses being simultaneously tested increases (Pike 2011). SEM analyses were performed with AMOS 18.0 software (AMOS Development Corp., Mount Pleasant, South Carolina, USA).

Results

Population structure and vegetative variables

Age structures were similar between sites and microsites (**Fig. 3**). A recruitment peak was evident in 2006, particularly in open areas at the high-stress site where 44.8 % of the sampled individuals established in 2006. Plants were older at the high-stress site (mean, lower 2.5%-upper 97.5%; 7.3 years, 6.3-8.3) than at the low-stress site (5.5 years, 4.8-6.1). Plants in open areas had intermediate age values (high-stress site 6.4 years, 5.7-7.4; low-stress site 6.2 years, 5.6-7.0). At the high-stress site the oldest individuals were 19 and 22 years old, whereas at the low-stress site the oldest individual was only 11 years old. At the high-stress site *Helleborus* individual density was higher than expected under juniper canopies, and lower than expected in open areas (0.21 *vs*. 0.04 individuals m^{-2}; $\chi^2 = 70.555$, $P < 0.001$), whereas at the low-stress site densities did not differ significantly between microsites (0.20 under juniper canopies and 0.17 individuals m^{-2} in open areas; $\chi^2 = 0.630$, $P = 0.427$).

A significant portion of the variance in leaf number could be explained by the linear model ($R^2_{adj} = 0.292$, $P < 0.001$). Plants had more leaves at the low-stress site than at the high-stress site (36 ± 3 *vs*. 18 ± 1 leaves per plant; F = 45.499, $P < 0.001$), with additional positive effects of plant age (F=21.143, $P < 0.001$) and height (F=24.207, $P < 0.001$). Plant height was affected by microsite, the interaction between site and microsite and plant age (model $R^2_{adj} = 0.371$, model $P < 0.001$). Plants were taller under juniper canopies than in open areas (36.1 ± 1.5 *vs*. 22.1 ± 0.8 cm; F = 82.212, $P < 0.001$) and older individuals were taller (F = 17.309, $P < 0.001$). Under juniper canopies, plants were taller at the high-stress site, whereas in open areas there were no differences in height between sites (F = 12.562, $P < 0.001$). GAMM indicated that plants had higher secondary growth at the low-stress than at the high-stress site (mean ± SE; 536 ± 18 vs. 388 ± 10 μm $year^{-1}$; t = –4.963, $P < 0.001$, **Table S1**), with a steep increase with plant age (F = 91.560, $P < 0.001$, **Fig. S1**). No effect of microsite or its interaction with site on secondary growth was detected.

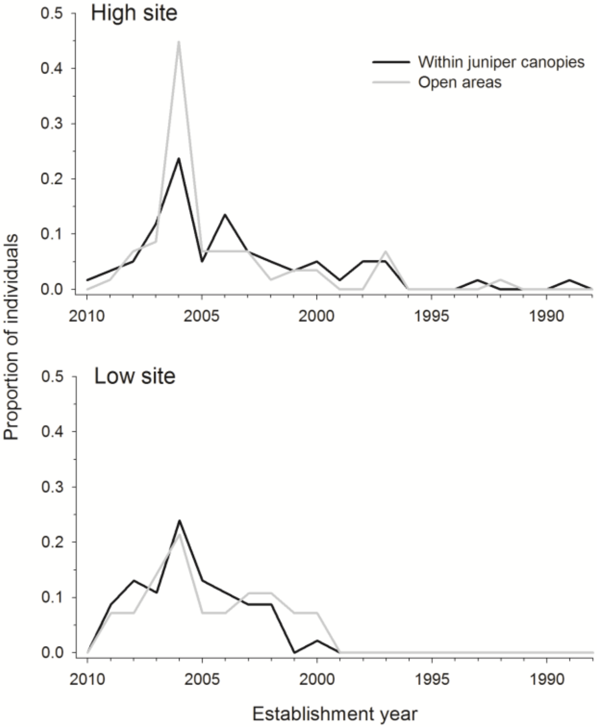

Figure 3. Age structure at each of the site by microsite combinations.

Reproductive variables

A total of 2061 flowers, 5266 carpels and 29,708 seeds were counted. The percentage of plants flowering at the low-stress site (45 %) was higher than at the high-stress site (16 %), where 29 % of plants growing in open areas flowered, but only 14 % of plants under juniper canopies did. At the low-stress site percentage of plants flowering at both microsites was similar (47 % and 45 % respectively). Logistic regression indicated that probability of flowering was higher for taller plants ($z = 7.239$, $P < 0.001$) and for plants growing in open areas ($z = 4.134$, $P < 0.001$).

Flower production was higher at the low-stress site than at the high-stress site even when the effect of plant height was removed (**Table S2, Fig. 4**). At the low-stress site plants produced more flowers in open areas than under juniper canopies, whereas at the high-stress site there was no effect of microsite. Flowers produced more carpels under juniper canopies than in open areas, with no additional effect of site. Fruit set was higher at the high-stress site than at the low-stress site, and under juniper canopies than in open areas. The number of developed seeds per carpel was higher at the high-stress site than at the low-stress site. Fecundity was affected by site, microsite, their interaction and plant height. After controlling the effect of differential plant height, plants had more seeds at the low-stress than at the high-stress site and under juniper canopies than in open areas. At the low-stress site there were no differences on fitness by microsite, whereas at the high-stress site fitness was higher under juniper canopies than in open areas.

Reproductive process

The structural equation model for the effects of facilitation on the reproductive process showed good fit to the data (**Fig. 2**). The number of flowers was the main factor affecting fitness at both sites. At the high-stress site, microsite affected fecundity indirectly through its effects on number of carpels per flower and on fruit set (standardized path coefficient of the indirect effect of microsite on fitness = 0.41), whereas at the low-stress site, microsite only affected the number of carpels per flower. Multigroup analysis showed that fruit set had a stronger effect on fecundity at the high-stress site. The degree of variance explained by number of flowers, number of carpels per flower and number of seeds per carpel was also higher at the high-stress site (**Table S3**).

Discussion

We explored the interaction between the perennial forb *Helleborus foetidus* and the nurse plant *Juniperus sabina* in two populations under contrasting levels of abiotic stress, and considered multiple and sequential effects on different vital traits. The net impact of the nurse plant was positive at the high-stress site, but neutral at the low-stress site. In both cases, this outcome was the result of the combination of multiple positive and negative effects acting on vegetative and reproductive variables.

Differences in individual density between sites (with more individuals per m^2 at the low-stress site) and between open areas and nurse canopies at the high-stress site (more individuals per m^2 under nurse canopies) probably reflect variations in effective recruitment rates. Summer water stress constrains seedling emergence and survival in Mediterranean environments, triggering demographic bottlenecks (Gómez-Aparicio et al. 2008, Caldeira et al. 2014) and leading to a population structure that reflects recruitment peaks associated with favorable years (Olano et al. 2011). This pattern has been seen in *H. foetidus*, for which emerged seedlings and seedling mortality are the most important determinants of recruitment and shape the spatial variation in recruitment more than pre-dispersal losses or post-dispersal removal processes (Garrido et al. 2002). In our case, low adult density and the high concentration of same-aged individuals in open areas at the high-stress site may reflect the difficulties of establishment at this site. This limitation would be reduced under nurse canopies due to the greater soil depth and water availability

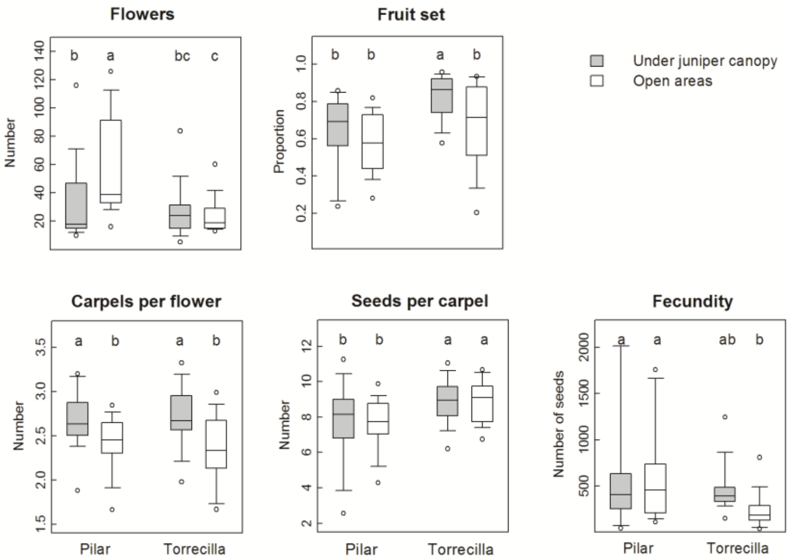

Figure 4. Boxplots of the reproductive variables. Boxes represent median, 25th and 75th percentiles, lines indicate 10th and 90th percentiles, and dots mark 5th and 95th percentiles. Treatments with the same letter do not differ significantly.

(Verdú and García-Fayos 2003, García-Cervigón et al. in press), leading to higher adult density and a more even age structure under nurse canopies, similar to those observed at the low-stress site.

Plants at the low-stress site were taller, had greater secondary growth, more leaves, flowered at a younger age and produced more seeds than at the high-stress site, altogether reflecting a better performance. Under these milder environmental conditions nurse plant presence caused no net effect on *Helleborus* performance; in fact, nurse plants were associated with a decrease in flower production. This negative effect might be a result of competition for nutrients between protégée and nurse plant, since although deeper soils under nurse canopies provided increased water and nutrient availability, nutrient levels in *H. foetidus* leaves did not actually increase (García-Cervigón et al. in press, Matías et al. 2011). At the high-stress site, on the contrary, nurse plants had both positive and negative effects on different vital traits of *Helleborus*, but the net effect was positive (Eckstein 2005). For example, nurse canopies simultaneously diminished secondary growth and increased seed production. The negative impact of shrub cover on secondary growth was probably related to a change in plant architecture associated with a reduction in light availability (García-Cervigón et al. 2013): under nurse canopies plants invest more in primary growth, becoming taller than in open areas in order to receive enough light for photosynthesis and thus promoting primary growth at the expense of secondary growth (Huang et al. 2014). This response was also reflected in leaf morphology, with a larger petiole to lamina ratio recorded under nurse canopies (García-Cervigón et al. in press). Whilst acknowledging the limitations of working with just two contrasting sites rather than with a gradient, our results nonetheless met the expectations of the Stress Gradient Hypothesis, by which positive effects of biotic interactions are expected to prevail under more stressful environments (Bertness and Callaway 1994, He et al. 2013).

The number of flowers was the main determinant of seed production at both sites, as previously reported for this species (Rey et al. 2006) and other Mediterranean plants (Herrera 1993, Gómez and Zamora 2000, Gómez 2003). However, flower production and seed development were modulated by site and microsite. At the low-stress site, the higher number of flowers produced by plants growing in open areas was compensated by a decrease in the number of viable carpels per flower, leading to seed production rates similar to those found under nurse canopies. Competition for stored resources between flowers of the same plant is one of the mechanisms regulating the number of carpels in Ranunculaceae (Johnson and Cook 1968, Zhigang et al. 2006). According to this, if the amount of stored resources were insufficient for all the produced flowers, reducing the number of carpels per flower would maximize the reproductive success at an individual plant scale. At the high-stress site, however, the net effect of nurse plant canopies on seed production was positive and resulted from the combination of positive effects on the number of carpels per flower and on fruit set. Our study species is an autonomous self-pollinated plant, but the exclusion of pollinators causes a decrease in fruit set (Herrera et al. 2001); the variation in fruit set that we recorded could therefore be related to microsite effects on pollinator service. Phenology varies between our study sites, with plants flowering and fructifying earlier at the low-stress (and low altitude) site (pers. obs.). Additional differences on flowering phenology between microsites might also exist, with plants under nurse canopies flowering later due to the slower soil heating, as a result of thermal inertia caused by the interception of sunlight by juniper in contrast to the more sun-exposed open areas (Verdú and García-Fayos 2003, García-Cervigón et al. 2012). *Helleborus foetidus* flowers very early in the season, when temperatures are very low, thus the availability of pollinators may be reduced. In fact, flower life is long (up to 20 days) probably as a mechanism to help ensure pollination (Herrera et al. 2001). A delayed phenology may increase the pollination success of this species, but this result might depend on particular annual conditions.

Variations in the effect of the nurse plant on *Helleborus foetidus* under contrasting environmental conditions and at different moments of the life cycle highlight the complexity and context-dependency of plant-plant interactions. Our results emphasize the need to evaluate entire processes and not only final outcomes when studying plant-plant interactions, and reinforce the role of facilitation as an important source of environmental variability affecting population dynamics (Brooker et al. 2008, McIntire and Fajardo 2014). Negative changes in populations imposed by stressful environmental conditions may be buffered by compensatory changes in demographic rates, which are known as demographic compensation (Doak and Morris 2010). In our case, increases in the number of viable carpels per flower, fruit set and effective recruitment under nurse canopies at the high-stress site may compensate the lower flower production compared to those observed for plants living at the low-stress site. Longer-term demographic studies would be necessary to evaluate the population growth rates at both sites, but our results point to facilitation as an additional source of environmental variability driving demographic compensation at a small scale in Mediterranean mountains (Doak and Morris 2010, García-Camacho et al. 2012).

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Supplementary material

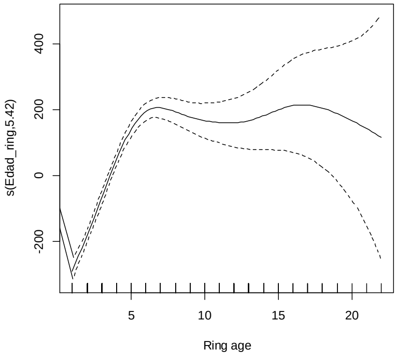

Figure S1. Spline curve adjusted in the generalized linear mixed model (GAMM) for age as explanatory variable for secondary growth.

Table S1. Results of the generalized additive mixed model (GAMM) for secondary growth ($R^2_{adj} = 0.208$).

	Estimate	SE	t	*P*
Intercept	588.462	53.459	11.008	<0.001
Site	-217.408	43.803	-4.963	<0.001
Microsite	-51.650	44.544	-1.160	0.246
Site*Microsite	103.584	60.777	1.704	0.089
Height	0.474	1.380	0.344	0.731
	edf			
Age	5.422		91.560	<0.001
	SD intercept	SD residual		
ID (random)	177.952	266.586		

Table S2. Mean values of the reproductive variables number of flowers (log) number of carpels per flower, fruit set (%), number of developed seeds per carpel and fitness (total number of seeds, log). Only values for significant differences found in the linear models ($P < 0.05$) are shown.

	Model fit		Site		Microsite		Site*Microsite				Height
	R^2_{adj}	*P*	High	Low	Within	Open	High W	High O	Low W	Low O	
No. flowers	0.487	<0.001	23 ± 2	48 ± 5			27 ± 3	19 ± 3	38 ± 6	58 ± 8	+
No. carpels / flower	0.141	0.005			2.69 ± 0.05	2.39 ± 0.05					
Fruit set	0.198	<0.001	75 ± 3	61 ± 3	74 ± 3	63 ± 3					
No. seeds / carpel	0.152	0.004	8.84 ± 0.18	7.68 ± 0.28							
Fecundity	0.365	<0.001	349 ± 37	607 ± 99	534 ± 87	416 ± 63	460 ± 53	239 ± 41	611 ± 169	603 ± 109	+

Table S3. Multigroup comparison between the two study sites of path coefficients of the structural equation model relating microsite to different levels of the reproductive process from number of flowers to fitness. Maximum likelihood χ^2 of different models constraining all free parameters (first row) and releasing each single free parameter one at a time are shown, as well as the probability of that parameter to improve the fully constrained model significantly (last column). Significant *P*-values at the classical one-stage method based on false discovery rates of Benjamini and Hochberg (1995) are highlighted in bold.

Free parameters	ML χ^2	ΔML χ^2	*P* ΔML χ^2
None	134.302		
Path flower number to fecundity	132.009	2.293	0.130
Path fruit set to fecundity	**123.762**	**10.540**	**0.001**
Path carpels per flower to fecundity	133.592	0.710	0.400
Path seeds per carpel to fecundity	130.106	4.196	0.041
Path microsite to fruit set	133.344	0.958	0.328
Path microsite to carpels per flower	133.977	0.325	0.569
Path microsite to flower number	132.841	1.461	0.227
Path microsite to seeds per carpel	134.300	0.002	0.963
Variance microsite	134.302	0.000	1.000
Covariance flowers and carpels per flower	133.353	0.949	0.330
Error fruit set	134.285	0.017	0.897
Error flower number	**85.639**	**48.663**	**0.000**
Error fecundity	133.661	0.641	0.423
Error seeds per carpel	**127.688**	**6.614**	**0.010**
Error carpels per flower	**87.421**	**46.881**	**0.000**

Universities operate one of the few survivors of the old apprenticeship system in their programs for awarding doctoral degrees. In some fields, particularly those with large and expensive laboratories dedicated to the solution of definite problems, you must abandon all thought of independence, and work upon an assigned topic for a dissertation (choice in research is a luxury of later postdoctoral appointments). In more genial and individualistic fields, you are usually given fair latitude in choosing a topic, and may emerge with a project uniquely your own. But in any case you are an apprentice, and you are under your mentor's thumb –more securely than at any time since the early years of primary school. It's a strange system with much to criticize, but it works in its own odd way. At some point, you just can't proceed any further with courses and books; you have to hang around someone who is doing research well. (And you need to be on hand, and ready to assimilate, all the time, every day.) The system does produce its horrors, but when it works (as it does rather more often than a cynic might expect, given the lack of checks and balances), I cannot imagine a better training.

Stephen Jay Gould

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* * *

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