IMPORTANCIA DE LA ALELOPATÍA EN LA ESTRUCTURA Y DINÁMICA DE LA VEGETACIÓN EN ECOSISTEMAS SEMIÁRIDOS

EL CASO DE *ARTEMISIA HERBA-ALBA* ASSO. EN LA DEPRESIÓN MEDIA DEL EBRO

Antonio Ignacio Arroyo Martínez

TESIS DOCTORAL
GRANADA 2017

Universidad de Granada

Instituto Pirenaico de Ecología
IPE CSIC

Editor: Universidad de Granada. Tesis Doctorales
Autor: Antonio Ignacio Arroyo Martínez
ISBN: 978-84-9163-443-0
URI: http://hdl.handle.net/10481/48113

TESIS DOCTORAL / DOCTORAL THESIS

Importancia de la alelopatía en la estructura y dinámica de la vegetación en ecosistemas semiáridos: el caso de *Artemisia herba-alba* Asso. en la depresión media del Ebro

Importance of allelopathy on vegetation structure and dynamics in semiarid ecosystems: the case study of *Artemisia herba-alba* Asso. in the Middle Ebro Valley

Antonio Ignacio Arroyo Martínez

DIRIGIDA POR / DIRECTED BY

Yolanda Pueyo Estaún

Concepción López Alados

El doctorando Antonio Ignacio Arroyo Martínez y los directores de la tesis Yolanda Pueyo Estaún y Concepción López Alados garantizamos, al firmar esta tesis doctoral, que el trabajo ha sido realizado por el doctorando bajo la dirección de los directores de la tesis y, hasta donde nuestro conocimiento alcanza, en la realización del trabajo se han respetado los derechos de otros autores a ser citados cuando se han utilizado sus resultados o publicaciones.

Zaragoza, 8 de Mayo de 2017

Director/es de la Tesis: Doctorando

Fdo.: Yolanda Pueyo Estaún

Fdo.: Antonio Ignacio Arroyo Martínez

Fdo.: Concepción López Alados

A mis padres y hermanos, por haberme apoyado tanto

y a Cristina, por estar siempre a mi lado.

Agradecimientos

Suena a tópico pero lo cierto es que este trabajo no sería lo que es sin todas aquellas personas que, de alguna u otra forma, me han acompañado durante estos últimos cuatro años y pico. Por lo tanto, debo mi gratitud a cada una de ellas.

No puedo comenzar si no es dándole las gracias a mis directores de tesis: Yolanda e Inma. Gracias por todo. Por darme la oportunidad sin conocerme de nada, por vuestra confianza y por estar ahí durante ese segundillo que abarcaba varios minutos y ese minutillo que duraba una hora. Gracias por haberme enseñado tanto y por vuestro apoyo constante. Quiero agradecérselo también a los compañeros de grupo con los que he compartido gran parte de mi trabajo y de mi tiempo. A Ana, hermana de tesis, gracias por compartir las penas en los malos momentos y por llenar el despacho de alegría con tus risas inesperadas. ¡Pregunta sin pedir permiso! A Mª Luz, muchas gracias por tu ayuda con el infernal (lo digo por el calor que hace en verano) trabajo de campo y por todas las veces que hablamos sobre palmeras de chocolate de camino a Belchite. A los compañeros del IPE: Fergus, Clara, Carlos, Natalia, Marina, Manuel, Elena G., María, Miguel B., Ángela. Bendita hora del *tupper*, no entiendo cómo pude pasarme casi la mitad de todo este tiempo sin ese ratico todos los días. Ahora que andaré un poco más libre me comprometo a materializar una liguilla de ping-pong, interinstitucional si es menester. También a los que estuvieron: Hugo, Paloma, Yacine, Ángel, Josu, Iker, Carmela, Guille. Y en general, a todos los servicios y personas del IPE que con su trabajo han contribuido a que haya tenido el mío listo a tiempo, en especial a los Pedros y a las chicas del laboratorio de material vegetal, Victoria y Elena L.

Tengo mucho que agradecer también a varias personas, ajenas al IPE, que han colaborado en el trabajo aquí presentado. A Jesús de “Viveros Ejea” le agradezco su dedicación para ofrecernos todo el material vegetal que hemos necesitado para montar los experimentos. A Ramón, por haberme transmitido su pasión por el banco de semillas. A Javier, por las charlas tan interesantes que hemos mantenido durante las vueltas del café. Y a François, por haberme hecho sentir como en casa durante los tres meses que pasé en Chambéry. Sin duda ese acento granaíno tuyo tuvo mucho que ver.

También le tengo que agradecer al Departamento de Ecología de la Universidad de Granada la oportunidad que me brindó al permitirme impartir algunas prácticas y poder sentir que te llamen “profe”. En especial le agradezco a Jorge su labor como tutor.

Creo que no está de más acordarse también de las entidades que han apoyado económicamente este trabajo. Fundamentalmente debo agradecer al Ministerio de Educación, Cultura y Deporte por la concesión de las ayudas FPU (AP-2012-4126) y de estancias breves y al Ministerio de Economía, Industria y Competitividad por su ayuda a través de los proyectos CGL-2012-37508 y CGL-2016-80783-R.

Debo un agradecimiento especial a toda mi familia. Particularmente a mis abuelos, por tener siempre preparado un buen plato de tortilla y de porra esperando a que yo vaya. A mis hermanos, Rafa y Mario, por haberme apoyado tanto, cada uno a su manera. Y a mis padres, Antonio y Lourdes, por no haber dudado en cruzarse media España varias veces al año. Gracias por vuestro apoyo.

No me puedo olvidar de las amistades que he hecho durante este periodo en Zaragoza: Sergio, Gito, Javi, Victor, los mañoluces Pino, Rafa, Eugenio, Alex y un largo etc. que han ocupado gran parte de mi tiempo fuera del IPE. En gran medida por vuestra culpa los fines de semana se pasaban tan rápido. Gracias también a Fran Oi, Pepe y Tena, por ayudarme a desconectar y que las vacaciones fueran de verdad unas vacaciones.

Por último, pero no menos importante, le agradezco a Cristina haberse aventurado a vivir esta experiencia conmigo. Por, sencillamente, haber estado a mi lado durante todo este tiempo. Durante los buenos momentos y los menos buenos. Sin ti, este trabajo no hubiera sido posible. Gracias por todo gordi. Sé que no siempre te he contado lo que andaba haciendo tanto como te hubiera gustado, así que no veo mejor oportunidad que esta para para que por fin puedas conocer todas las frikadas que he hecho en el IPE.

Supongo que, pese a todo, me habré dejado a muchas personas en el tintero. No obstante, simplemente por el hecho de estar leyendo esto, ¡GRACIAS! a ti también.

Índice

Resumen

Las zonas áridas y semiáridas ocupan aproximadamente el 41% de la superficie terrestre y son habitadas por un tercio de la población mundial. Las predicciones para el próximo siglo señalan un endurecimiento de las condiciones climáticas en estas regiones. Para poder realizar una correcta gestión y conservación de sus recursos y afrontar los cambios futuros de manera más eficaz, resulta fundamental conocer el funcionamiento de los ecosistemas de estas regiones. En este sentido, las interacciones bióticas entre plantas constituyen una pieza clave para el funcionamiento de los ecosistemas áridos y semiáridos, ya que determinan su productividad, diversidad y capacidad de recuperación. Dentro de las interacciones bióticas, el papel que tiene tanto la competencia por los recursos como la facilitación ha sido ampliamente estudiado. Sin embargo, a menudo se olvida un tercer tipo de interacción entre plantas, la alelopatía. La alelopatía es un tipo de interferencia al igual que la competencia, pero que no implica el consumo de recursos, sino la producción y liberación de compuestos químicos al medio con capacidad para inhibir la germinación, el crecimiento y la reproducción de las plantas vecinas. La importancia de la alelopatía ha sido reconocida en ecosistemas gestionados (p. ej. sistemas agrícolas) y más recientemente como mecanismo para explicar el tremendo éxito de algunas especies invasoras en las comunidades invadidas. Sin embargo, su papel en comunidades naturales está mucho menos estudiado. En ecosistemas áridos y semiáridos, la interferencia alelopática puede jugar un papel especial, por varias razones. Por un lado, en estos ecosistemas son muy abundantes especies potencialmente alelopáticas, como las plantas aromáticas. Por otra parte, las duras condiciones ambientales de las zonas áridas y semiáridas favorecen la producción y liberación de compuestos alelopáticos y hacen que las especies sean más susceptibles a su efecto. Además, el estudio de la alelopatía se suele dar en condiciones aisladas de otros mecanismos de interacción y en condiciones de laboratorio, lo que pone de manifiesto la necesidad de un mayor número de estudios de campo para obtener evidencias realistas de la importancia de la alelopatía en condiciones naturales.

En esta tesis doctoral se examina la importancia relativa de la alelopatía en la estructura y la dinámica de una comunidad vegetal semiárida con respecto a otras interacciones bióticas entre plantas (p. ej. facilitación) bajo diferentes condiciones de estrés ambiental. Para ello, se ha combinado el análisis del patrón espacial de la vegetación y la diversidad alrededor de las plantas alelopáticas con experimentos realizados tanto en condiciones de laboratorio como en la propia comunidad vegetal (condiciones naturales). Como especie alelopática se ha seleccionado a *Artemisia herba-alba* Asso., un caméfito aromático de poca altura que se distribuye por toda la cuenca Mediterránea. Las comunidades vegetales estudiadas se localizan en el sector central de la depresión del Ebro, que se caracteriza por tener clima mediterráneo semiárido con una marcada continentalidad, y constituye junto con el sudeste ibérico una de las regiones más áridas de la península ibérica.

En los **capítulos 1 y 2** se analiza la relación espacial entre las plantas alelopáticas y el resto de especies de la comunidad vegetal. Específicamente, en el **capítulo 1**, se presenta el estudio de la relación entre el patrón espacial del establecimiento de las plantas en una comunidad vegetal semiárida y las propiedades hidrológicas y microclimáticas de los sitios donde se establecen. Para ello, se analiza el establecimiento de especies de matorral y de gramíneas bajo tres especies potencialmente nodrizas (*S. vermiculata*, *A. herba-alba* y *L. spartum*), así como la germinación, la supervivencia y el crecimiento de *S. vermiculata* y *L. spartum* bajo individuos adultos de estas dos especies y en suelo desnudo, en un experimento de cuatro años. También se analiza el contenido de agua en el suelo, la infiltración de agua tras las lluvias, la concentración de nutrientes y la radiación solar bajo las tres especies potencialmente nodrizas y el suelo desnudo para identificar los procesos responsables de los patrones observados. En el **capítulo 2**, se presenta el efecto que tienen las interacciones bióticas de tres especies dominantes de una comunidad vegetal semiárida: la gramínea *L. spartum*, el arbusto alelopático *A. herba-alba* y el arbusto facilitador *S. vermiculata*, en la diversidad y la composición de especies, bajo diferentes condiciones de aridez y pastoreo. Para ello, se cuantifica el balance neto de las interacciones de las tres especies de estudio basándose en datos de co-ocurrencia de especies. También, se analiza el patrón espacial de la diversidad alrededor de los individuos de las tres especies de estudio

calculando el *individual species-area relationship* (ISAR) y los cambios en la composición de especies calculando el índice de similaridad de Chao-Jaccard.

En los **capítulos 3 y 4** se analiza el alcance de la interferencia química en distintos estadios del ciclo vital de las especies que conviven con *A. herba-alba*, incluyendo la propia especie alelopática (autotoxicidad). Concretamente, en el **capítulo 3**, se investiga si los efectos en la vegetación, observados en las inmediaciones de los individuos de *A. herba-alba* en los dos capítulos anteriores, se deben a una heterogeneidad espacial en la estructura del banco de semillas entre *A. herba-alba* y el arbusto no alelopático *S. vermiculata*. Para ello, se analiza la riqueza de especies y la densidad de semillas del banco de semillas en estos dos micrositios y en el suelo desnudo. También, se evalúa el efecto que tiene el extracto acuoso de *A. herba-alba* en la tasa y en el tiempo de emergencia de las plántulas desde el banco de semillas, así como también en la mortalidad de las plántulas emergidas. En el **capítulo 4**, se investiga el efecto alelopático y autotóxico de los compuestos volátiles y solubles en agua liberados por *A. herba-alba* sobre la germinación y el crecimiento temprano de un conjunto de especies de interés (*S. vermiculata*, *L. spartum*, *P. halepensis* y la propia *A. herba-alba*) que coexisten con *A. herba-alba* en comunidades vegetales semiáridas. También, se identifican y cuantifican los compuestos fenólicos presentes en el extracto acuoso de *A. herba-alba* y se comprueba el efecto de una mezcla de los compuestos identificados en la germinación de las especies de interés.

En el **capítulo 5**, se examina la importancia de la interferencia alelopática de *A. herba-alba* en condiciones naturales. Para ello, se analiza el crecimiento, la vitalidad, la germinación y la supervivencia de especies que co-existen con *A. herba-abla* en una comunidad vegetal semiárida (*S. vermiculata*, *L. spartum* y *A. herba-alba*) con y sin la presencia de interferencia química mediante el uso de carbón activo, en un experimento de campo de dos años. También, se evalúa el balance neto de interacciones de *A. herba-alba* en comparación con el balance neto de *S. vermiculata*, y cómo la aridez puede modular el balance neto.

A diferencia de otros estudios que a menudo se refieren al potencial alelopático de una especie vegetal basándose en la evaluación de la fitotoxicidad de determinados compuestos en condiciones de laboratorio y sobre especies que no siempre coexisten con la

especie alelopática, los distintos capítulos que componen esta tesis doctoral ofrecen un enfoque integral que permite mejorar nuestro entendimiento acerca del significado de la alelopatía en la organización de las comunidades naturales semiáridas. Se ha observado que *A. herba-alba* genera en su entorno un patrón espacial de la vegetación más disperso de lo que es habitual en las comunidades vegetales de zonas áridas y semiáridas. Además, se ha encontrado que *A. herba-alba* es rica en metabolitos secundarios, de los cuales se han identificado algunos compuestos fenólicos, que pueden ser liberados tanto por volatilización como disueltos en agua y que actúan sobre todo inhibiendo la germinación de las especies que coexisten con *A. herba-alba*, incluyendo sus propias semillas. Una de las mayores aportaciones de la tesis ha sido la realización de experimentos en el campo. Estos experimentos indicaron que los compuestos alelopáticos producidos y emitidos por *A. herba-alba* se distribuyen y acumulan en concentraciones suficientes como para que tengan un efecto en las plantas vecinas. Además, han permitido comprender que la actividad alelopática de *A. herba-alba* es, al margen del resto de interacciones bióticas, una interacción relevante en las comunidades naturales semiáridas estudiadas, aunque sus efectos en la vegetación pueden ser fácilmente sobreestimados basándose exclusivamente en experimentos de laboratorio. Futuros trabajos deben ampliar este conocimiento cuantificando la importancia relativa de la interferencia alelopática frente a la interferencia causada por la competencia, integrando los microrganismos del suelo como parte fundamental de la interacción química entre plantas e investigando como la alelopatía puede constituir una adaptación evolutiva para evitar convertirse en plantas facilitadoras en estos ecosistemas.

Summary

Arid and semiarid environments occupy approximately the 41% of terrestrial surface and are the habitat for a third of the global population. The predictions for the next century point out that climatic conditions in those environments will become even harsher. In order to manage and conserve their resources, and face future changes more effectively, it is essential to know the functioning of arid and semiarid ecosystems. In this sense, biotic interactions among plants are crucial to the functioning of arid and semiarid ecosystems, since they determine their productivity, diversity and resilience. Within plant-plant interactions, the role of both resource competition and facilitation has been widely studied. However, allelopathy, a third kind of interaction is often overlooked. Allelopathy is a kind of chemical interference that differs from competition because it does not involve the depletion of resources, but the production and release to the environment of chemical compounds that inhibit the germination, growth and reproduction of neighboring plants. The role of this chemical interaction has been recognized in managed ecosystems (*e.g.* agricultural systems), and more recently as a mechanism to explain the success of some invasive plant species in the invaded communities. However, the role of allelopathy in natural communities is much less understood. In arid and semiarid environments allelopathy may be especially relevant due to several reasons. On the one hand, in those environments many species, such as aromatic shrubs, are expected to show allelopathic activity. On the other hand, harsh environmental conditions in arid and semiarid ecosystems favor the production and release of allelopathic compounds, and make species more susceptible to their action. Moreover, allelopathy is usually studied under laboratory conditions and isolated from other mechanism of interaction, which underscores the need for new field studies in order to obtain realistic evidences of the significance of allelopathy in natural conditions.

This thesis examines the relative importance of allelopathy in driving the structure and dynamic of a semiarid plant community with respect to other biotic interactions among plants (*e.g* facilitation) under different environmental stress conditions. To this end, the

analysis of the vegetation patterning and diversity around the allelopathic plants has been combined with experiments performed in both the laboratory and the natural community itself (*i.e.* natural conditions). *Artemisia herba-alba* Asso., an aromatic dwarf shrub distributed throughout the Mediterranean basin, has been selected as allelopathic species. The plant communities under study are located in the Middle Ebro Valley region, which is characterized by a semiarid Mediterranean climate with a pronounced continentality, and constitutes, together with the Iberian southeast, one of the most arid regions of the Iberian Peninsula.

In **chapters 1 and 2** the spatial relationship between allelopathic plants and the rest of species in the plant community is analyzed. **Chapter 1** presents the study of the relationship between the spatial pattern of seedling establishment in a semiarid plant community and the hydrological and microclimatic properties of the microsites where they are established. Specifically, the establishment of shrubs and perennial grasses species is analyzed under three potential nurse species (*S. vermiculata*, *A. herba-alba* and *L. spartum*), as well as the seed germination, survival and growth of *S. vermiculata* and *L. spartum* seedlings beneath adult individuals of these two species, along a four-year field experiment. In addition, the soil water content, water infiltration rates after a rainfall, soil nutrients concentration and solar radiation under the three potentially nurse plants species and in the bare soil, are also analyzed to identify the processes responsible for the observed patterns of establishment. **Chapter 2** presents the effect of the net interaction outcome of three dominant species in a semiarid plant community: the perennial grass *L. spartum*, the allelopathic shrub *A. herba-alba* and the nurse shrub *S. vermiculata*, on diversity and species composition in their vicinity, under different conditions of aridity and grazing. The net interaction balance of the three study species is quantified based on species co-occurrence data. Furthermore, the spatial pattern of diversity in the surroundings of the three study species individuals is analyzed by computing the individual species-area relationship (ISAR), and changes in species composition is analyzed by calculating the Chao-Jaccard similarity index.

In **chapters 3 and 4** the extent of the chemical interference at different vital stages of species coexisting with *A. herba-alba* is analyzed, including the allelopathic species itself (autotoxicity). In particular, **chapter 3** assesses whether the vegetation patterning observed in the vicinity of *A. herba-alba* individuals in the two previous chapters is due to a spatial heterogeneity in seed bank structure with respect to the non-allelopathic shrub *S. vermiculata*. To this end, seedbank richness and density in these two microsites and in the bare soil are assessed. In addition, the effects of *A. herba-alba* aqueous extract on the rate and timing of seedling emergence from the seed bank, as well as the mortality of emerged seedlings, are evaluated. **Chapter 4** investigates the allelopathic and autotoxic effects of volatile and water soluble chemicals released by *A. herba-alba* on seed germination and early seedling growth of a set of species of interest (*S. vermiculata*, *L. spartum*, *P. halepensis* and *A. herba-alba* itself) that coexist with *A. herba-alba* in natural semiarid plant communities. Also, the phenolic compounds present in the *A. herba-alba* aqueous extract are quantified and identified, and the effects of a mixture of those identified are tested on the germination of study species.

In **chapter 5**, the importance of the allelopathic interference of *A. herba-alba* is examined under natural conditions. Specifically, the growth, vitality, germination and survival of a number of species (*S. vermiculata*, *L. spartum* y *A. herba-alba*) that coexist with *A. herba-alba* in a semiarid plant community are analyzed with and without the presence of chemical interference by the use of activated carbon, along a 2-years field experiment. Also, the net interaction balance of *A. herba-alba* is assessed in comparison with the net balance of *S. vermiculata*, and how allelopathy may modulate its interaction outcome under different drought stress conditions.

Unlike other studies of allelopathy that often refer to the allelopahtic potential of a species, based solely on the evaluation of the phytotoxicity of the allelopathic compounds in laboratory bioassays with species that do not always coexists with the allelopathic species, the different chapters of this thesis offer an integrated approach that allows to improve the understanding of the significance of allelopathy in the organization of semiarid plant communities. It has been found that the vegetation patterning in the vicinity of *A. herba-alba* is less aggregated than the usual for plant communities of arid and semiarid environments. In addition, it has been observed that *A. herba-alba* is rich in secondary metabolites that can act as allelopathic compounds. These compounds can be released

through volatilization and dissolved in water, and inhibit mainly the seed germination of the species that coexists with *A. herba-alba,* and also its own seeds. Some phenolic compounds have been identified as allelochemicals involved in its autotoxicity. One of the major contributions of this thesis has been the performing of field experiments. These experiments indicated that allelopathic compounds produced and released by *A. herb-alba* are distributed and concentrated enough to have an effect on neighboring plants. Furthermore, these experiments indicated that the allelopathic activity of *A. herba-alba* is, besides to the rest of biotic interactions, a relevant interaction in the semiarid plant communities studied, although its effects in vegetation can be easily overestimated based only on laboratory experiments. Future works should expand this knowledge by quantifying the relative importance of the allelopathic interference versus the interference caused by resources competition, including soil microorganisms as a central part in the chemical interaction between plants and investigating how allelopathy can be an evolutionary adaptation to avoid becoming nurse plants in these ecosystems.

Introducción general

La vegetación y el funcionamiento de los ecosistemas áridos y semiáridos

Las zonas áridas y semiáridas (denominadas "*drylands*" en inglés) son, por definición, regiones donde la precipitación es escasa y la evapotranspiración muy elevada (Whitford 2002). Estas regiones se pueden clasificar en varios subgrupos en función de su índice de aridez (IA; precipitación / evapotranspiración; Whitford 2002; Safriel & Adeel 2005) como regiones subhúmedas (0.65>IA>0.50), semiáridas (0.50>IA>0.20), áridas (0.20>IA>0.05) e hiperáridas (IA<0.05). Se estima que estas regiones ocupan aproximadamente el 41% de la superficie terrestre emergida, ascendiendo al 74% en la península ibérica, y son habitadas por aproximadamente un tercio de la población mundial (más de dos mil millones de personas; Safriel & Adeel 2005; Reynolds *et al.* 2007; MAGRAMA 2016; Figs. 1 y 2). Los usos del suelo en las zonas áridas y semiáridas están relacionados principalmente con la producción agrícola y su uso como pastos para el ganado (Safriel & Adeel 2005). Por ello, la subsistencia de la población depende del mantenimiento de la productividad y la diversidad de los ecosistemas áridos y semiáridos y de los servicios que estos proporcionan. Las predicciones climáticas para el próximo siglo auguran que las zonas áridas y semiáridas sufrirán un aumento de la aridez motivada por la reducción de las precipitaciones y el aumento de las temperaturas (IPCC 2014). Además, se prevé que para finales de este siglo, la extensión de las regiones áridas y semiáridas a escala global habrá aumentado entre el 11 y el 23% (Huang *et al.* 2015). Esto, junto con otros factores de origen antrópico como malas prácticas agrícolas y el posterior abandono de los cultivos o un uso inadecuado de los recursos pastables (sobrepastoreo) puede conducir a un aumento de los procesos de degradación y el riesgo de desertificación de las zonas áridas y semiáridas (Safriel & Adeel 2005), que en muchos casos son irreversibles (al menos a escala humana; Allington & Valone 2010). Por lo tanto, el conocimiento del funcionamiento de los ecosistemas áridos y semiáridos resulta fundamental para una correcta gestión y conservación de sus recursos y para afrontar los cambios futuros de manera más eficaz (Maestre *et al.* 2016).

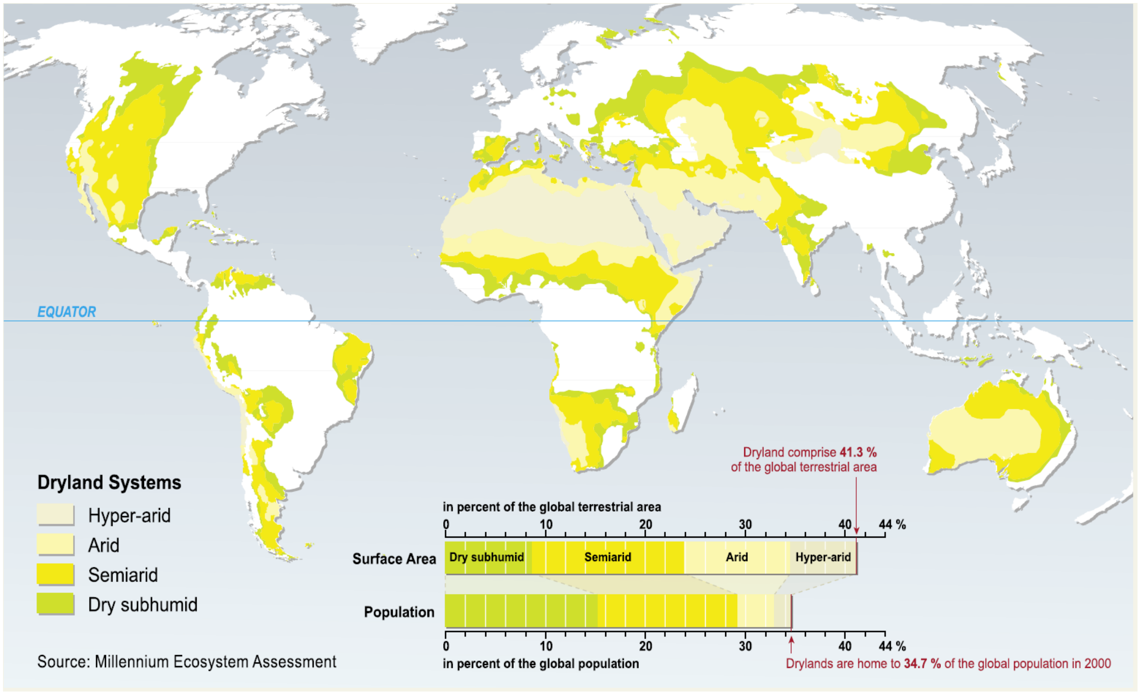


Fig. 1. Distribución de las zonas áridas y semiáridas en el mundo. Fuente: Millennium Ecosystem Assessment 2005

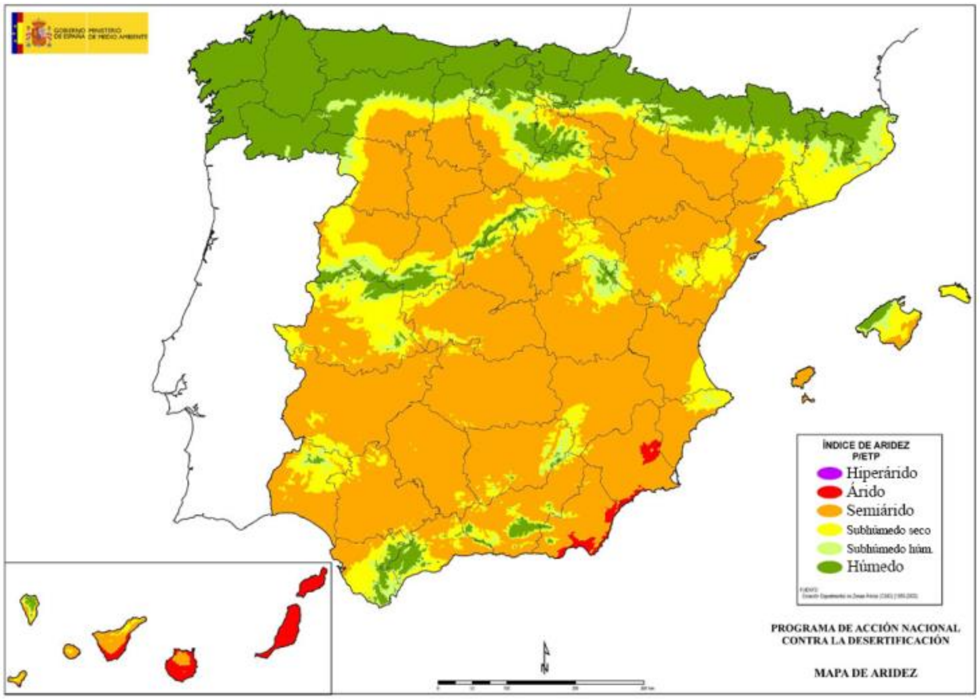


Fig. 2. Distribución de las zonas áridas y semiáridas en España. El color morado, rojo, naranja y amarillo indican regiones hiperáridas, áridas, semiáridas y subhúmedas respectivamente. Fuente: adaptada de MAGRAMA 2016.

La escasa y episódica disponibilidad de agua en las zonas áridas y semiáridas provoca que la vegetación se encuentre sometida periódicamente a un elevado estrés hídrico (Noy-Meir 1973). No es de extrañar por tanto que las plantas de estos ecosistemas presenten numerosas adaptaciones morfológicas y fisiológicas para hacer frente a las condiciones de sequía. En general, se diferencian dos tipos de estrategias vitales en las plantas que habitan en estos ecosistemas (Levitt 1980; Whitford 2002; Ward 2009). Por un lado, hay plantas que directamente escapan de las condiciones de mayor estrés. Este sería el caso de las especies anuales, que responden muy rápidamente a las condiciones favorables de disponibilidad de agua y nutrientes, y cuyo periodo de actividad para crecer y reproducirse ocurre cuando esta disponibilidad es mayor, normalmente en primavera (Levitt 1980; Whitford 2002; Ward 2009). Por otro lado, hay plantas cuya estrategia pasa por evitar el estrés hídrico. Estas plantas poseen una combinación de características morfológicas tales como raíces profundas o una alta relación de biomasa radicular en comparación con la biomasa aérea, hojas pequeñas a menudo con pilosidad o una cutícula cerúlea, cierto grado de suculencia, distinto tipo de fotosíntesis (C_4 o CAM) o control estomático de la pérdida de agua, que les permiten soportar periodos de mayor escasez de agua (Levitt 1980; Whitford 2002; Ward 2009). Muchos arbustos y algunas gramíneas perennes son ejemplos de plantas típicas de comunidades vegetales de zonas áridas y semiáridas con adaptaciones para tolerar los periodos menos favorables por la sequía. Además, estas plantas se caracterizan por tener un crecimiento relativamente lento y hacer un uso eficiente de los recursos (nutrientes y agua; Grime 1977).

Tal y como señala Noy-Meir (1973), aparte de ser infrecuentes, las precipitaciones en las zonas áridas y semiáridas se caracterizan por una gran variabilidad temporal a lo largo del año y por ser altamente impredecibles. La escasez de agua es, sin duda, uno de los factores que más fuertemente condiciona todos los procesos que ocurren en estos ecosistemas. Por ejemplo, Muldavin et al. (2008) encontró que la tasa de producción primaria neta de la vegetación está positivamente correlacionada con la precipitación anual en el Desierto de Chihuahua. Del mismo modo, la mayor o menor humedad del suelo controla directamente los ciclos biogeoquímicos de carbono (C) y nutrientes en los ecosistemas áridos y semiáridos (Austin *et al.* 2004). Se hace referencia a que estos sistemas están controlados por los pulsos de agua (paradigma pulso-reserva), en el que un evento de precipitación desencadena un pulso de actividad, como el crecimiento de la vegetación o la descomposición de la hojarasca, que cesa cuando la disponibilidad de agua es menor (Noy-Meir 1973; Chesson *et al.* 2004).

Además de los factores climáticos, cada vez existen más evidencias acerca de la importancia de los atributos bióticos determinando la estructura y el funcionamiento de los ecosistemas áridos y semiáridos (Maestre *et al.* 2016). En estos ecosistemas donde el agua es el principal elemento limitante, la cobertura vegetal no es continua sino que está organizada en un "mosaico de dos fases", con parches de vegetación densos que se alternan con zonas de suelo desnudo (Noy-Meir 1973; Aguiar & Sala 1999). Estos parches toman la forma de bandas o manchas de diferente tamaño, y están compuestos por especies leñosas y herbáceas (Aguiar & Sala 1999). Aunque se hace referencia a las zonas con menor densidad de vegetación como suelo desnudo, éste no está realmente desnudo, sino que a menudo aparece cubierto por una costra biológica formada por líquenes y cianobacterias entre otros organismos (Belnap 2003; Maestre *et al.* 2011). La distribución parcheada de la vegetación juega un papel muy importante en la redistribución de la escorrentía en estos ecosistemas (Cerdà 1997b; Ludwig *et al.* 2005). En concreto, los parches de vegetación, debido a que las raíces modifican y mejoran la textura del suelo creando macroporos, actúan como sumideros favoreciendo una mayor infiltración del agua de lluvia y de la escorrentía que procede desde el suelo desnudo, donde la infiltración es normalmente más baja debido a la costra biológica y a la compactación del suelo (Noy-Meir 1973; Cerdà 1997b; Ludwig *et al.* 2005; Wang *et al.* 2007; Pueyo *et al.* 2013). Además, la mayor cantidad de sombra debajo de las plantas reduce la evaporación en los parches de vegetación (Valiente-Banuet & Ezcurra 1991). Como consecuencia de ambos procesos, la disponibilidad de agua y nutrientes es mayor y por un espacio de tiempo más amplio en los parches de vegetación que en el suelo desnudo (Schlesinger *et al.* 1995; Macek *et al.* 2016; Fig. 3). Por ello, se denomina a estas manchas como "islas de fertilidad" (Schlesinger *et al.* 1995). Este hecho posibilita un mayor establecimiento y crecimiento de la vegetación en los propios parches de vegetación, lo que a su vez permite que una mayor cantidad de agua esté disponible para las plantas, generando así un proceso de retroalimentación positiva esencial para mantener la dinámica y la organización de los ecosistemas áridos y semiáridos (Rietkerk *et al.* 2004; D'Odorico *et al.* 2007).

Fig. 3. Aspecto que muestran los parches de vegetación de una comunidad vegetal semiárida varias horas después de una lluvia. A simple vista se puede observar como la humedad del suelo es sustancialmente mayor en los parches de vegetación que en el suelo desnudo.

La herbivoría es otro elemento biótico importante que determina la estructura y el funcionamiento de los ecosistemas áridos y semiáridos (Maestre *et al.* 2016; Fig. 4). El efecto de la herbivoría en estos ecosistemas depende de varios factores como su intensidad, el tipo de herbívoro y el nivel de productividad de las plantas (Eldridge *et al.* 2016). Herbívoros de pequeño tamaño, como insectos o pequeños mamíferos, no tienen la capacidad suficiente para causar efectos significativos (Olff & Ritchie 1998). Herbívoros más grandes, o con mayor capacidad para crear perturbaciones como el ganado doméstico, son más eficientes transportando semillas, creando una mayor heterogeneidad en los recursos del suelo y disminuyendo la capacidad competitiva de las especies más dominantes, lo que puede repercutir de forma positiva en el ecosistema, por ejemplo favoreciendo un aumento de la diversidad (Collins 1998; Olff & Ritchie 1998; Fensham, Silcock & Firn 2014). El efecto positivo del pastoreo en el mantenimiento de la diversidad es más probable cuanto mayor sea la productividad (Olff & Ritchie 1998; Osem, Perevolotsky & Kigel 2002). Sin embargo, los ecosistemas áridos y semiáridos son por lo general poco productivos (co-limitados por la escasez de agua y la baja concentración de N en el suelo; Hooper & Johnson 1999; James, Tiller & Richards 2005). En estos casos, la sostenibilidad del pastoreo se basa en el uso de cargas ganaderas adecuadas que permitan la regeneración de la vegetación y una adecuada composición de la comunidad vegetal. La introducción de un pastoreo más intenso (sobrepastoreo) puede conducir a la desaparición de especies de interés en el pasto (p. ej. las más palatables), causando cambios en la composición vegetal y disminución de la diversidad de espcies (Puigdefábregas & Mendizabal 1998; Olff & Ritchie 1998; Alados *et al.* 2003). Además, el sobrepastoreo altera los procesos hidrológicos, aumenta la compactación del suelo y conduce a una reducción en la biomasa y la cobertura vegetal (Castellano & Valone 2007; Pueyo *et al.* 2013; Eldridge *et al.* 2016). Conforme la vegetación se pierde, el suelo pierde su protección y, como consecuencia, se favorece la erosión, lo que dificulta aún más el establecimiento de nuevas plantas y promueve una mayor pérdida de vegetación (Cerdà 1997a; D'Odorico *et al.* 2013). Este proceso de retroalimentación positiva es el que domina en sistemas desertificados (Schlesinger *et al.* 1990; D'Odorico *et al.* 2013), haciéndolos bastante estables y difíciles de recuperar.

Fig. 4. Imagen de un pequeño rebaño de ovejas (*Rasa aragonesa*) pastando en una comunidad vegetal semiárida de la depresión media del Ebro.

Numerosos estudios han puesto de manifiesto la relación que existe entre la diversidad de especies y el funcionamiento de los ecosistemas (Hooper *et al.* 2005). Por ejemplo, suele existir una relación positiva entre la riqueza de especies y los niveles de productividad (Mittelbach *et al.* 2001). Ruijven et al. (2003) encontraron que en una comunidad de plantas experimental el número de plantas invasoras decrece conforme aumenta la diversidad de especies. Santonja et al. (2017) encontraron que la descomposición de la hojarasca formada por una mayor diversidad de especies se relaciona con una mayor abundancia de microrganismos y una mayor liberación de C y nitrógeno (N). Recientemente, Maestre et al. (2012b) encontraron en un análisis a escala global que la diversidad de especies es un predictor importante para distintas variables del funcionamiento de los ecosistemas áridos y semiáridos relacionadas con los ciclos del C, N y fósforo (P). Junto con la diversidad específica, la combinación de determinados atributos bióticos como la composición de especies y el patrón de la vegetación resulta necesaria para maximizar el funcionamiento de estos ecosistemas (Maestre *et al.* 2012a). Por lo tanto, el mantenimiento de la diversidad debería ser un objetivo de las estrategias de conservación y gestión en las zonas áridas y semiáridas para evitar una reducción de su funcionamiento y los servicios que éstos proveen (Maestre *et al.* 2012b).

Papel de las interacciones entre plantas en zonas áridas y semiáridas

Algunos de los atributos bióticos que condicionan la estructura y el funcionamiento de los ecosistemas áridos y semiáridos, como la diversidad y el patrón de la vegetación, están ligados y determinados a su vez por las interacciones bióticas entre plantas que ocurren en las comunidades vegetales (Soliveres & Maestre 2014; Maestre *et al.* 2016). Tradicionalmente, se ha considerado a la competencia por los recursos como el principal tipo de interacción responsable de la estructura de las comunidades vegetales de estos ambientes (Noy-Meir 1973; Tilman 1982; Chesson *et al.* 2004). Sin embargo, la importancia que las interacciones positivas tienen en la organización de las comunidades vegetales, en particular de aquellas sometidas a un elevado nivel de estrés ambiental, ha sido ampliamente reconocida en las últimas dos décadas (Bertness & Callaway 1994; Bruno, Stachowicz & Bertness 2003; Callaway 2007; Brooker *et al.* 2008; McIntire & Fajardo 2014). Las interacciones positivas entre plantas (agrupadas bajo el término facilitación) ocurren cuando la presencia de una planta beneficia directa o indirectamente la supervivencia, el crecimiento o la reproducción de otras plantas vecinas (Callaway 2007). En estos ecosistemas, los arbustos se comportan frecuentemente como plantas facilitadoras para el resto de especies vegetales de la comunidad (Gómez-Aparicio *et al.* 2004; Gómez-Aparicio 2009), sobretodo, durante la fase de establecimiento de las plántulas (Miriti 2006). Entre los mecanismos directos de facilitación destacan el aumento de la cantidad de agua disponible para las plantas bajo el dosel de los arbustos, bien porque interceptan el agua de escorrentía que procede desde el suelo desnudo, condensan el gua de la niebla o la movilizan desde zonas más profundas hacia la superficie ("*hydraulic lift*" en inglés), la reducción de la intensa radiación solar, lo que amortigua las altas temperaturas y reduce los niveles de evapotranspiración debajo de las plantas facilitadoras, y el aumento de los nutrientes con respecto al suelo desnudo (Dawson 1993; Callaway 2007; Sotomayor, Lortie & Lamarque 2014). Algunos de los mecanismos indirectos de facilitación incluyen la protección frente a la acción de los herbívoros, la atracción de polinizadores y la asociación con microorganismos del suelo como las micorrizas (Callaway 2007; Graff, Aguiar & Chaneton 2007; Smit *et al.* 2007). De esta manera, las plantas facilitadoras permiten que especies sensibles, que no toleran bien tales niveles de estrés, puedan persistir en la comunidad, expandiendo su nicho ecológico (Bruno *et al.* 2003; Liancourt, Callaway & Michalet 2005; McIntire & Fajardo 2014; Soliveres *et al.* 2015). Las interacciones positivas son cruciales para mantener una alta riqueza de especies (Le Bagousse-Pinguet *et al.* 2014; Soliveres & Maestre 2014), lo que a su vez permite el correcto funcionamiento de los ecosistemas áridos y semiáridos (Maestre *et al.* 2012b, 2016). Aunque la facilitación supone enormes beneficios para las plantas facilitadas, se ha encontrado que una alta densidad de plantas facilitadas puede generar un impacto negativo en el *fitness* de las plantas facilitadoras (Schöb *et al.* 2014; pero ver Tirado, Bråthen & Pugnaire 2015). Además, se ha documentado que las plantas facilitadas pueden llegar a reemplazar a los arbustos facilitadores a lo largo de la sucesión vegetal (Armas & Pugnaire 2005).

El patrón espacial de la vegetación ha sido utilizado en numerosos trabajos como un indicador de las interacciones bióticas que predominan entre las distintas especies la comunidad vegetal (Tirado & Pugnaire 2005; Kikvidze *et al.* 2005; Ziffer-Berger *et al.* 2014; Chacón-Labella, de la Cruz & Escudero 2015; López *et al.* 2016). Dado que las especies vegetales son organismos sésiles, cuando dos o más especies están asociadas positivamente en el espacio, es decir, presentan un patrón de coexistencia más frecuente que cabría esperar, se puede interpretar que existe una interacción positiva entre ellas. Por el contrario, cuando dos o más especies están asociadas negativamente en el espacio, es decir, coexisten menos veces de lo que cabría esperar, se puede interpretar como una interacción negativa entre ellas. Por último, cuando dos o más especies presentan un patrón de coexistencias al azar, se puede interpretar como una ausencia de interacción entre ellas (o bien que el resultado neto de las interacciones positivas y negativas es neutral; Fig. 5). Cabe destacar que además de las interacciones bióticas, existen otros mecanismos capaces de generar el patrón espacial de asociación observado entre las especies tales como una distribución parcheada de los recursos favorables, una dispersión limitada de las semillas, la propagación vegetativa o la captura de semillas (Aguiar & Sala 1997; Flores & Jurado 2003; Bestelmeyer, Ward & Havstad 2006; Pueyo *et al.* 2008; Alados *et al.* 2010; López *et al.* 2016). Muchos de estos mecanismos, junto con un predominio de las interacciones positivas, operan simultáneamente en los parches de vegetación de las comunidades áridas y semiáridas generando procesos de retroalimentación positiva que ayudan a mantener esa estructura parcheada (Rietkerk *et al.* 2004; Scanlon *et al.* 2007).

El balance neto entre la facilitación y la competencia de las especies vegetales ha sido un intenso objeto de estudio (Pugnaire, Haase & Puigdefabregas 1996; Tielbörger & Kadmon 2000; Armas & Pugnaire 2005; Valiente-Banuet & Verdú 2008) especialmente, desde que Bertness y Callaway (1994) propusieran la hipótesis del gradiente de estrés (SGH, de sus siglas en inglés). Básicamente, la SGH predice que las interacciones positivas serán más frecuentes en ambientes con unas elevadas condiciones de estrés biótico (p. ej. pastoreo) o abiótico (p. ej. aridez), mientras que bajo condiciones ambientales relativamente benignas las interacciones positivas serán raras y las comunidades estarán gobernadas por la competencia (Fig. 5). Muchos estudios han comprobado la generalidad de las predicciones de la SGH (Holzapfel *et al.* 2006; Ziffer-Berger *et al.* 2014; López *et al.* 2016). A nivel global, se acepta que ocurre un cambio hacia el predominio de las interacciones positivas conforme se incrementa el nivel de estrés (Cavieres *et al.* 2014; He & Bertness 2014). En concordancia, se estima que aproximadamente el 30% de las especies vegetales en comunidades de zonas áridas y semiáridas del planeta se encuentran asociadas con una especie facilitadora (Soliveres & Maestre 2014). Sin embargo, otros trabajos encuentran un colapso de la facilitación bajo un estrés ambiental extremo (Tielbörger & Kadmon 2000; Michalet *et al.* 2006, 2014), lo que ha llevado a refinar las predicciones de la SGH (Maestre *et al.* 2009). Así, la relación de las interacciones positivas con el estrés no sería lineal, sino unimodal, es decir, que la frecuencia de las interacciones positivas es máxima bajo condiciones elevadas de estrés, pero no extremas (Michalet *et al.* 2006). Esta es la relación que cabría esperar cuando el estrés está gobernado por un recurso (p. ej. el agua), mientras que cuando el factor de estrés no está gobernado por un recurso (p. ej. temperatura) la relación esperada es más lineal (Maestre *et al.* 2009).

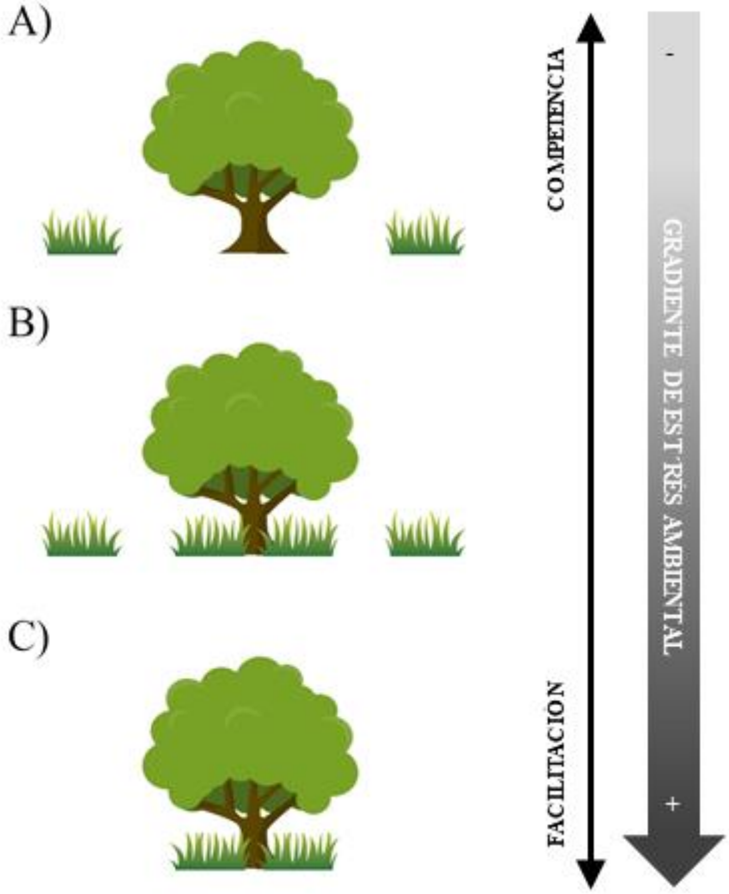


Fig. 5. Tipos de asociación espacial entre especies como resultado de las interacciones bióticas. A) Asociación espacial negativa. Las dos especies coexisten menos frecuentemente de lo que cabría esperar por azar, lo que puede interpretarse como un predominio de interacciones negativas. B) Las dos especies coexisten con la frecuencia que cabría esperar por azar, lo que indica un balance neutral de interacciones. C) Asociación espacial positiva. Las dos especies coexisten más frecuentemente de lo que cabría esperar por azar, lo que puede interpretarse como un predominio de interacciones positivas entre ellas.

Alelopatía, una interacción entre plantas mediada químicamente

El hecho de que unas plantas afecten a otras a través de la emisión de compuestos químicos al medio es conocido desde hace mucho tiempo (Willis 2007). Pero no fue hasta hace relativamente poco (1937) cuando el austriaco Hans Molisch, un fisiólogo vegetal, acuñó formalmente el término alelopatía para referirse a este fenómeno (Molisch 2001; Mallik 2008). Posteriormente, Rice (1984) definió la alelopatía como la influencia, directa o indirecta, que una planta (incluyendo microorganismos) ejerce sobre la germinación, crecimiento o supervivencia de otras plantas debido a la emisión de compuestos químicos (denominados aleloquímicos, del inglés "*allelochemicals*") al medio ambiente. Esta definición es una de las más usadas actualmente, pero resulta un tanto amplia, ya que puede abarcar casi cualquier aspecto de las interacciones entre plantas mediadas químicamente. Por esta razón, a menudo se restringe el uso del término alelopatía para hacer referencia únicamente a la influencia negativa sobre otras plantas (Inderjit & Duke 2003). Dicha influencia, puede estar causada directamente por los efectos biológicos de los aleloquímicos en la división celular, la permeabilidad de la membrana celular, la respiración o la actividad fotosintética (Gniazdowska & Bogatek 2005; Duke & Dayan 2006), pero también indirectamente, debido a sus efectos en la actividad de los microorganismos del suelo (Castaldi *et al.* 2009; Chomel *et al.* 2016). Por tanto, la alelopatía se considera un tipo de interacción negativa, que se diferencia de la competencia en que no implica la reducción de un recurso necesario y constituye junto con la competencia por los recursos lo que conjuntamente se denomina como interferencia (Muller 1969).

Los aleloquímicos son incorporados al medio principalmente por exudados radiculares, lavado de las hojas (p.ej. lluvia, niebla, rocío y también riego), volatilización o descomposición de la hojarasca (Friedman & Waller 1985; Inderjit & Duke 2003). En su mayoría, los aleloquímicos son sustancias producidas por las plantas como metabolitos secundarios (Inderjit & Duke 2003; Gniazdowska & Bogatek 2005). Rice (1984) estableció hasta 14 categorías de aleloquímicos en función de su naturaleza química: (1) derivados del ácido cinámico, (2) cumarinas, (3) fenoles simples o derivados del ácido benzoico, gálico y protocatechuico, (4) flavonoides, (5) taninos, (6) terpenoides y esteroides, (7) ácidos orgánicos solubles en agua, (8) ácidos grasos de cadena lineal, (9) quinonas complejas, (10) lactonas, (11) aminoácidos y polipéptidos, (12) alcaloides y cianohidrinas, (13) glucosinolatos y (14) purines y nucleósidos (Fig. 6). Su actividad alelopática depende de la concentración, de las condiciones ambientales, de las tasas de emisión y acumulación en el suelo o de la sensibilidad de las especies afectadas (Inderjit & Duke 2003; Kobayashi 2004; Inderjit *et al.* 2011). Aunque se ha encontrado que muchos metabolitos secundarios tienen propiedades fitotóxicas, no todos los aleloquímicos son metabolitos secundarios. Se ha sugerido que las plantas pueden usar también otras sustancias de una forma alelopática. Por ejemplo, algunas plantas serían capaces de aumentar la disponibilidad de determinados elementos inorgánicos como metales pesados, sales o sulfuros mediante hiperacumulación o exudados radiculares, y tolerar estos mayores niveles mejor que las plantas vecinas, lo que daría lugar a efectos alelopáticos. Este fenómeno se conoce como alelopatía elemental (ver Morris, Grossl & Call 2008 para una revisión sobre el tema).

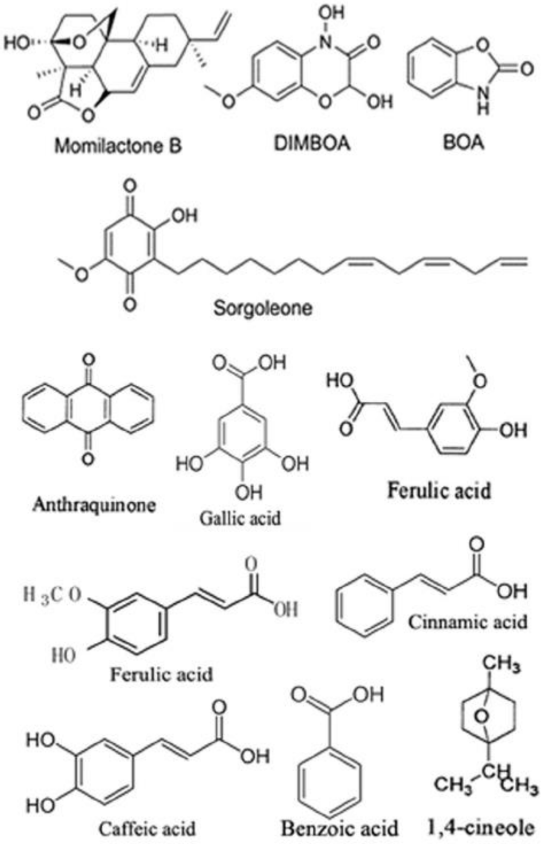


Fig. 6. Estructura de algunos aleloquímicos producidos por las plantas. Fuente: Cheng & Cheng 2015.

La alelopatía es un campo de estudio muy fructífero en agricultura (Cheng & Cheng 2015). Uno de los principales propósitos de su investigación es la aplicación de los efectos alelopáticos de muchas especies de cultivo (p. ej. arroz; *Oryza sativa* L.; Amb & Ahluwalia 2016) como una alternativa ecológica para el control de malas hierbas, o incluso plagas, permitiendo así limitar el uso de herbicidas y plaguicidas (Einhellig & Leather 1988;

Macias *et al.* 2007; Farooq *et al.* 2011; Narwal & Haouala 2013). También, en los últimos años, ha surgido un renovado interés por el estudio de la alelopatía en el campo de las invasiones biológicas (Callaway & Aschehoug 2000; Bais *et al.* 2003; Callaway & Ridenour 2004; Gómez-Aparicio & Canham 2008; Fabbro, Güsewell & Prati 2013). Se ha propuesto que la actividad alelopática explicaría parte del tremendo éxito de algunas plantas invasoras. Concretamente, la hipótesis de las armas novedosas ("*Novel weapons hypothesis*"; Callaway & Ridenour 2004) establece que algunas de estas plantas liberan compuestos alelopáticos que, aunque prácticamente no tienen ningún efecto sobre las especies vegetales que conviven con ellas en sus comunidades de origen, resultan altamente eficientes inhibiendo las especies vegetales en las comunidades invadidas (Callaway & Aschehoug 2000; Callaway & Ridenour 2004; Thorpe *et al.* 2009; Ledger *et al.* 2015). La diferencia biogeográfica en el efecto de los compuestos alelopáticos revela que algunas especies pueden adaptarse a su "vecino químico" (Callaway & Aschehoug 2000; Grøndahl & Ehlers 2008) y ha suscitado un interesante debate sobre el papel de la alelopatía como fuerza de co-evolución (Grøndahl & Ehlers 2008; Jensen & Ehlers 2010; Thorpe *et al.* 2011).

Los compuestos producidos y liberados por las plantas alelopáticas juegan un papel importante en la regulación de distintos aspectos del funcionamiento de los ecosistemas naturales (Muller 1969; Wardle *et al.* 1998; Inderjit *et al.* 2011; Koocheki, Lalegani & Hosseini 2013). Muchos metabolitos secundarios con potencial alelopático actúan como defensas químicas frente a la herbivoría, modificando la población y alimentación de los herbívoros (Wardle *et al.* 1998; War *et al.* 2012). También, estos compuestos modifican la disponibilidad de nutrientes en el suelo. Por ejemplo, los compuestos fenólicos interactúan con el ciclo del N y pueden limitar su disponibilidad (Hättenschwiler & Vitousek 2000). Además, los compuestos alelopáticos alteran las comunidades microbianas del suelo, por ejemplo reduciendo la colonización por micorrizas, y por tanto su actividad (Cipollini, Rigsby & Barto 2012; Wang *et al.* 2012). Los compuestos producidos y liberados por las plantas alelopáticas pueden conducir a que éstas se mantengan en las diferentes etapas de la sucesión vegetal (Gant & Clebsch 1975). Por último, como se ha comentado anteriormente, numerosos estudios han demostrado la importancia de la actividad alelopática en la invasión y dominancia de las especies exóticas en las comunidades invadidas (Callaway & Aschehoug 2000; Bais *et al.* 2003).

A escala más local, la presencia de compuestos alelopáticos determina qué especies pueden o no co-existir con la planta alelopática (Ehlers, Charpentier & Grøndahl 2014; Linhart *et al.* 2015), modificando por tanto el patrón espacial de la vegetación (Fig. 7). En un trabajo clásico de alelopatía, Muller et al. (1964) atribuyeron las zonas de inhibición observadas alrededor de arbustos aromáticos como *Adenostoma fasciculatum*, *Artemisia califórnica* y *Salvia leucophylla* a la acción de los compuestos volátiles que éstos liberan. En un trabajo más reciente, Armas y Pugnaire (2011) encontraron que *Stipa tenacissima* L. inhibe el establecimiento de especies anuales y otras gramíneas a su alrededor a través de exudados radiculares. El efecto que tienen las plantas alelopáticas en la organización espacial de la vegetación está mediado en gran medida por los efectos inhibitorios de los compuestos alelopáticos en la germinación de las semillas (Gniazdowska & Bogatek 2005). Sin embargo, resulta sorprendente que no existan trabajos en la literatura que estudien las consecuencias que tienen las plantas alelopáticas sobre el banco de semillas del suelo (pero ver Fabbro *et al.* 2013). Por otra parte, la existencia de una zona de inhibición no debe tomarse como única prueba de la presencia de compuestos alelopáticos, ya que otros mecanismos, como una fuerte competencia por los recursos, podrían conducir un patrón de la vegetación parecido (Inderjit, Weston & Duke 2005; Armas & Pugnaire 2011; Pescador *et al.* 2014). En otras ocasiones, se ha observado que el efecto de las plantas alelopáticas ocurre debajo del suelo, en forma de segregación de las raíces entre las plantas alelopáticas y otras plantas de la comunidad (Mahall & Callaway 1992; Schenk, Callaway & Mahall 1999). También, se ha observado como en un matorral mediterráneo rico en especies, la presencia de una planta alelopática puede conducir, indirectamente, a un incremento de la diversidad, debido a sus efectos negativos sobre una especie muy competitiva (Ehlers *et al.* 2014) lo que se conoce como facilitación indirecta (Brooker *et al.* 2008). Sin embargo, no está claro si en zonas áridas y semiáridas, donde la facilitación y no la competencia por el espacio es la interacción predominante (Soliveres & Maestre 2014), las plantas alelopáticas podrían tener el mismo efecto en la diversidad.

Fig. 7. Ejemplos de patrones de inhibición alrededor de plantas con actividad alelopática. (a) Inhibición de anuales alrededor de *S. tenacissima* (Fuente: Armas & Pugnaire 2011). (b) Espacios concéntricos a los individuos de *Medicago sativa* L. con poca o ninguna vegetación (Fuente: Weston & Mathesius 2013). (c) Anillo de inhibición formado alrededor de *S. leucophylla* (Muller 1966).

En muchas ocasiones se toma el efecto fitotóxico *in vitro* que tienen determinados compuestos químicos liberados por las especies vegetales potencialmente alelopáticas en la germinación, crecimiento o supervivencia de otras plantas, como única evidencia de alelopatía (Gniazdowska & Bogatek 2005). Sin embargo, las condiciones experimentales en laboratorio, como por ejemplo el tipo de suelo usado y la presencia o ausencia de sus microorganismos, las concentraciones y el nivel de exposición a los compuestos alelopáticos o la mezcla específica de compuestos alelopáticos que se usa, están lejos de las que pueden encontrarse en la naturaleza (Inderjit & Weston 2000). Además, a menudo en estos estudios el efecto de los compuestos alelopáticos se comprueba en especies modelo como la lechuga *(Lactuca sativa* L.; Escudero *et al.* 2000; Scognamiglio *et al.* 2013; Zhang *et al.* 2014), que no coexisten en los ecosistemas naturales con las plantas alelopáticas, limitando así el significado ecológico que tienen los resultados obtenidos. En este sentido, el uso del banco de semillas puede constituir una buena alternativa para solventar tales limitaciones. En cualquier caso, más estudios de campo son necesarios para obtener evidencias realistas sobre el significado de la alelopatía en condiciones naturales (Inderjit & Weston 2000).

La metodología necesaria para evaluar la interferencia alelopática en condiciones naturales resulta desafiante cuanto menos, sobre todo a la hora de separar los efectos alelopáticos de otros factores como la competencia por los recursos (Wardle *et al.* 1998; Inderjit & Callaway 2003). Esto añadido a las dificultades encontradas para relacionar los resultados de estudios de laboratorio con observaciones del patrón de la vegetación en condiciones naturales, ha generado que el papel de la alelopatía determinando la estructura y composición de las comunidades vegetales haya sido incluso puesto en duda (Harper 1975; Wardle *et al.* 1998). El carbón activo (CA) se usa como una manera efectiva de reducir la interferencia química de las plantas alelopáticas en condiciones de campo, debido a su alto poder para secuestrar los compuestos bioquímicos (Callaway & Aschehoug 2000; Inderjit & Callaway 2003; Hille & den Ouden 2005). Aun así, el CA puede modificar también la concentración de nutrientes en el suelo, el pH o la capacidad para retener agua, generando una serie de efectos secundarios que pueden confundirse con los efectos beneficiosos que resultan de reducir la interferencia química (Inderjit & Callaway 2003; Lau *et al.* 2008; Weißhuhn & Prati 2009). Esta posibilidad debe ser cuidadosamente evaluada cuando se usa CA (Lau *et al.* 2008). Además, la mayoría de los pocos estudios de alelopatía en condiciones de campo han sido realizados en sistemas gestionados (Kulmatiski 2011; Li, Nie & Zhang 2016; Zhang *et al.* 2016) o en comunidades vegetales invadidas por especies exóticas (Kulmatiski & Beard 2006; Gómez-Aparicio & Canham 2008; Fabbro *et al.* 2013), mientras que casi ninguno se ha centrado en comunidades de plantas no perturbadas (pero ver Nilsson 1994; Nilsson *et al.* 2000; da Silva *et al.* 2015).

Importancia de la alelopatía en zonas áridas y semiáridas

Las interacciones alelopáticas podrían ser especialmente relevantes determinando la estructura y dinámica de las comunidades vegetales de zonas áridas y semiáridas, por varias razones. En primer lugar, en estas regiones predominan las plantas aromáticas, que son consideradas como especies potencialmente alelopáticas (Muller *et al.* 1964; Thompson 2005; Araniti *et al.* 2012). De hecho, se ha sugerido que en estos ambientes tan duros, la alelopatía puede constituir una estrategia evolutiva en contra de convertirse en plantas facilitadoras (van der Putten 2009) y evitar así la competencia por los recursos escasos. Por otra parte, se ha observado como los compuestos químicos liberados por las plantas alelopáticas pueden interferir también en la germinación, el crecimiento y la supervivencia de la propia especie que los produce. Este fenómeno es conocido como autotoxicidad (Friedman & Waller 1985; Singh, Batish & Kohli 1999). En comunidades vegetales de zonas áridas y semiáridas, donde los recursos son escasos (p.ej. agua), la autotoxicidad puede representar un mecanismo adaptativo de las especies para autorregular su densidad poblacional y evitar la competencia intraespecífica (Armas & Pugnaire 2011). Así, una planta autotóxica puede reducir el reclutamiento de individuos conspecíficos inhibiendo la germinación cerca de los individuos parentales, o retrasándola hasta que las condiciones sean más benignas (p.ej. tras las lluvias; Friedman & Waller 1985; Singh *et al.* 1999). Por lo tanto, en comunidades vegetales áridas y semiáridas, la autotoxicidad podría tener también un papel relevante determinando el patrón espacial y dinámica de las especies alelopáticas.

En segundo lugar, se sabe que la producción, liberación, acumulación e incluso la fitotoxicidad de los compuestos alelopáticos se ven favorecidas en presencia de un mayor estrés ambiental de origen abiótico como altas temperaturas, escasez de agua o una intensa radiación solar (Tang *et al.* 1995; Pedrol, González & Reigosa 2006). Lo mismo puede ocurrir bajo un mayor nivel de estrés biótico. Por ejemplo, Karban (2007) encontró que la capacidad de los compuestos volátiles emitidos por la especie *Artemisia tridentata* Nutt. para inhibir la germinación de algunas hierbas aumentó cuando las hojas fueron dañadas experimentalmente, lo que sugiere que la herbivoría incrementa la actividad alelopática (pero ver Kong, Hu & Xu 2002). Y, en tercer lugar, las plantas sensibles al efecto de los compuestos alelopáticos son más vulnerables bajo el efecto de múltiples factores de estrés (Pedrol *et al.* 2006). Por lo tanto, el balance neto de interacciones de una planta alelopática podría no seguir las predicciones de la SGH, ya que, independientemente de la intensidad del efecto facilitador, el potencial efecto positivo de las plantas alelopáticas de zonas áridas y semiáridas podría verse contrarrestado de alguna manera por una mayor intensidad del efecto alelopático bajo mayores condiciones de estrés ambiental. Sin embargo, la alelopatía ha sido olvidada en la mayoría de los trabajos que examinan el efecto neto de las interacciones entre plantas (Holmgren, Scheffer & Huston 1997; Miriti 2006) en comunidades vegetales de zonas áridas y semiáridas, o bien se refieren a interferencia en sentido genérico (Callaway & Walker 1997; Holzapfel & Mahall 1999), pero su estudio resulta necesario para poder predecir adecuadamente la dinámica de estas comunidades ante condiciones cambiantes de aridez y pastoreo.

En la literatura se asume que las plantas alelopáticas de comunidades vegetales de zonas áridas y semiáridas liberan sobre todo compuestos químicos volátiles (p. ej. terpenoides), mientras que los compuestos alelopáticos solubles en agua (p. ej. muchos fenoles) son más frecuentes en plantas de regiones con un clima más frío (Moral & Cates 1971; Chou 1999; Reigosa, Sánchez-Moreiras & González 1999). Pero lo cierto es que las plantas adaptadas a las duras condiciones ambientales de las zonas áridas y semiáridas producen y acumulan una gran variedad de compuestos fenólicos para evitar los daños por el estrés oxidativo inducido por la escasez de agua, entre otros factores de estrés, y por lo tanto son muy ricas en este tipo de compuestos (Bautista *et al.* 2016; Varela *et al.* 2016). Además, aunque la escasez de agua en estas regiones puede actuar como una barrera física para la difusión de compuestos alelopáticos solubles en agua, los parches de vegetación incrementan la cantidad de agua disponible para las plantas (Noy-Meir 1973; Cerdà 1997b; Ludwig *et al.* 2005), y en consecuencia, la disponibilidad de los compuestos solubles en agua se ve favorecida en los parches de vegetación. Por lo tanto, el papel de los compuestos solubles en agua, y en especial el de los fenoles, en la interferencia química de las plantas alelopáticas de zonas áridas y semiáridas, puede haber sido pasado por alto.

Objetivos e hipótesis

El **objetivo general** de esta tesis doctoral es determinar la importancia relativa de la alelopatía en la estructura y la dinámica de una comunidad vegetal natural semiárida con respecto a otras interacciones bióticas entre plantas (p. ej. facilitación). Para ello, se combina el análisis de datos recogidos directamente en el campo con experimentos realizados tanto en laboratorio como en condiciones naturales, lo que permitirá obtener distinta información sobre el papel que juega la alelopatía, incluyendo la autotoxicidad, en los diferentes estadios del ciclo vital de las plantas.

En concreto, se abordaron los siguientes objetivos específicos e **hipótesis** de trabajo:

1. Establecer la relación espacial entre las plantas alelopáticas y el resto de especies que componen la comunidad vegetal semiárida (capítulos 1 y 2):

   H1.1: La actividad alelopática favorece un patrón menos agregado de la vegetación de lo que es habitual para los arbustos de comunidades semiáridas. Independientemente de si se mejoran las condiciones microclimáticas e hidrológicas con respecto al suelo desnudo, las plantas se establecerán preferentemente fuera de los parches de vegetación formados por plantas alelopáticas.

H1.2: La alelopatía favorece una disminución de la diversidad alrededor de los arbustos alelopáticos en comunidades vegetales de zonas semiáridas. Se espera que las plantas alelopáticas generen una "zona de inhibición" a su alrededor en la que tan solo las especies capaces de tolerar la presencia de compuestos alelopáticos podrán establecerse.

2. Evaluar el alcance de la interferencia química de distintos tipos de compuestos alelopáticos en diferentes estadios del ciclo vital de las plantas (germinación, establecimiento y crecimiento) de las especies vegetales que coexisten con la especie alelopática en la comunidad vegetal semiárida, incluyendo la propia especie alelopática (existencia de autotoxicidad) (capítulos 1, 3, 4 y 5).

H2.1: Los efectos alelopáticos y autotóxicos en ambientes semiáridos están mediados tanto por compuestos volátiles, como por compuestos solubles en agua.

H2.2: La alelopatía es un fenómeno especialmente relevante inhibiendo la germinación, reduciendo el establecimiento y la supervivencia de especies susceptibles que emergen desde el banco de semillas, explicando así el patrón de la vegetación observado alrededor de las plantas alelopáticas.

H2.3: La autotoxicidad es un mecanismo relevante en comunidades vegetales semiáridas. Se espera que los compuestos químicos producidos y liberados por las plantas alelopáticas inhibirán de manera sustancial la germinación, la supervivencia o el crecimiento de sus propia plántulas.

H2.4: Algunas especies vegetales de la comunidad están adaptadas a las plantas alelopáticas. Estas especies tolerarán los efectos fitotóxicos de los compuestos químicos producidos y liberados por las plantas alelopáticas.

3. Determinar el balance neto de interacciones de un arbusto alelopático (facilitación – interferencia, donde interferencia = competencia + alelopatía) bajo distintas condiciones de estrés ambiental, y sus consecuencias en la estructura y dinámica de la comunidad vegetal semiárida (capítulos 2 y 5).

H3.1: La alelopatía es una interacción relevante en ambientes semiáridos que ocurre simultáneamente con la facilitación y la competencia.

H3.2: El balance neto de interacciones de las plantas alelopáticas en comunidades vegetales semiáridas es negativo. La presencia de compuestos alelopáticos contrarrestará los efectos beneficiosos de la facilitación. Sin embargo, este balance será netamente positivo para las especies tolerantes.

H3.3: La importancia relativa de la alelopatía aumenta conforme se incrementan las condiciones de aridez y pastoreo. Se espera que bajo condiciones de mayor estrés aumente la producción de los compuestos alelopáticos, al mismo tiempo que las especies son más vulnerables a sus efectos, y por tanto, el balance de interacciones de las plantas alelopáticas siga siendo negativo.

Metodología general

Para dar respuesta a los objetivos y las hipótesis planteados en esta tesis doctoral se ha combinado el análisis de datos recogidos en campo con experimentos tanto de laboratorio como de campo. En cada capítulo se incluye información detallada sobre la metodología que se ha empleado y los análisis estadísticos que han sido utilizados en cada caso. Por tanto, en este apartado se presenta únicamente el área de estudio (sector central de la depresión del Ebro) y la especie alelopática de estudio (*Artemisia herba-alba* Asso.).

Área de estudio: depresión media del Ebro

El trabajo de campo realizado durante esta tesis doctoral (que incluye muestreos de vegetación y experimentos de campo en condiciones naturales) se ha llevado a cabo en el sector central de la depresión del Ebro (noreste de España; Fig. 8). El clima en el área es mediterráneo semiárido, y constituye junto con el sudeste ibérico una de las regiones más áridas de la península. La precipitación media anual varía entre 300 y más de 400 mm, desde el centro hacia el norte y el sur de la depresión. Las lluvias, que muchas veces son torrenciales, ocurren principalmente en primavera y en otoño, mientras que los veranos y algunos inviernos suelen ser secos. La temperatura media anual en el área de estudio se sitúa en torno a los 15 ºC, pero con una fuerte oscilación estacional. Así, las temperaturas máximas en verano pueden alcanzar los 42 ºC, mientras que las mínimas en invierno pueden ser de varios grados bajo cero (datos de precipitación y temperatura obtenidos del Atlas Climático Digital de Aragón; http://anciles.aragon.es/AtlasClimatico/). Además, en el área de estudio son muy característicos los días de “cierzo”, un viento del oeste-noroeste, frio y de considerable velocidad (rachas de hasta 70 km/h), que tiene un alto poder desecante (Longares 1997). La litología del área de estudio está constituida fundamentalmente por afloramientos yesíferos, que se alternan con margas, calizas y arcillas. Esta litología confiere a la depresión media del Ebro un relieve muy característico, compuesto por fondos de valle planos separados por pequeñas colinas (Pueyo 2005). La principal actividad antrópica en la zona ha sido tradicionalmente el aprovechamiento agro-pastoral de los recursos, con cultivos de cereal de secano y ganadería extensiva de ovejas (*Rasa aragonesa*; <0.7 cabezas ha^{-1} y año^{-1}; Pueyo 2005).

Se seleccionaron dos zonas de estudio a lo largo del amplio gradiente de aridez que existe desde el río Ebro hasta el prepirineo. La mayor parte del trabajo de campo se desarrolló en la Reserva Ornitológica de "El Planerón" (41º22´09´´N, 00º37´50´´O; Fig. 8). Geográficamente, se localiza en el término municipal de Belchite (Zaragoza), en la margen derecha de la depresión del Ebro, entre el Barranco de Lopín y la Muela de La Pedriza (Longares 1997). Topográficamente se trata de una zona cerrada, de escasa pendiente, con un marcado carácter endorreico. Las condiciones climáticas de esta zona son las de mayor aridez de la región. La precipitación media anual es de 315 mm y la temperatura media anual es de 15.4 ºC (Fig. 9; IA = 0.26). La segunda zona de estudio se sitúa más al norte, en el término municipal de Castejón de Monegros (Huesca), cerca de la sierra de Alcubierre (41º41´36´´N, 00º09´49´´O, Fig. 8). La precipitación media anual es de 377 mm y la temperatura media anual de 14.25 ºC (Fig. 9). Esta zona se utilizó en contraposición a la anterior por su menor grado de aridez (IA = 0.33). Los suelos son margo-arcillosos en las zonas de estudio, siendo especialmente ricos en arcillas en la Reserva Ornitológica de "El Planerón", lo que favorece un drenaje deficitario, y ligeramente salinos.

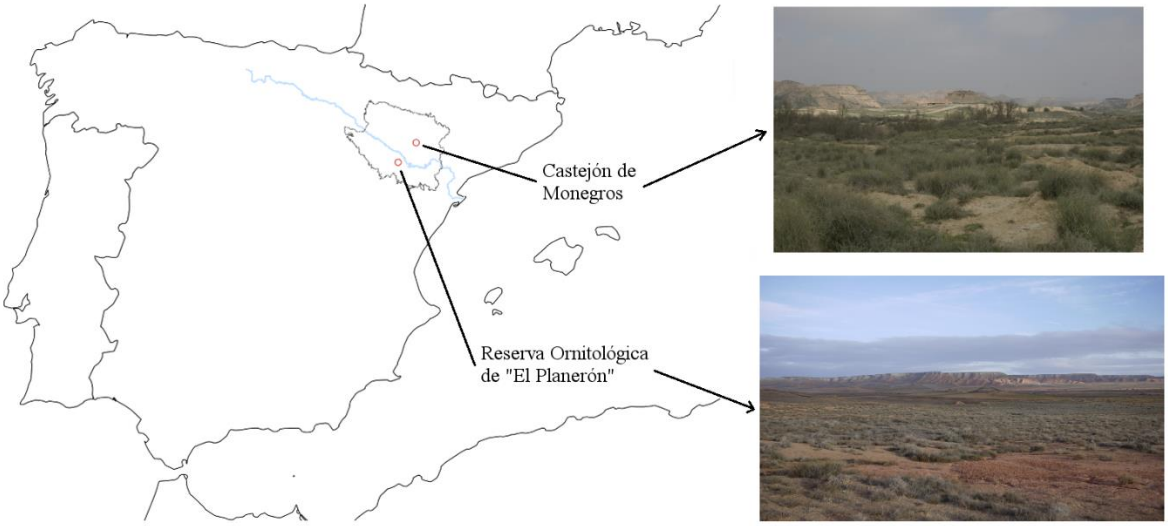


Fig. 8. Mapa de localización e imágenes de las zonas de estudio. Fotografías: Yolanda Pueyo.

En ambas zonas de estudio aparece la misma comunidad vegetal en las áreas no cultivadas. Dicha comunidad está dominada por matorrales bajos como *Salsola vermiculata* L., *Artemisia herba-alba* Asso., *Peganum harmala* L., *Atriplex halimus* L. o *Camphorosma monspeliaca* L., gramíneas perennes como *Lygeum spartum* L., *Stipa parviflora* Desf., *Dactylis glomerata* L. subsp. *hispanica* (Roth) Nyman o *Brachypodium retusum* (Pers.) P.Beauv. y una gran variedad de especies anuales como *Aizoon hispanicum* L., *Frankenia pulverulenta* L. o *Plantago afra* L. (Braun-Blanquet & Bolòs 1957). La composición florística de la comunidad vegetal varía en función de diversos factores topográficos y litológicos. Por ejemplo, las zonas de mayor salinidad aparecen dominadas por especies halófitas como *Suaeda vera* J.F.G.mel y *Limonium* sp. La vegetación en estas zonas aparece estructurada en parches en una matriz de suelo desnudo (Fig. 8). En estas comunidades, el uso pastoral sostenido ha favorecido a los arbustos nitrófilos en detrimento de las gramíneas (Puigdefábregas & Mendizabal 1998; Pueyo *et al.* 2013).

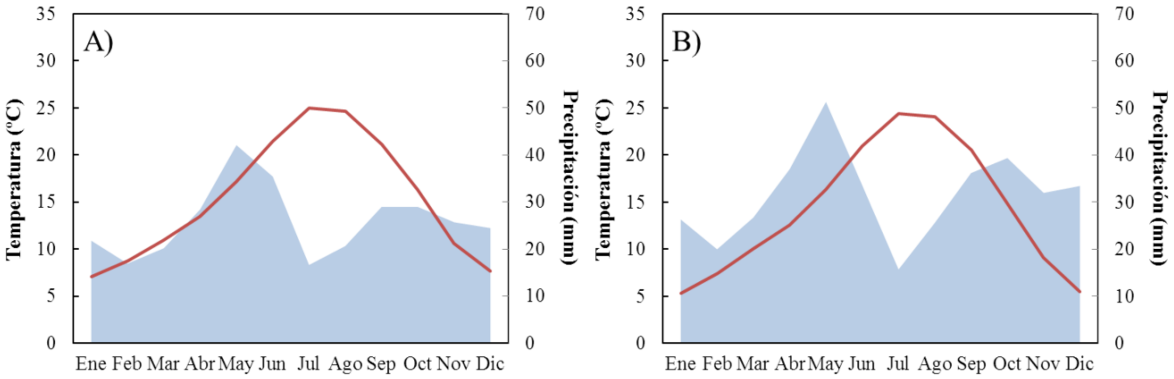


Fig. 9. Temperatura (línea roja) y precipitación mensual (área sombreada) durante el periodo 1970-2000 en A) la reserva ornitológica “El Planerón” (Belchite) y B) Castejón de Monegros. Datos obtenidos del Atlas Climático digital de Aragón (http://anciles.aragon.es/AtlasClimatico/).

Especie de estudio: *Artemisia herba-alba* Asso.

Artemisia herba-alba Asso. (Fam. *asteraceae*; ontina; “*white wormwood*” en inglés) es un caméfito aromático, de poca altura (aprox. 30 cm), que se caracteriza por tener hojas pequeñas y cortas de un color verde-blanquecino y ramas rígidas y erectas (aunque a veces están arqueadas hacia abajo; Fig. 10A). Florece entre Septiembre y Diciembre y sus flores son de un color marrón-amarillento. *Artemisia herba-alba* se distribuye por toda la cuenca Mediterránea, fundamentalmente por la región del Mediterráneo occidental (Fig. 10B; Mohamed *et al.* 2010). En España es abundante en la mitad oriental donde aparece frecuentemente formando parte de matorrales xerófilos pastados más o menos nitrófilos.

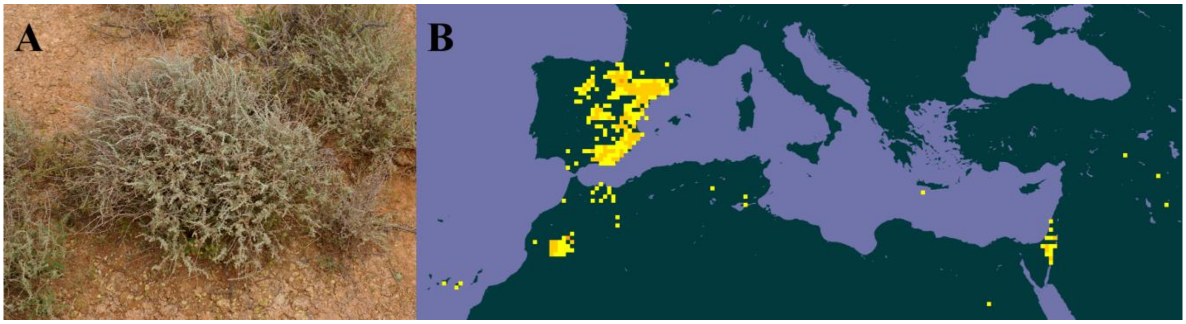


Fig. 10. A) Imagen de *A. herba-alba* (Fotografía: Yolanda Pueyo) y B) mapa de distribución (Fuente: GBIF).

En muchas regiones del norte de África y Oriente Próximo *A. herba-alba* se ha utilizado tradicionalmente como planta medicinal para el tratamiento, por ejemplo, de la hipertensión o la diabetes (Tahraoui *et al.* 2007) o en infusiones para el dolor de estómago (Friedman *et al.* 1986). Además, *A. herba-alba* es considerada como una especie forrajera muy importante para el ganado doméstico en algunas regiones del Argelia, donde crece abundantemente (Mohamed *et al.* 2010).

Al igual que muchas otras especies del género *Artemisia*, *A. herba-alba* posee un marcado carácter alelopático bien documentado (Friedman, Orshan & Ziger-Cfir 1977; Escudero *et al.* 2000; Mohamed *et al.* 2010; Chon & Nelson 2010). Estudios previos han identificado, tanto en sus partes aéreas como en las raíces, multitud de compuestos fenólicos (en su mayoría ácidos fenólicos, p.ej. ácidos vanílico, caféico, chlorogénico y *p*-Coumárico; Seddik *et al.* 2010; Bourgou *et al.* 2016), flavonoides (p.ej. luteolin, quercetin y apigenin Saleh *et al.* 1985; Mohamed *et al.* 2010) y aceites esenciales (p.ej. alfa/beta-thujones, camphor, chrysanthenone y 1,8-cineole; Salido *et al.* 2004; Mohsen & Ali 2009; Tilaoui *et al.* 2015) que tienen capacidad para inhibir la germinación, el crecimiento y la supervivencia de especies sensibles en experimentos de laboratorio (Friedman *et al.* 1977; Escudero *et al.* 2000). Además, estos compuestos tienen otros efectos biológicos de interés, tales como antibióticos, antioxidantes o antiinflamatorios (Seddik *et al.* 2010; Khlifi *et al.* 2013). La presencia y abundancia relativa de estos compuestos varía entre regiones geográficas (Salido *et al.* 2004; Mohamed *et al.* 2010). Por otra parte, también se ha sugerido que *A. herba-alba* es una especie autotóxica, es decir, que inhibe el establecimiento de sus propias plántulas cerca de los adultos (Friedman & Orshan 1975). Sin embargo, el papel ecológico que juega *A. herba-alba* en la comunidad vegetal como arbusto alelopático y autotóxico prácticamente no ha sido estudiado. En la literatura tan solo se pueden encontrar un par de trabajos en este sentido. Por un lado, Friedman y colaboradores (1975; 1977) observaron que *A. herba-alba* causa una supresión de anuales, y de otras plántulas de *A. herba-alba* cerca de plantas adultas de *A. herba-alba* en sendos trabajos en el desierto de Néguev en Israel. En otro estudio, Escudero et al. (2000) concluyó que el ecotono tan abrupto observado en una comunidad semiárida en el centro de España puede estar controlado, al menos parcialmente, por la interferencia alelopática de *A. herba-alba* en *Helianthemum squamatum* (L.) Dum. Cours., aunque sus conclusiones están basadas únicamente en resultados de experimentos de laboratorio. Por otro lado, *A. herba-alba* es una especie muy común en las comunidades vegetales semiáridas de la depresión media del Ebro. Por lo general, estas comunidades están dominadas por dos especies arbustivas, *A. herba-alba* y *S. vermiculata*, y una gramínea perenne de gran porte, *L. spartum*, que aparecen acompañadas por una gran variedad de especies anuales. Son comunidades muy sencillas que permiten entender fácilmente los procesos que operan en ellas. Por tanto, se considera que *A. herba-alba* y estas comunidades constituyen un marco ideal para la consecución de los objetivos y las hipótesis que se plantean en esta tesis doctoral.

Estructura de la tesis

Esta tesis doctoral se encuentra organizada en cinco capítulos que abordan distintos aspectos de los objetivos planteados. Cada capítulo se corresponde con un artículo de investigación que se encuentra publicado, o en revisión, en una revista científica de ámbito internacional. Por esta razón, los capítulos están escritos en inglés y presentan una estructura dividida en introducción, material y métodos, resultados y discusión.

En el **capítulo 1**, *Plant nurse effects rely on combined hydrological and ecological components in a semiarid ecosystem* (Ecosphere 2016), se presenta el estudio de la relación entre el patrón espacial del establecimiento de las plantas en una comunidad vegetal semiárida y las propiedades hidrológicas y microclimáticas de los sitios donde se establecen. Para ello, se analiza el establecimiento de especies de matorral y de gramíneas bajo tres especies potencialmente nodrizas (*S. vermiculata*, *A. herba-alba* y *L. spartum*) en ocho parcelas de 6 x 6 m^2 a lo largo de dos años, así como la germinación, la supervivencia y el crecimiento de *S. vermiculata* y *L. spartum* bajo individuos adultos de estas dos especies y en suelo desnudo, en un experimento de cuatro años. También se analiza el contenido de agua en el suelo, la infiltración de agua tras las lluvias y la radiación solar bajo las tres especies potencialmente nodrizas y el suelo desnudo para identificar los procesos responsables de los patrones observados.

En el **capítulo 2**, *Plant-plant interactions as a mechanism structuring plant diversity in a Mediterranean semi-arid ecosystem* (Ecology and Evolution 2015), se presenta el efecto que tienen las interacciones bióticas de tres especies dominantes de una comunidad vegetal semiárida: la gramínea *L. spartum*, el arbusto alelopático *A. herba-alba* y el arbusto facilitador *S. vermiculata*, en la diversidad y la composición de especies, bajo diferentes condiciones de aridez y pastoreo. Para ello, se cuantifica el balance neto de las interacciones de las tres especies de estudio basándose en datos de co-ocurrencia de especies. También, se analiza el patrón espacial de la diversidad alrededor de los individuos de las tres especies de estudio calculando el *individual species-area relationship* (ISAR) y los cambios en la composición de especies calculando el índice de similaridad de Chao-Jaccard.

En el **capítulo 3**, *Effects of the allelopathic plant* Artemisia herba-alba *Asso. on the soil seed bank of a semi-arid plant community* (Journal of Plant Ecology 2016), se investiga si los efectos en la vegetación, observados en las inmediaciones de los individuos de *A. herba-alba* en los dos capítulos anteriores, se deben a una heterogeneidad espacial en la estructura del banco de semillas entre *A. herba-alba* y el arbusto no alelopático *S. vermiculata*. Para ello, se analiza la riqueza de especies y la densidad de semillas del banco de semillas en estos dos micrositios y en el suelo desnudo. También, se evalúa el efecto que tiene el extracto acuoso de *A. herba-alba* en la tasa y en el tiempo de emergencia de las plántulas desde el banco de semillas, así como también en la mortalidad de las plántulas emergidas.

En el **capítulo 4**, *Autotoxic and allelopathic effects of volatile and water soluble chemicals of* Artemisia herba-alba *Asso.* (en revisión), se investiga, en una serie de experimentos de laboratorio, la actividad alelopática y autotóxica de los compuestos volátiles y solubles en agua liberados por *A. herba-alba*. Para ello, se analiza el efecto de estos compuestos sobre la germinación y el crecimiento temprano de un conjunto de especies de interés (*S. vermiculata*, *L. spartum*, *P. halepensis* y la propia *A. herba-alba*) que coexisten con *A. herba-alba* en comunidades vegetales semiáridas. También, se identifican y cuantifican los compuestos fenólicos presentes en el extracto acuoso de *A. herba-alba* y se comprueba el efecto de una mezcla de los compuestos identificados en la germinación de las especies de interés.

En el **capítulo 5**, *Evidence for allelopathic interference of neighboring plant species by* Artemisia herba-alba *Asso: a field study* (en revisión), se examina la importancia de la interferencia alelopática de *A. herba-alba* en condiciones naturales. Para ello, se analiza el crecimiento, la vitalidad, la germinación y la supervivencia de especies que co-existen con *A. herba-alba* en una comunidad vegetal semiárida (*S. vermiculata*, *L. spartum* y *A. herba-alba*) con y sin la presencia de interferencia química mediante el uso de carbón activo, en un experimento de dos años. También, se evalúa el balance neto de interacciones de *A. herba-alba* en comparación con el balance neto de *S. vermiculata*, y cómo la aridez puede modular el balance neto.

En la **Discusión general** se discuten en conjunto los resultados más importantes obtenidos en los capítulos anteriores en relación con las hipótesis planteadas en la introducción. Además, se presentan nuevas perspectivas y futuras líneas de investigación para ampliar el conocimiento sobre el papel que juega la alelopatía en la organización de las comunidades vegetales semiáridas, así como también las principales **conclusiones** obtenidas en esta tesis doctoral.

Capítulo 1 / Chapter 1

Plant nurse effects rely on combined hydrological and ecological components in a semiarid ecosystem

Yolanda Pueyo, David Moret-Fernández, Antonio I. Arroyo, Angel de Frutos, Sonia Kéfi, Hugo Saiz, Raquel Charte, Mª Luz Giner, Concepción L. Alados

Ecosphere (2016), 7, e01514

Plant nurse effects rely on combined hydrological and ecological components in a semiarid ecosystem

Yolanda Pueyo[1], David Moret-Fernández[2], Antonio I. Arroyo[1], Angel de Frutos[1], Sonia Kéfi[3], Hugo Saiz[4,5], Raquel Charte[1], Mª Luz Giner[1], Concepción L. Alados[1]

[1] Instituto Pirenaico de Ecología, Consejo Superior de Investigaciones Científicas, Av. Montañana 1005, 50059 Zaragoza, Spain.

[2] Estación Experimental Aula Dei, Consejo Superior de Investigaciones Científicas, Av. Montañana 1005, 50059 Zaragoza, Spain.

[3] Institut des Sciences de l´Évolution. BioDICée Team. CNRS - Université de Montpellier. Place Eugène Bataillon, 34095 Montpellier, France.

[4] UMR 6553 Ecobio. CNRS - Université de Rennes. Avenue du General Leclerc, 35042 Rennes, France.

[5] Departamento de Biología y Geología, Física y Química Inorgánica. Universidad Rey Juan Carlos. C/Tulipán s/n, 28933 Móstoles, Spain.

Abstract

Plant establishment in semiarid ecosystems is affected by the limited spatial and temporal availability of resources and adequate microsites provided by nurse plants. There has been little research on plant establishment in these ecosystems that consider both the ecological roles of different plant types and the abiotic properties of their microsites. Such studies could provide important insights about the functioning of semiarid ecosystems. Here, we investigated the links between the patterns of plant establishment and the hydrological and microsite properties of shrubs and grasses in a semiarid ecosystem northeastern Spain. For ecological experiments, we measured the spatial patterns of the establishment of shrubs and grasses in eight 6x6 m^2 quadrats over two years; we also sowed seeds of *Salsola vermiculata* (a dominant shrub) and *Lygeum spartum* (the dominant perennial grass) under adult shrubs (*S. vermiculata*) and grasses (*L. spartum*) and in bare soil, and then examined seedling germination, survival, and growth over four years. For hydrological experiments, we analyzed soil water content under the two co-dominant shrubs (*S. vermiculata* and *Artemisia herba-alba*), the dominant perennial grass (*L. spartum*), and in bare soil over 18 months; we also measured water infiltration and solar radiation at the same four microsites to identify the hydrological processes responsible for the observed eco-hydrological patterns. The three potential nurse plants greatly improved the hydrological and microsite conditions. They increased soil water content after rainfall relative to bare soil. Moreover, *S. vermiculata* and *L. spartum* slowed the drying process. However, only *S. vermiculata* acted as a nurse plant. It improved plant density, diversity, performance, and survival during the whole study period. *L. spartum* facilitated plant establishment during early stages, but interfered with seedling performance and survival during later stages, probably because of the increasing competition for water with seedlings. *A. herba-alba* did not facilitate plant establishment at any stage, most likely because of water scarcity during prolonged dry periods and its allelopathic effects. We conclude that the ecological role of a plant cannot be directly inferred from its hydrological or microsite properties. Long-term eco-hydrological studies are required to understand the role of nurse plants on seedlings establishment.

Keywords

Eco-hydrology, facilitation, infiltration, interference, plant establishment, shrub-grass interactions, soil water content.

Introduction

Plant establishment (i.e. seedling emergence and survival) is a critical stage for the maintenance of plant diversity and productivity in arid and semiarid ecosystems, and it is mostly conditioned by water availability (Noy-Meir 1973). Most recruitment occurs during exceptionally wet years, and most seedling mortality occurs during drought periods (Beatley 1974). Water availability in these ecosystems has significant spatial variability, because of the redistribution of rainfall through surface run-off and sub-surface water flows, and because infiltration varies at different locations (Ludwig et al. 2005). The significant spatial and temporal heterogeneity of water availability in these semiarid ecosystems has led to their classification as “source-sink” systems (Cerdà 1997, Imeson and Prinsen 2004). These ecosystems typically have patches of vegetation within a bare soil matrix, with substantial water run-off from bare soil and water infiltration beneath established plant canopies. Thus, most new seedlings are established near plant canopies. The ecological and hydrological dynamics of semiarid ecosystems are well known (Ludwig et al. 2005, Bautista et al. 2007, Pueyo et al. 2013). However, few studies have simultaneously examined the spatial dynamics of hydrology and patterns of plant establishment within these plant communities (Drezner 2007). Such studies are clearly necessary because they can provide important information for conservation by identifying environmental factors that determine successful plant establishment in semiarid ecosystems (Gomez-Aparicio et al. 2005).

Soil water content, or soil water storage (*sensu* Reynolds et al., 2004), is the most important hydrological component for plant dynamics. It accounts for spatial and temporal variability due to water gains (by infiltration after rainfall and run-on) and losses (by evaporation, uptake and transpiration by plants, leakage, and run-off; Figure 1). Thus, this hydrological component best characterizes the spatio-temporal availability of water for plant establishment and dynamics (Reynolds et al. 2004, Jenerette et al. 2012). However, resources such as nutrients and availability of light can also affect plant establishment, as can non-resource factors related to the microsite environment (Figure 1), such as maximum temperatures during the warm season and minimum temperatures during the cold season (Callaway 2007).

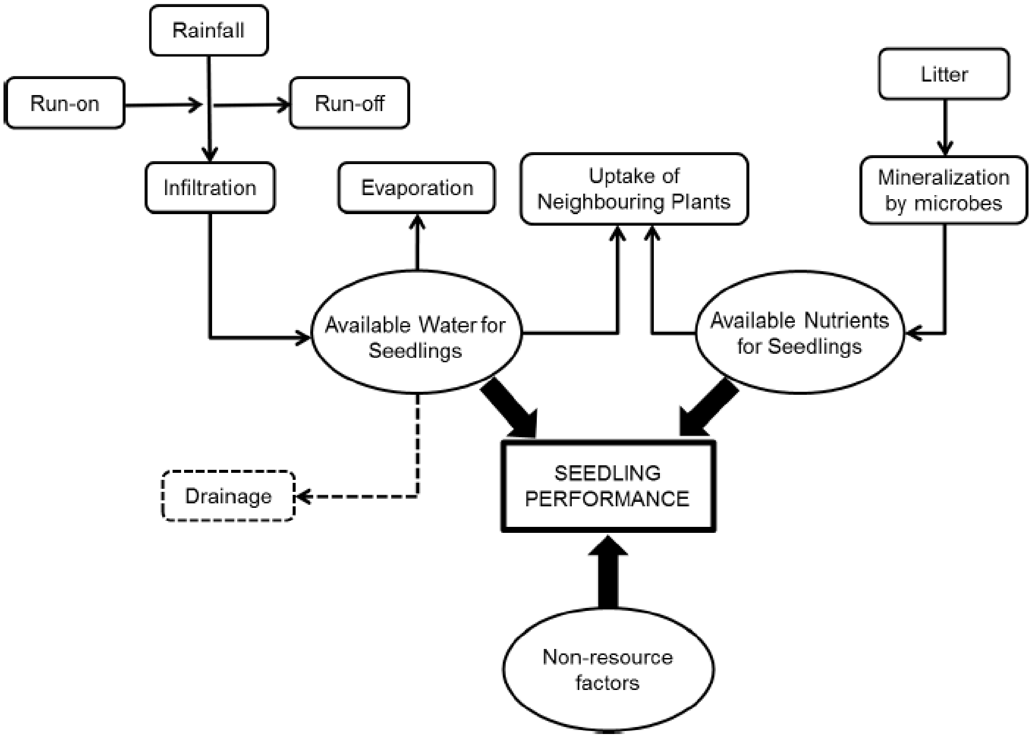


Figure 1. The most relevant factors related to seedling performance in a semiarid "source-sink" ecosystem. Dotted arrow: water losses by drainage are negligible in ecosystems where infiltration is low and evaporation is high.

Nurse plants can improve the resource and non-resource factors that are important for plant establishment in semiarid environments (Flores and Jurado 2003). While it is clear that non-resource microsite environmental conditions for plant establishment are generally improved by a wide array of nurse plant types, nurse plants can either facilitate or interfere with seedlings for resources (i.e. competing for water) (Michalet 2007, Maestre et al. 2009b). The net outcome of the interaction between a nurse plant and a seedling depends on the morpho-functional traits of both the benefactor and beneficiary species (Gómez-Aparicio 2009, Maestre et al. 2009b). In semiarid plant communities, coexisting shrubs and perennial grasses account for most of the perennial biomass. In these ecosystems, shrubs usually act as nurse plants that improve plant establishment (Gómez Aparicio et al. 2004, Maestre et al. 2009a, Poulos et al. 2014), because they can ameliorate harsh abiotic conditions providing shade and increasing the availability of water and nutrients without exerting strong competition for water (Callaway 2007). This is because they usually have deep roots that do not compete directly for water with established plants in the upper layers of soil (Soriano and Sala 1983). Previous research in experimental plantations indicated that perennial grasses facilitate establishment by providing more favorable abiotic conditions than bare soil. However, in natural ecosystems there is long-term competition of the roots of grasses with those of annuals and seedlings of woody plants (Aguilera and Lauenroth 1993, Jurena and Archer 2003), because, in contrast to shrubs, their root systems are mostly in the upper layers of soil (Soriano and Sala 1983, Armas and Pugnaire 2011). Thus the depletion of water from the upper layers of soil (Aguiar et al. 1996) prevents the establishment of shallow-rooted plants (Armas and Pugnaire 2011), such as seedlings and annuals (Jurena and Archer 2003). Still, grass-shrub interactions at establishment stage are not yet well understood (Browning et al. 2014), because there may be species-specific effects (Alados et al. 2006), and the outcome of the interactions may vary among different environmental conditions (Maestre et al. 2009b). Moreover, previous studies have examined interference and facilitative interactions among shrubs and grasses from an ecological perspective. However, given that water is the main limiting resource in semiarid environments, simultaneous examination of the ecological and hydrological properties of each plant type (together with other microsite properties under plant canopies) could provide important insights into the role of each plant type in community dynamics (Archer et al. 2012), and in the maintenance of plant diversity and productivity.

The general objective of this research is to examine the role of hydrological and microsite properties of different plants on the pattern of plant establishment in multi species “source-sink” semiarid ecosystems. The specific aim of this study was to assess the different roles of dominant plants (shrubs and grasses) on the dynamics of a semiarid plant community in north-eastern Spain, by investigating the effects of these dominant plants on soil moisture, microsite environment (solar radiation and soil nutrient content), and plant establishment at different life-cycle stages.

We hypothesized that (1) shrubs function as nurse plants in the establishment of plants in the studied ecosystem because of their physiognomy and water uptake patterns. Further, we hypothesize that perennial grasses do not functions as nurse plants because, even though they improve some microsite conditions related to plant establishment, they compete with seedlings for access to water. (2) We also expected that the different ecological roles of these two plant types would predominantly derive from their differing effects on the spatial-temporal patterns of soil moisture (with higher water availability in the upper layers under shrubs than under perennial grasses; Figure S1) and not from differences in nutrient availability or other microsite properties. Finally, we measured cumulative water infiltration, and we related this hydrological property and the microsite environmental properties (solar radiation) to observed soil moisture dynamics, as (3) they are expected to be important driving forces for the observed eco-hydrological processes.

Material and methods

Study area

The study area is located in "El Planerón", a nature reserve in the Middle Ebro Valley (NE Spain, 41º22'N 0º37'W) that has a semiarid Mediterranean climate. The average annual temperature is 15.3ºC, and the average annual precipitation 308.6 mm (Cuadrat et al. 2007), most of which occurs in spring and autumn. The soils are predominantly silty clay, and have low water infiltration in the bare areas because of high compaction (Pueyo et al. 2009, Moret et al. 2011). The landscape is predominantly flat (elevation 240 m a.s.l), and is composed of a mosaic of dry cereal croplands and uncultivated lands, that consist of an open plant community of small shrubs (*Salsola vermiculata* L. and *Artemisia herba-alba* Asso), perennial grasses (*Lygeum spartum* L., *Stipa parviflora* Desf. and *Dactylis glomerata* L.), and numerous forbs and ephemeral plants. The vegetation occurs in patches, and the non-vegetated areas are mostly bare with compacted clay soil and some scattered biological soil crusts (i.e. lichen species *Diploschistes diacapsis*, *Squamarina lentigera* and *Fulgensia* sp.). Traditional land use in the area was based on a traditional agro-pastoral system involving extensive sheep (*Rasa aragonesa*) production. Despite the fact that grazing in the study site is restricted since 1990, past land use is still patent on plant communities, that are more open and less dominated by perennial grasses than original plant communities (Pueyo 2005).

Patterns of plant establishment

We investigated the spatial pattern of perennial plants establishment (all the dominant shrubs and perennial grasses), by the study of eight 6x6 m^2 quadrats that were established in spring 2011 in a well-preserved plant community that was dominated by two shrubs (*S. vermiculata* and *A. herba-alba*) and three perennial grasses (*L. spartum*, *S. parviflora* and *D. glomerata*). A quadrat size representative of the plant community was selected taking into account that the average size of adult plants was 0.36 ± 0.02 m^2 (n=246; Wiegand and Moloney 2014). In each quadrat, we located the central point of the canopy, and the identity, phenology (seedling, juvenile, or adult) and status (alive or dead) of all shrubs and perennial grasses. We recorded plant survival and new seedling establishment in autumn 2011, spring and autumn 2012, and spring 2013.

Additionally, we assessed the role of shrubs and grasses on overall plant establishment of perennials and annuals in spring 2011. To do so, we identified the number of individuals or each species that occurred under the canopy of adult individuals of *S. vermiculata*, *A. herba-alba,* and *L. spartum* in each quadrat. For these measurements, we randomly selected 15 individuals of *S. vermiculata* and *A. herba-alba* per quadrat; we selected all individuals of adult *L. spartum* in the quadrats (5 individuals in total), because adults of this species were very scarce in the plots. We measured the canopy cover of the adult plants to compute the density and the species richness per surface unit, by measuring the largest radius of the canopy (r_1), and the perpendicular radius (r_2), and estimating the area of canopy cover as: $A=\pi \cdot r_1 \cdot r_2$.

In 2007, we began a sowing experiment in the same plant community to quantify the emergence and survival of seeds of the dominant shrub (*S. vermiculata*) and perennial grass (*L. spartum*) at three different interspersed microsites: bare soil (BS), under the canopy of *S. vermiculata* (SA) and under the canopy of *L. spartum* (LY). For each microsite and plant species, 200 seeds were soaked in water for 24 h and planted (200 seeds per microsite x 3 microsites x 2 seed species=1200 seeds in total) in February 2007. In the BS microsite, seeds were planted in a regular grid, with 20 cm between adjacent seeds. In the SA and LY microsites, seeds were planted around the border of the canopy of randomly selected adults of *S. vermiculata* and *L. spartum* (~25 individuals per species), with 10 cm between adjacent seeds. In all microsites, pre-existing seedlings were removed from the vicinity of the planted seeds to prevent interactions. The location of each planted seed was marked with a nail to ensure that germinated seedlings were not confused with naturally occurring seedlings. Germination rates were determined in April 2007. Seedling survival and growth were recorded in May and September 2007, March 2008, June 2010, and June 2011. Growth was estimated by seedling height (*S. vermiculata* and *L. spartum*) and the number of leaves (*S. vermiculata*).

Hydrological and environmental properties of microsites

Concurrent to the plant establishment measurements, we investigated the spatio-temporal patterns of soil water availability for seedlings. We installed 16 soil moisture probes (EC-5 Decagon Soil Moisture Probe, Decagon Devices, Inc., Washington, USA; accuracy ± 3% for most soil types) in January 2012; these probes were buried obliquely, 10 cm in the soil, to register soil moisture at depths of 5 to 10 cm. We avoided the top 5 cm of the soil, because the volume of influence (0.3 L) would include air volume. There were 4 probes per microsite, in the base soil (at least 50 cm away from all perennial plants), and under the canopies of *A. herba-alba*, *S. vermiculata*, and *L. spartum* adults. Plants were randomly selected in the same area as the vegetation experimental plots. Volumetric water content (VWC) was recorded every hour from January 19, 2012 to July 5, 2013. Probability density functions (PDFs) were built with the hourly VWC records from the four microsites to assess soil water content during the study period. Later, to evaluate the mechanism underlying the VWC patterns on the PDFs, wetting and drying processes after rainfall events were studied separately. The comparison of wetting processes among the four microsites allowed the evaluation of the soil water recharge as a net balance between canopy interception of rainfall, water redistribution processes between bare soil and plants, and the infiltration capacity at each microsite. Analysis of the drying process after rainfall allowed the estimation of the width of the window of high water availability at each microsite. The differences in the drying process beneath plants relative to bare soil allowed the assessment of the relevance of water uptake (*via* transpiration and that required for growth) and evaporation to total soil water loss. To investigate the wetting and drying processes at the four microsites, after each rainfall event larger than 1 $L \cdot m^{-2}$, we computed the maximum VWC, the time required to reach that maximum, the minimum VWC, and the time required to reach that minimum. Data from the first two months was excluded to assure stable measurements by the probes (Chamizo et al. 2013). Rainfall was obtained from a meteorological station that was about 1500 m from the experimental area. We defined a rainfall event as the sum of rainfall pulses with inter-pulses no longer than 12 h; after 12 h without rainfall, the next rainfall event was considered separate. Based on these criteria, we registered 56 rainfall events (and thus, wetting periods) during the study period. In the study of drying processes, we only considered periods of 10 consecutive days in which there was no rainfall as a drying period. There were 18 drying periods during the study period.

In order to better understand the potential drivers of changes in soil water content, we experimentally measured infiltration rates and solar radiation at the four microsites. With the infiltration measurements, we compared the water cumulative infiltration, I (mm), at different times at each microsite without considering the magnitude of the input water. Thus, this measurement excluded factors such as canopy interception and lateral water redistribution. Infiltration was experimentally measured in the field with a tension infiltrometer. A total of 11, 9, 7, and 8 infiltration measurements were performed at random sites on bare soil, and adult individuals of *S. vermiculata*, *A. herba-alba* and *L. spartum* respectively. The cumulative infiltration curves on *S. vermiculata*, *A. herba-alba,* and bare soil were measured with a hat infiltrometer (HI) (Moret Fernandez et al. 2015), a modification of the tension infiltrometer that allows measurement of the transient infiltration curve on the soil surface with undisturbed plants. The high porosity between the surface stems of *L. spartum* prevented formation of the vacuum in the HI hat, making these measurements impossible; thus, on this microsite a conventional disc infiltrometer (Perroux and White 1988) with a base radius of 50 mm was employed. Measurements with these different tension infiltrometers are similar (Moret Fernandez et al. 2015). To setup the disc infiltrometer, the *L. spartum* stalks were cut at ground level and a circular thin layer of commercial sand (80–160 µm diameter), with the same radius as the disc base, was layered on the soil surface. Only infiltration measurements at soil saturation conditions (which last up to 15 min) were conducted. Flow readings were automatically recorded every 5 seconds based on the decline of water level in the water supply reservoir. Water infiltration values were measured at 0.5, 1, 5, and 10 min.

Solar radiation was assumed to be directly correlated to potential water evaporation (Ritchie 1972, Franco and Nobel 1989, Valiente-Banuet and Ezcurra 1991), and thus, in order to estimate the relevance of potential water evaporation at the four microsites, solar radiation was measured on the bare soil, and under the canopies of adults of *A. herba-alba, S. vermiculata,* and *L. spartum*. Photosynthetic photon flux (PPF, solar radiation from 400 to 700 nm) was measured 10 times in bare areas and under the canopies of adults of *A. herba-alba*, *S. vermiculata* and *L. spartum* adult plants, on a cloudy day (April 25, 2012) and a sunny day (May 25, 2012) using a portable Apogee MQ-200 quantum meter.

Soil temperature was monitored at the four microsites as a complementary measurement to better understand the potential drivers of changes in soil water content (see Appendix 1 for details on complementary analyses).

Finally, soil chemical analyses were performed in order to control for the effects of differences in soil nutrient content among the microsites. We collected 16 soil samples from the upper 15 cm of soil from the four microsites in October 2013 (four samples per microsite; samples collected in the north aspect of the canopy). Soil samples were dried in the laboratory and passed through a 2 mm sieve. The soil pH in water following a dilution of 1:2.5, electrical conductivity of the saturated soil paste extract, organic matter content (Heanes 1984), and total carbon and nitrogen (Vario MAX CN elemental analyzer) were measured for all samples.

Statistical analysis

The spatial patterns of adults, juveniles, and seedlings of *S. vermiculata*, *A. herba-alba,* and perennial grasses were investigated with univariate and bivariate pair correlation functions (Stoyan and Stoyan 1994, Wiegand and Moloney 2014). The different species of perennial grasses (*L. spartum*, *S. parviflora* and *D. glomerata*) were analyzed together because the small number of individuals did not allow analysis of individual species. The pair correlation function is a second-order analysis similar to Ripley's K, but is a non-cumulative statistic; in other words, the pair correlation function does not integrate smaller scale effects into larger scales (Wiegand and Moloney 2004). We followed the procedure of Wiegand and Moloney (2004) for computation of pair-correlation functions correcting for edge effects. A significant departure of an observed pattern from the null random model was tested by comparison with the 5th largest and smallest simulation envelopes using 99 Monte Carlo simulations. The significance of the aggregation or segregation in a univariate patterns (i.e. univariate spatial patterns of adults, juveniles and seedlings of each plant type) was assessed with a heterogeneous Poisson null model with a kernel width of 20 cm (Wiegand and Moloney 2004). The spatial association between adults, juveniles and seedlings (i.e. bivariate patterns between adults of each plant type and juveniles and seedlings of each plant type: 9 adult *vs* juvenile patterns and 9 adult *vs* seedling patterns) was tested for independence with a toroidal shift null model (Wiegand and Moloney 2004). To facilitate interpretation and increase test power, the pair-correlation functions of the eight quadrats were combined into average functions as described by Wiegand and Moloney (2014).

Differences in the density of species and individuals of the three nurse species (*A. herba-alba*, *S. vermiculata* and *L. spartum*) were analysed with the Kruskal-Wallis test (ANOVA was not used due to the large difference in sample size between microsites). The Wilcoxon test with Holm correction was used for pairwise *post hoc* comparisons between the three microsites.

For the sowing experiment, differences in seedling emergence at the microsites were tested in April 2007 with a χ^2-test. Differences in seedling survival between microsites were tested after 1 month (May 2007), 5 months (September 2007), 11 months (March 2008), 38 months (June 2010), and 50 months (June 2011) with a χ^2-test. Fisher's exact test was used when there were fewer than 5 seedlings at a microsite. The Marascuilo procedure (Marascuilo 1966) was employed to simultaneously compare seedling emergence and survival at all pairs of microsites (SA *vs.* LY, SA *vs.* BS, and LY *vs.* BS microsites) on each census date. To investigate seedling growth at the three microsites, we performed a generalized linear mixed model (GLMM) for longitudinal data with repeated measures: height and number of leaves of *S. vermiculata* seedlings (Height_Salsola and Leaves_Salsola) and height of *L. spartum* seedlings (Height_Lygeum) were the response variables. The explanatory variables in the three models were the sowing microsite and the number of months after seedling emergence. The identity of each individual seedling measured over time was included in the model as a random factor. The best final model for each response variable was selected by comparing Akaike information criterion (AIC) values with ANOVA, following the procedure described by Zuur et al. (2009).

Soil wetting and drying processes after rainfall events were analysed with GLMMs. Responses variables in the wetting-process analysis were (1) the difference between the VWC before a rainfall event and the maximum VWC after rainfall (Increment_VWC), and (2) the rate of the wetting process (Wetting_slope). This was calculated by dividing Increment_VWC by the time required to reach maximum VWC. The main explanatory variable in the wetting-process analysis was the microsite (nurse species *vs*. bare soil, four levels). The covariables were the total amount of rainfall in the event (Amount_rainfall) and the soil water content before the rainfall event (VWC_before). We also included the effect of the interaction between the microsite and the covariables. The response variable in the drying process was the VWC value after ten days without rainfall (VWC_10days). The main explanatory variable in the drying-process analysis was the microsite (four levels). We also included the mean temperature during the 10 days of drying (Mean_temp; obtained from the meteorological station) and the initial VWC after rainfall (Initial_VWC) as covariables, and the interactions between microsites and covariables. We included a random factor in all of the models: each set of four probes (one per microsite) connected to the same data-logger (Box) that were closer between them than with the rest of the probes. Temporal autocorrelation was controlled by incorporating initial soil water content and temperature as covariables (Berdugo et al. 2014). To obtain the most suitable and parsimonious models for the wetting and the drying processes, we followed the procedure described by Zuur et al. (2009).

To detect differences among microsites in water infiltration at different times, photosynthetic photon flux and soil chemical properties, a one-way ANOVA was employed, with a logarithmic or arcsine transform when necessary to assure normality. Holm *post hoc* tests were employed to detect differences between pairs of microsites.

Point pattern analysis was performed with Programita software (Wiegand and Moloney 2004, 2014). All other statistical analysis was performed with R (R_Core_Team 2014); for GLMM, we employed the nlme R-package (Pinheiro et al. 2014).

A summary of the research questions and the different methodological approaches employed is provided as Supplementary Material (Table S1).

Results

Patterns of plant establishment

Univariate point pattern analysis showed that *A. herba-alba* adults and juveniles had segregated patterns, and seedlings of this species had small-scale aggregation. *S. vermiculata* adults had a segregated pattern at a small scale (less than 5m), but juveniles and seedlings were aggregated at a small scale (Table 1 and Figure S2). Perennial grass adults and juveniles were segregated, but the pattern for seedlings appeared random (Table 1 and Figure S2). Thus, there was a tendency for aggregation in early life stages and for segregation at adult stages for all studied plants, although *A. herba-alba* and perennial grasses had segregation already at the juvenile stages. Bivariate point pattern analysis showed that *S. vermiculata* juveniles and seedlings and *A. herba-alba* juveniles were spatially segregated from *A. herba-alba* adults, but that perennial grass adults, juveniles, and seedlings (marginally) were spatially associated with *A. herba-alba* adults. Perennial grass adults, juveniles, and seedlings, and *S. vermiculata* juveniles were spatially associated with *S. vermiculata* adults. Only *A. herba-alba* juveniles were negatively associated with *S. vermiculata* adults (Table 1 and Figure S2). *S. vermiculata* adults, *A. herba-alba* adults, and perennial grass juveniles were spatially associated with perennial grass adults, but *S. vermiculata* juveniles were spatially segregated from them (Table 1 and Figure S2). Consideration of all juveniles and seedlings together indicated negative associations with *A. herba-alba* adults and positive associations with *S. vermiculata* adults (Table 1, columns Juveniles ALL, Seedlings ALL and Juveniles & Seedlings ALL; Figure S2).

The nurse plants had significant differences in the density of plant species beneath their canopies (Kruskal-Wallis $\chi^2 = 143.36$, $p < 0.001$). A *post hoc* test showed that *S. vermiculata* had significantly more species per m^2 than *A. herba-alba* and *L. spartum*. *A. herba-alba* also had significantly more species per m^2 than *L. spartum*. The density of individual plants was significantly dependent upon the nurse plant (Kruskal-Wallis $\chi^2 =$

127.948, $p < 0.001$). A *post hoc* test showed that *S. vermiculata* had significantly more plants per m^2 than *A. herba-alba* and *L. spartum*.

Table 1. Univariate and bivariate spatial patterns between adults, juveniles, and seedlings of *Artemisia herba-alba* (A), *Salsola vermiculata* (S), and perennial grasses (PG) based on the pair-correlation function. -, significant small-scale segregation (<1 m); +, significant small-scale aggregation; 0, random small-scale pattern; 0(-) and 0(+), marginally significant small-scale segregation and aggregation. The univariate patterns were compared with a heterogeneous Poisson null model (intensity function estimated with a kernel width of 20 cm). The bivariate patterns were tested for independence with a toroidal shift null model. Figure S2 shows the plots for each analysis. Empty cells indicate associations that were not evaluated. Average number of points per quadrat was 625±90 (n=8).

	Adults A	Adults S	Adults PG	Juv. A	Juv. S	Juv. PG	Juv. All
Adults A	-	0	+	-	-	+	-
Adults S		-	+	-	+	+	+
Adults PG			-	0(+)	-	+	0
Juv. A				-			
Juv. S					+		
Juv. PG						-	
Seedl. A							
Seedl. S							
Seedl. PG							
Juv.&Seedl. A							
Juv.&Seedl. S							
Juv.&Seedl. PG							

	Seedl. A	Seedl. S	Seedl. PG	Seedl. All	Juv.&Seedl. A	Juv.&Seedl. S	Juv.&Seedl. PG	Juv.&Seedl. All
Adults A	-	0	0 (+)	0(-)	-	-	+	-
Adults S	0	0	+	+	0(-)	+	+	+
Adults PG	0(+)	0	0(-)	0	+	0(-)	0(-)	0
Juv. A								
Juv. S								
Juv. PG								
Seedl. A	+							
Seedl. S		+						
Seedl. PG			0					
Juv.&Seedl. A					0			
Juv.&Seedl. S						+		
Juv.&Seedl. PG							0	

The results of the sowing experiment indicated no significant differences between microsites in *S. vermiculata* seedling emergence in April 2007 ($\chi^2 = 4.73$, $p = 0.094$; Figure 2a), but significant differences in *L. spartum* seedling emergence ($\chi^2 = 20.95$, $p < 0.001$).

The Marascuilo procedure showed that *L. spartum* seedling emergence was significantly greater at the BS microsite than at the SA and LY microsites (Figure 2b). Analysis of seedling survival indicated no significant differences in *S. vermiculata* in May 2007 ($\chi^2 = 1.91$, $p = 0.369$), September 2007 ($\chi^2 = 4.54$, $p = 0.103$), March 2008 ($\chi^2 = 0.15$, $p = 0.925$) and June 2010 ($\chi^2 = 1.78$, $p = 0.411$), although there were significant differences in June 2011 (Fisher's exact test, $p = 0.004$), presumably due to new germination at SA microsite (Figure 2a). There were marginally significant differences in *L. spartum* seedling survival in May 2007 ($\chi^2 = 5.70$, $p = 0.058$), and significant differences in September 2007 ($\chi^2 = 23.761$, $p < 0.001$), March 2008 ($\chi^2 = 16.2$, $p < 0.001$), June 2010 ($\chi^2 = 63.402$, $p < 0.001$) and June 2011 ($\chi^2 = 40.495$, $p < 0.001$; Figure 2b). The Marascuilo procedure showed that in September 2007, *L. spartum* seedling survival was higher at the SA and LY microsites than at the BS microsite. In March 2008, *L. spartum* seedling survival was higher at the SA microsite than at the BS microsite; after that time, survival at the SA microsite was significantly higher than at the LY and BS microsites (Figure 2b).

Our investigation of the role of the different microsites on seedling growth only considered data until March 2008, because seedling survival at some microsites was very low after this date. In this period, the height of *S. vermiculata* seedlings was greater at the SA and LY microsites than at the BS microsite (Table 2a). Although the LY microsite accounted for the greatest seedling growth until September 2007, the average height of *S. vermiculata* seedlings at this microsite drastically decreased to the levels at the BS microsite in March 2008; at the SA microsite, the average growth was greatest at the end of the analysed period (Figure S3a). *Salsola vermiculata* seedlings had significantly more leaves at the SA microsite (Table 2b). There was an interaction between SA microsite and time explained by the similar number of leaves between microsites until September 2007. At the end of the analysed period (March 2008) the difference between the SA microsite and the other microsites became more apparent (Figure S3b). With respect to *L. spartum* seedlings, they were taller at the SA and LY microsites than at the BS microsite (Table 2c). There was also an interaction between the SA and LY microsites and time: only at the SA microsite taller plants were present at the end of the studied period (March 2008; Figure S3c).

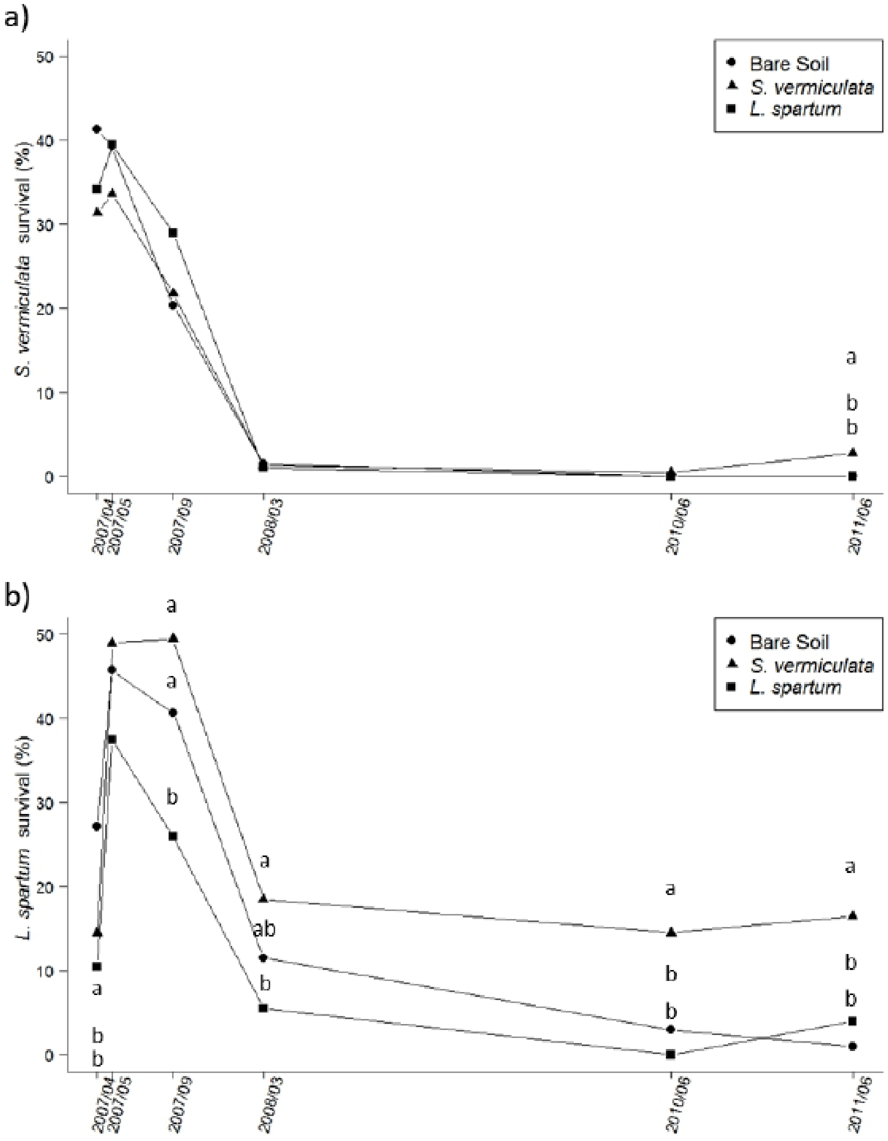


Figure 2. Percent germination (April 2007) and survival (May 2007 to June 2011) of (a) *S. vermiculata* and (b) *L. spartum* on bare soil (circles), under the canopies of *S. vermiculata* (triangles), and under the canopies of *L. spartum* (squares). Different letters indicate significant differences in a *post hoc* test between microsites on a measurement date.

Hydrological and environmental properties of microsites

The probability density functions (PDFs) showed similar trends for VWC at the four microsites, because they all had the same rainfall patterns (Figure 3). However, the bare soil and *A. herba-alba* microsites had peak PDFs at lower VWC values than the *S. vermiculata* and *L. spartum* microsites (Figure 3).

Table 2. Generalized linear mixed model for repeated measures of (a) height, and (b) number of leaves of *Salsola vermiculata* seedlings, and (c) height of *Lygeum spartum* seedlings measured in May 2007, September 2007, and March 2008 relative to bare soil (reference). SA_microsite, *S. vermiculata* microsite; LY_microsite, *L. spartum* microsite. Random effects were chosen between ~1|Individual, ~Microsite|Individual, and ~Time|Individual based on the Akaike information criterion.

a) Response variable: Height_Salsola

Random effects: ~Microsite|Individual			AIC	BIC	logLik
			1809.568	1852.764	-893.784
Variable	Value	Std.Error	DF	t-value	p-value
Intercept	2.564	0.201	228	12.773	**<0.001**
SA_microsite	2.836	0.351	228	8.079	**<0.001**
LY_microsite	4.382	0.491	228	8.930	**<0.001**
Time	0.217	0.044	147	4.879	**<0.001**

b) Response variable: Leaves_Salsola

Random effects: ~Time|Individual			AIC	BIC	logLik
			1496.012	1535.228	-738.006
Variable	Value	Std.Error	DF	t-value	p-value
Intercept	4.972	0.218	228	22.806	**<0.001**
SA_microsite	1.105	0.314	228	3.524	**<0.001**
LY_microsite	0.174	0.312	228	0.557	0.578
Time	0.196	0.069	145	2.824	**0.005**
SA_microsite:Time	-0.200	0.096	145	-2.084	**0.039**
LY_microsite:Time	0.045	0.095	145	0.478	0.634

c) Response variable:Height_Lygeum

Random effects: ~Microsite|Individual			AIC	BIC	logLik
			2432.505	2488.791	-1203.252
Variable	Value	Std.Error	DF	t-value	p-value
Intercept	4.949	0.221	305	22.396	**<0.001**
SA_microsite	0.892	0.335	305	2.662	**0.008**
LY_microsite	1.491	0.376	305	3.964	**<0.001**
Time	-0.052	0.036	256	-1.464	0.1444
SA_microsite:Time	0.130	0.046	256	2.817	**0.0052**
LY_microsite:Time	-0.150	0.061	256	-2.466	**0.0143**

The increment in soil VWC (increment_VWC) after rainfall was substantially larger under the canopy of the three plant species than in bare soil (Table 3a). A Holm *post hoc* test indicated no differences between *A. herba-alba*, *S. vermiculata* and *L. spartum* in the increment_VWC after rainfall ($p > 0.005$ for all pairwise comparisons). The amount of rainfall was positively related to the increment_VWC, but the antecedent VWC (ant_VWC) was negatively related to increment_VWC. This indicates that more water infiltrated into dry soils than soils that were already wet. The interaction between ant_VWC and the three plant microsites was significant and negative: the negative relationship between ant_VWC and increment_VWC was less pronounced under plants than in bare soil (Figure S4a).

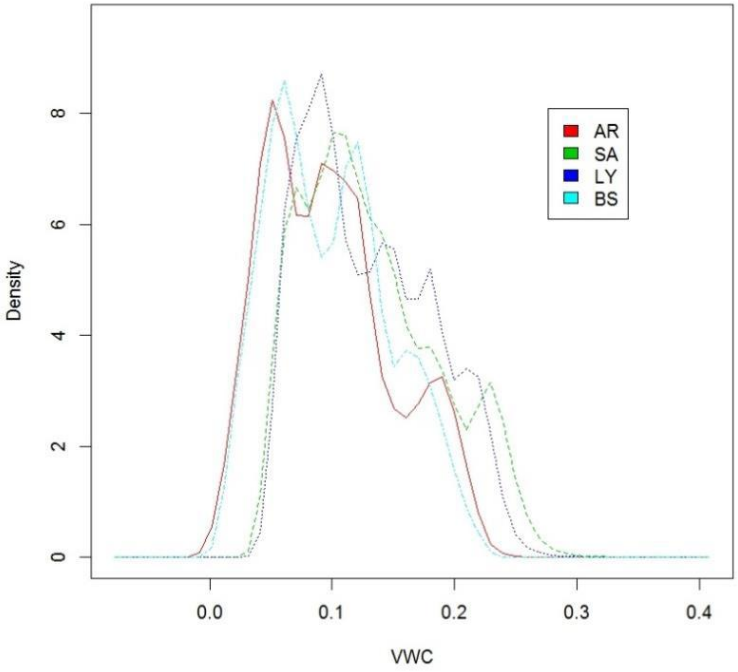


Figure 3. Probability density function (PDF) of soil volumetric water content (VWC) measured hourly from January 2012 to July 2013 under the canopies of *A. herba-alba* (AR), *S. vermiculata* (SA), and *L. spartum* (LY), and on bare soil (BS).

The slope of the wetting process (Wetting_slope) was greater under the plant canopies (Table 3b), indicating that the water infiltration was faster under plant canopies than in bare soil. However, there were no significant differences between the three plant species in the slope of the wetting process. There was a negative relationship between the ant_VWC and the slope of the wetting process, indicating that water infiltrated into drier soils more quickly than into wet soils. Moreover the amount of water in a rainfall event had a positive effect on the slope of the wetting process (Table 3b).

The amount of soil water after 10 days without rainfall (VWC_10days) was negatively associated with mean temperature during the drying period and positively associated with initial VWC after rainfall (Table 3c). In addition, VWC_10days was greater under *S. vermiculata* and *L. spartum* than in bare soil (Table 3c). VWC_10days under *A. herba-alba* was not significantly different from the bare soil value, which implies faster drying under *A. herba-alba* than under the other two species. This explains the VWC pattern found for *A. herba-alba* at the PDF (Figure 3). The interaction between initial VWC and microsite was significant. In particular, under the canopy of the three species, the relationship between initial VWC and VWC_10days had a shallower slope than on the bare soil (Table 3c; Figure S4b). This means that when sufficient water is available in the soil, water uptake by the three plant species is a relevant phenomenon.

Table 3. Generalized linear mixed model of (a) increment in volumetric water content (Increment_VWC), (b) slope of wetting process after rainfall (Wetting_slope), and (c) VWC 10 days after rainfall (VWC_10days) for 56 wetting events (a and b) and 18 drying events (c) from Janurary 19, 2012 to July 5, 2013 relative to bare soil (reference). AR_microsite, *Artemisia herba-alba* microsite; SA_microsite, *Salsola vermiculata* microsite; LY_microsite, *Lygeum spartum* microsite.

a)

Response variable: Increment_VWC			AIC	BIC	logLik
Random effects: ~Microsite|box			-2373.7	-2281.9	1206.83
Explanatory variable	Value	Std.Error	DF	t-value	p-value
Intercept	0.027	0.011	723	2.411	**0.016**
VWC_before	-0.201	0.072	723	-2.805	**0.005**
Amount_rainfall	0.003	$1.78\cdot10^{-4}$	723	19.421	**<0.001**
AR_microsite	0.053	0.014	723	3.893	**<0.001**
SA_microsite	0.066	0.018	723	3.608	**<0.001**
LY_microsite	0.061	0.018	723	3.447	**0.001**
VWC_before:AR_microsite	-0.357	0.105	723	-3.391	**0.001**
VWC_before:SA_microsite	-0.262	0.094	723	-2.801	**0.005**
VWC_before:LY_microsite	-0.315	0.105	723	-2.999	**0.003**

b)

Response variable: Increment_VWC			AIC	BIC	logLik
Random effects: ~Microsite|box			-2373.7	-2281.9	1206.83
Explanatory variable	Value	Std.Error	DF	t-value	p-value
Intercept	0.027	0.011	723	2.411	**0.016**
VWC_before	-0.201	0.072	723	-2.805	**0.005**
Amount_rainfall	0.003	$1.78\cdot10^{-4}$	723	19.421	**<0.001**
AR_microsite	0.053	0.014	723	3.893	**<0.001**
SA_microsite	0.066	0.018	723	3.608	**<0.001**
LY_microsite	0.061	0.018	723	3.447	**0.001**
VWC_before:AR_microsite	-0.357	0.105	723	-3.391	**0.001**
VWC_before:SA_microsite	-0.262	0.094	723	-2.801	**0.005**

VWC_before:LY_microsite	-0.315	0.105	723	-2.999	**0.003**

c)

Response variable: VWC_10days			AIC	BIC	logLik
Random effects: ~Microsite|box			-1065.1	-995.8	552.5
Variable	Value	Std.Error	DF	t-value	p-value
Intercept	0.081	0.011	233	7.224	**<0.001**
Mean_temp	-0.004	$2.5\cdot10^{-4}$	233	-14.518	**<0.001**
Initial_VWC	0.513	0.060	233	8.621	**<0.001**
AR_microsite	0.016	0.012	233	1.330	0.185
SA_microsite	0.035	0.014	233	2.526	**0.012**
LY_microsite	0.041	0.014	233	2.833	**0.005**
Initial_VWC:AR_microsite	-0.178	0.076	233	-2.352	**0.020**
Initial_VWC:SA_microsite	-0.175	0.070	233	-2.505	**0.013**
Initial_VWC:LY_microsite	-0.211	0.074	233	-2.837	**0.005**

The microsites had significant differences in cumulative water infiltration as measured with the tension infiltrometer at different times ($F_{3,31} = 5.213$, p = 0.005 at 0.5 minutes; $F_{3,31} = 9.648$, $p < 0.001$ at 1 minute; $F_{3,31} = 42.953$, $p < 0.001$ at 4 minutes; $F_{3,31} = 20.628$, $p < 0.001$ at 10 minutes). The Holm *post hoc* test showed that cumulative water infiltration for short periods of time (< 1 min) was significantly greater under *L. spartum* and *S. vermiculata* than in the bare soil, with infiltration under *A. herba-alba* being intermediate (Figure 4a). For longer infiltration times (> 5 min), cumulative water infiltration was much greater under *L. spartum*. *A. herba-alba* and *S. vermiculata* were not significantly different, but had significantly greater infiltration than the bare soil. For even longer infiltration times (> 10 min), cumulative water infiltration under *L. spartum* was so high that it obscured the differences in cumulative water infiltration between the shrubs and the bare soil (Figure 4), which were only significant when removing *L. spartum* from the analysis (data not shown).

There were significant differences in the Photosynthetic Photon Flux (PPF) between microsites both on the cloudy day ($F_{3,36}$ =1 81.57, $p < 0.001$) and the sunny day ($F_{3,36}$ = 381,86, $p < 0.001$). The Holm *post hoc* test showed that PPF was significantly greater in bare open areas than under the canopy of *A. herba-alba*, under the canopy of *S. vermiculata* and under the canopy of *L. spartum*, on the cloudy day and the sunny day (Figure 4b).

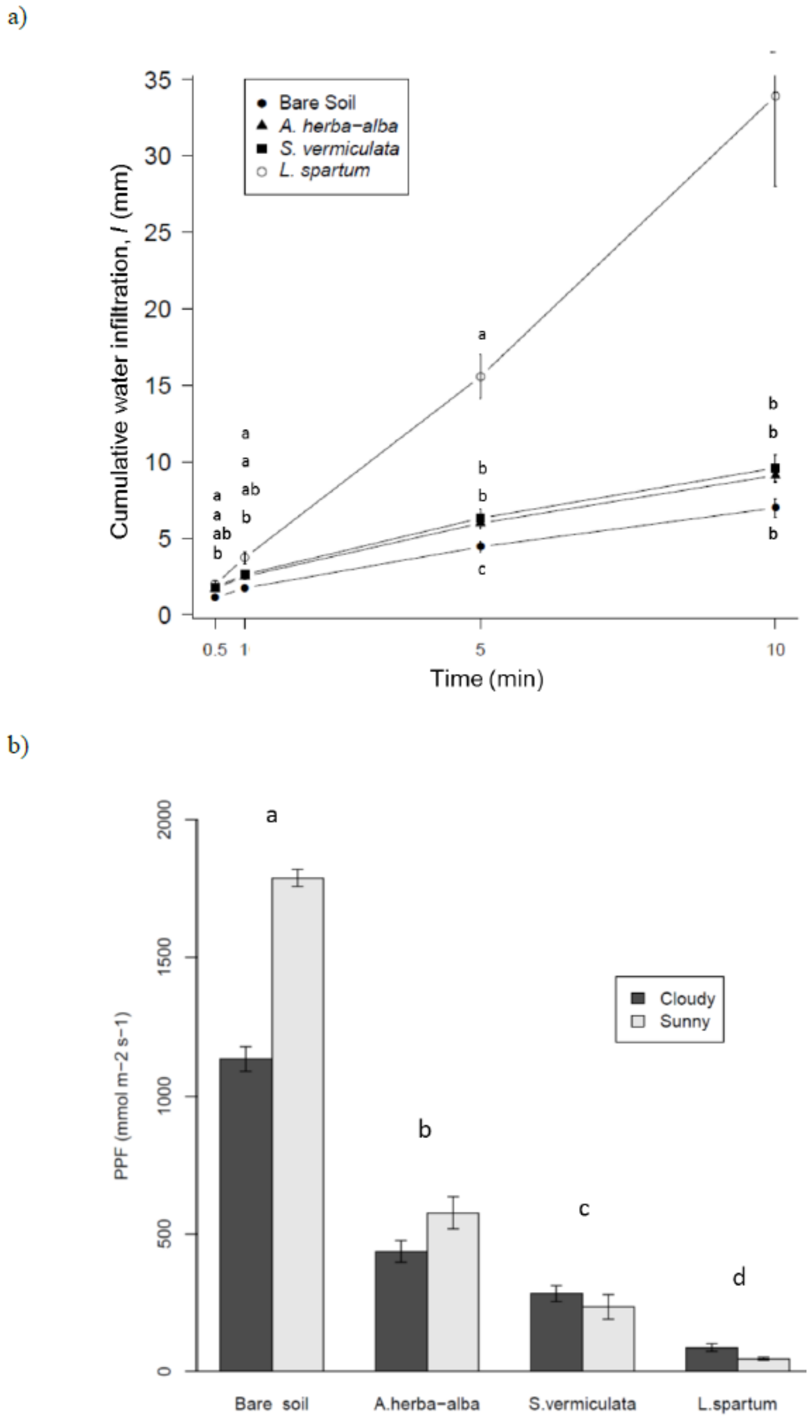


Figure 4. a) Means and standard errors of cumulative water infiltration (mm) from 0.5 to 10 min, in bare soil (n=11, filled circles), and under the canopies of *A. herba-alba* (n=7, filled triangles), *S. vermiculata* (n=9, filled squares), and *L. spartum* (n=8, open circles). Different letters indicate significant differences in a *post hoc* test between microsites on a measurement date. b) Means and standard errors of photosynthetic photon flux (PPF, □mol m^{-2} s^{-1}) in bare soil and under the canopies of *A. herba-alba*, *S. vermiculata,* and *L. spartum* on a cloudy day (April 25, 2011) and a sunny day (May 25, 2011). Values are from 10 measurements per microsite. Different letters indicate significant differences in a *post hoc* test between microsites on a measurement date. Data from sunny and cloudy days were analyzed separately, but the results were the same for a combined analysis.

Maximum daily summer temperatures were substantially lower under the canopies of the three species than in the bare soil (*L. spartum*: 6.7ºC lower; *S. vermiculata*: 3.2ºC lower; *A. herba-alba*: 2.5ºC lower; Table S2a; Figure S5a). The Holm *post hoc* test showed that the reduction of maximum daily temperatures under *L. spartum* was significantly greater than that under *A. herba-alba* ($p < 0.001$) and *S. vermiculata* ($p < 0.001$). *A. herba-alba* and *S. vermiculata* had no significant differences in maximum daily temperatures in summer ($p = 0.69$). Minimum daily winter temperatures were higher under the canopies of the three species than in the bare soil (*L. spartum*: 2.2ºC higher; *S. vermiculata*: 1.5ºC higher; *A. herba-alba*: 1.0ºC higher; Table S2b; Figure S5b). The Holm *post hoc* test showed that the minimum daily temperatures were significantly higher under *L. spartum* than under *A. herba-alba* ($p < 0.001$). The minimum temperatures in winter were not different for *S. vermiculata* and *L. spartum* ($p = 0.18$) or *A. herba-alba* ($p = 0.24$). The daily temperature ranges were smaller under the canopies of the three species than in bare soil (*L. spartum*: 5.9ºC smaller; *S. vermiculata*: 3.6ºC smaller; *A. herba-alba*: 2.7ºC smaller; Table S2c; Figure S5c). The range of daily temperatures was significantly different for all the microsites ($p < 0.001$ for all).

Organic matter, C, and N soil content were significantly greater under the canopies of *A. herba-alba* and *S. vermiculata* than in bare soil (Table 4); *L. spartum* had intermediate values and was not significantly different from other microsites. The C/N ratio was significantly greater in *S. vermiculata* than in the bare soil, and *A. herba-alba* and *L. spartum* had intermediate values.

Table 4. Soil chemical analysis in bare soil, and under the canopies of *Artemisia herba-alba*, *Salsola vermiculata* and *Lygeum spartum* (4 samples per microsite). Different letters indicate significant differences between microsites in a *post hoc* test. *EC*, electrical conductivity; *OM*, organic matter percentage; *C/N*, C/N ratio.

	Bare soil	*A. herba-alba*	*S. vermiculata*	*L. spartum*	$F_{3,12}$	p-value
pH	7.89±0.01	7.75±0.07	7.76±0.07	7.70±0.03	2.554	0.104
EC (mS cm^{-1})	1.756±0.034	1.757±0.040	1.849±0.050	1.855±0.034	1.909	0.181
OM (%)	0.523±0.047^{a}	1.762±0.474^{b}	2.386±0.581^{b}	1.378±0.135^{ab}	6.708	**0.007**
C (%)	1.262±0.038^{a}	2.399±0.314^{b}	2.848±0.327^{b}	2.072±0.198^{ab}	9.371	**0.002**
N (%)	0.047±0.002^{a}	0.130±0.031^{b}	0.158±0.028^{b}	0.105±0.012^{ab}	7.183	**0.005**
C/N	6.39±0.33^{a}	7.72±0.23^{ab}	8.46±0.69^{b}	7.654±0.158^{ab}	4.867	**0.019**

Discussion

We investigated the link between the ecological and the hydrological roles of shrubs and grasses in a semiarid ecosystem by quantifying their effects on plant establishment and the spatio-temporal dynamics of soil water availability beneath their canopies. Thus, this work simultaneously studied the ecological role of potential nurse plants and their hydrological and microsite characteristics, and highlighted the importance of an integrative eco-hydrological approach to fully understanding the functioning of semiarid ecosystems.

Overall, adult plants greatly improved the abiotic conditions for plant establishment (although there were inter-species differences) compared to the bare soil (Figure S1). This finding confirms the high spatial heterogeneity of environmental suitability for plant establishment in a semiarid ecosystem (Callaway 2007, Ward 2009). However, even though abiotic conditions beneath the species investigated were quite similar, there were large differences in their performance as nurse plants. *S. vermiculata* had a preeminent role as a nurse plant, and it facilitated intra- and inter-specific survival and growth of seedlings. *S. vermiculata* is a tall shrub, with deep roots and a large and moderately dense canopy, traits that are characteristic of benefactor species (Gómez-Aparicio 2009). Our analysis of soil water content indicated that *S. vermiculata* infiltrated water efficiently after rainfall events compared to the bare soil, and that soil beneath this species took longer to dry than bare soil. Moreover, the soil beneath *S. vermiculata* had an enriched chemical composition compared to the bare soil and the soil beneath perennial grasses. All these factors together made *S. vermiculata* a good nurse plant species for plant establishment.

Artemisia herba-alba might seem to have a similar role as *S. vermiculata*, given its similar physiognomy (both species are shrubs) and similar hydrological and chemical properties. However, our observational data showed that seedlings and juveniles seldom appeared under *A. herba-alba* canopies, and this led to low plant diversity and density beneath this species compared to *S. vermiculata*. Water scarcity during prolonged dry periods is a possible reason for the poor nurse plant function of *A. herba-alba* adults. Beneath *A. herba-alba,* wetting events were as efficient at refilling soil water content as under *S. vermiculata* and *L. spartum*. However, the dynamics of the drying process beneath *A. herba-alba* were not statistically different from those on bare soil. The deeper roots of *S.*

vermiculata relative to *A. herba-alba* (Guerrero-Campo 1998) could lead to a lower water uptake in the upper soil layers under *S. vermiculata.* It could also promote a “hydraulic lift” (Callaway 1995, Caldwell et al. 1998) in which water is taken from deep soil layers and released passively into shallow soil layers, thus increasing the window of water availability under its canopy. Alternatively, it could be argued that the faster drying process beneath *A. herba-alba* could be due to its sparser canopy, which would lead to greater solar radiation (as indicated by the PPF measurements), and greater evaporation rates after rainfall. However, our soil temperature measurements indicated no differences for *A. herba-alba* and *S. vermiculata,* so this explanation should be rejected. Moreover, *A. herba-alba*, as many other species of the genus *Artemisia*, has allelopathic properties (Friedman et al. 1977, Escudero et al. 2000). Previous studies have identified several sesquiterpenes lactones, flavonoids and essential oils in the roots and aerial parts of this species with inhibitory activity of seedling germination, growth and survival in greenhouse experiments (Salido et al. 2004, Abou et al. 2010). Here, we showed that under natural conditions, *A. herba-alba* interferes with seedlings, both intra- and interspecifically. This allelopathic activity, together with water scarcity during prolonged dry periods, may explain its poor function as a nurse plant. Given that allelopathy is a common phenomenon in stressful environments, such as arid, semiarid, and Mediterranean ecosystems (Schenk et al. 1999, Vila and Sardans 1999), our results underline that a simple study of the hydrological or the microsite characteristics of a plant is insufficient to determine its true role as a nurse plant. In addition, other soil factors not considered in this study such as the microbiota or limiting nutrients like phosphorous may also be relevant in determining the plant species role in the plant community.

The perennial grasses had only some characteristics that made them suitable as nurse plants. Our experimental study confirmed that seedling survival and growth in the first stages of development were enhanced by the presence of the perennial grass *L. spartum*. However, seedlings and juveniles were not found close to perennial grasses, and species richness and density beneath the canopy of *L. spartum* was smaller than under the two co-dominant shrubs. Moreover, long-term survival and growth of *S. vermiculata* and *L. spartum* seedlings were hindered in the *L. spartum* microsite in our sowing experiments. Taking together, these findings suggest that perennial grass adults facilitated plant establishment and performance during the first year after germination, but then interfered with these same plants after the first year (Soliveres et al. 2010). Contrary to our initial hypothesis, our study of the hydrological and microsite characteristics of *L. spartum* indicated no apparent reason for this shift from positive to negative interactions with seedlings. Together with *S. vermiculata*, *L. spartum* created the largest window of water availability for seedlings after rainfall, the maximum and minimum temperatures beneath its canopy were greatly modulated, and the nutrient content was higher than in bare soil (although not as favourable as beneath *S. vermiculata*). It could be argued that the reduction in solar radiation beneath *L. spartum* could be too extreme for optimal seedling performance (Holmgren et al. 2012), but then, there would be an initial interference with seedling survival and performance, and not a shift from facilitation to interference. Strong competition for water with seedlings in the upper soil layers of perennial grasses has been reported (Köchy and Wilson 2000), but, we found no signs that soil water depletion by grasses was faster than that by shrubs. We measured water content in the upper 15 cm of the soil, which may have caused evaporative processes to occur too fast to allow observation of depletion of soil water by grasses (Cavanaugh et al. 2011). Kambatuku et al. (2013) found strong competition by grasses in the upper 15 cm of soil, but reported that evaporation was not an important factor because they grew plants in containers under controlled conditions with regular watering. We argue that at shallow depths under field conditions, evaporation is more important than water uptake by neighbors (Potts et al. 2010, Cavanaugh et al. 2011). Water uptake by grasses would be relevant for seedling establishment only when seedling roots reach deeper into the soil. Moreover, non-resource microsite environmental properties could be more important for the early stages (germination and emergence) of plant establishment, and resource-related interactions (mainly for water availability) for the later stages of establishment (Jankju 2013). At these later stages of development, the root systems of established plants would interfere with nurse plants. This explanation clarifies the shift of function we observed in perennial grasses, which facilitated establishment of early-stage seedlings, but interfered with later-stage seedlings. This shift may also explain why some studies of perennial grasses in semi-arid regions reported positive effects whereas others reported negative effects of perennial grasses on seedlings (Maestre et al. 2001, Maestre et al. 2003, Armas and Pugnaire 2011), depending on when measurements were made (Soliveres et al. 2010). Thus, our combined ecological-hydrological results warn against making generalizations about the ecological role of plants based on consideration of the spatial pattern of seedlings at a single development stage, and oversimplifications about the ecological role of plants based on water infiltration patterns.

On the other hand, we found that below-ground interference by perennial grasses was also conspecific, a phenomenon described in previous studies (Aguilera and Lauenroth 1995). This restricts recruitment of seeds near a conspecific adult (Armas and Pugnaire 2011), and confers to shrubs the role of nurses of the perennial grasses in semiarid steppes. *Salsola vermiculata* facilitated *L. spartum* and other perennial grass seedlings, but not the inverse. Thus, perennial grasses may be replacing their own nurse plants during plant succession, as previously reported in other semiarid regions (Armas and Pugnaire 2005). This has important implications for restoration plans of these ecosystems (Pueyo et al. 2009).

We found that seed germination was independent of microsite (*S. vermiculata* seedlings) or even larger in bare soil than beneath adult plants (*L. spartum* seedlings), implying a fast response of seedlings to the rainy 2007 spring (Figure S6a) that was independent of other microsite conditions. However, summer 2007 was drier than average (Figure S6b), and subsequent survival and growth were associated with the presence of nurse plants. Later in the plant development, there was a shift from an aggregated pattern at the seedling stage to a segregated pattern when adults, that was most probably caused by a self-thinning process involving density-dependent interactions (Fonteyn and Mahall 1981, Phillips and MacMahon 1987, Wiegand et al. 2005). This ontogenetic shift is a common pattern in perennial plants in deserts (Miriti 2006, Armas and Pugnaire 2009). This is attributed to an increased interference between plants with well-developed root systems that overlap and colonize bare soil in the inter-patches. This depletes soil water and increases the mortality of neighbors that are less efficient in water uptake (Armas and Pugnaire 2009).

Our field measurements allowed some assessment of the hydrological processes behind the spatial-temporal dynamics of soil water content. Thus, the extremely high infiltration capacity of *L. spartum*, which was much greater than that of the shrubs *S. vermiculata* and *A. herba-alba*, contrasted with the dynamics of soil water content after rainfall, which was more modest and had a similar magnitude than for the shrubs. As previously reported, and in agreement with our measurements, *L. spartum* has an extraordinary infiltration potential (Cerdà 1997) due to soil modification (with macropores and channels in its root zone), but this is somehow counterbalanced by high rainfall interception from the canopy (Berdugo et al. 2014), resulting in a more restricted actual infiltration.

On the other hand, although we do not have direct measurements of evapotranspiration, we can evaluate the contribution of evaporation to loss of soil water based on measurements of soil water content at the different microsites. Thus, a higher soil water content during an inter-pulse period under the canopy of woody plants than in the bare soil indicates that evaporation (not plant use, including transpiration) is the predominant source of water loss (Breshears et al. 1997). Moreover, both the presence of greater soil water under the canopies of woody plants than in bare soil after a rainfall event and the greater infiltration into already wet soils imply that the redistribution of water from bare areas to vegetated patches is a relevant phenomenon (Greene 1992, Pueyo et al. 2013, Berdugo et al. 2014), more important in fact than rainfall interception by plant canopies (Breshears et al. 1997). Accumulation of water in the soil beneath plants is due to surface and sub-surface soil properties that affect infiltration rate and runoff, such as sediment accumulation, microtopography, the abundance of macropores, less crust development and soil compaction (Greene 1992, Galle et al. 1999, Gomez-Aparicio et al. 2005, Michaelides et al. 2009). In bare soil, low infiltration due to soil surface sealing and the absence of barriers cause run-off (Dunkerley et al. 1995, Arnau Rosalén et al. 2008, Mayor et al. 2009). Notably, drainage in this particular ecosystem is negligible because of the low infiltration of clay soils (Moret et al. 2011), but this should be considered in other semiarid ecosystems with more permeable soils.

Conclusions

Our integrated ecological-hydrological study of a semiarid ecosystem showed that shrubs and grasses improved the abiotic conditions under their canopies compared to the bare soil, because they increased the availability of soil moisture and reduced solar radiation. However, our study of the three most dominant species in this plant community indicated that only the shrub *S. vermiculata* had a long-term role as a nurse plant that favored seedlings establishment. The perennial grass *L. spartum* facilitated early plant establishment, and the shrub *A. herba-alba* did not facilitate establishment at any stage. Our experiments and observations suggest that competition for water and allelopathy may explain why these other two species do not serve as nurse plants, even though they improved the microsite environment. We conclude that the identification of a species as a nurse plant cannot simply be inferred by measurement of nearby abiotic conditions, but that long-term eco-hydrological studies are required to fully understand the underlying mechanisms.

Acknowledgements

This study was supported by research projects CGL2012-37508 and CGL2011-27259 (funded by MINECO and FEDER), and GA-LC-020/2010 and 2012/GA-LC-074 (funded by the Aragon regional government). We thank SEO/Birdlife for kindly allowing this research at "El Planerón" Ornithological Reserve. We also thank Paloma Nuche, Yacine Kouba, Maria Luisa Dehesa, Pedro Sanchez, and Pedro Bravo for their help with the field work, and Thorsen Wiegand for use of Programita software. AF and HS were supported by a JAE-DOC postdoctoral contract and a JAE-PRE predoctoral contract respectively, from CSIC and Fondo Social Europeo. AIA was supported by a FPU grant (reference AP-2012-4126) from the Spanish Ministry of Education, Culture and Sports.

References

Abou, E. H. M., M. A. El-Sayed, M. E. Hegazy, S. E. Helaly, A. M. Esmail, and N. S. Mohamed. 2010. Chemical constituents and biological activities of *Artemisia herba-alba*. Records of natural products 4:1-25.

Aguiar, M. R., J. M. Paruelo, O. E. Sala, and W. K. Lauenroth. 1996. Ecosystem responses to changes in plant functional type composition: An example from the Patagonian steppe. Journal of Vegetation Science 7:381-390

.

Aguilera, M. O., and W. K. Lauenroth. 1993. Seedling establishment in adult neighborhoods intraspecific constraints in the regeneration of the bunchgrass Bouteola gracilis. Journal of Ecology 81:253-261.

Aguilera, M. O., and W. K. Lauenroth. 1995. Influence of gap disturbances and types of microsites on seedling establishment in Bouteola gracilis. Journal of Ecology 83:87-97.

Alados, C. L., P. Gotor, P. Ballester, D. Navas, J. Escos, T. Navarro, and B. Cabezudo. 2006. Association between competition and facilitation processes and vegetation spatial patterns in alpha steppes. Biological Journal of the Linnean Society 87:103-113.

Archer, N. A. L., J. N. Quinton, and T. M. Hess. 2012. Patch vegetation and water redistribution above and below ground in south-east Spain. Ecohydrology 5:108-120.

Armas, C., and F. I. Pugnaire. 2005. Plant interactions govern population dynamics in a semi-arid plant community. Journal of Ecology 93:978-989.

Armas, C., and F. I. Pugnaire. 2009. Ontogenetic shifts in interactions of two dominant shrub species in a semi-arid coastal sand dune system. Journal of Vegetation Science 20:535-546.

Armas, C., and F. I. Pugnaire. 2011. Belowground zone of influence in a tussock grass species. Acta Oecologica-International Journal of Ecology 37:284-289.

Arnau Rosalén, E., A. Arnau Rosalén, C. Calvo Cases, H. Boix Fayos, P. Lavee, and Sarah. 2008. Analysis of soil surface component patterns affecting runoff generation. An example of methods applied to Mediterranean hillslopes in Alicante (Spain). Geomorphology 101:595-606.

Bautista, S., A. G. Mayor, J. Bourakhouadar, and J. Bellot. 2007. Plant spatial pattern predicts hillslope semiarid runoff and erosion in a Mediterranean landscape. Ecosystems 10:987-998.

Beatley, J. C. 1974. Phenological events and their environmental triggers in Mojave-desert ecosystems. Ecology 55:856-863.

Berdugo, M., S. Soliveres, and F. Maestre. 2014. Vascular Plants and Biocrusts Modulate How Abiotic Factors Affect Wetting and Drying Events in Drylands. Ecosystems:1-15.

Breshears, D. D., P. M. Rich, F. J. Barnes, and K. Campbell. 1997. Overstory-imposed heterogeneity in solar radiation and soil moisture in a semiarid woodland. Ecological Applications 7:1201-1215.

Browning, D. M., J. Franklin, S. R. Archer, J. K. Gillan, and D. P. Guertin. 2014. Spatial patterns of grassland–shrubland state transitions: a 74-year record on grazed and protected areas. Ecological Applications 24:1421-1433.

Caldwell, M. M., T. E. Dawson, and J. H. Richards. 1998. Hydraulic lift: Consequences of water efflux from the roots of plants. Oecologia 113:151-161.

Callaway, R. 1995. Positive interactions among plants. The botanical review 61:306-349.

Callaway, R. M. 2007. Positive interactions and interdependence in plant communities. Springer, Dordrecht, The Netherlands.

Cavanaugh, M. L., S. A. Kurc, and R. L. Scott. 2011. Evapotranspiration partitioning in semiarid shrubland ecosystems: a two-site evaluation of soil moisture control on transpiration. Ecohydrology 4:671-681.

Cerdà, A. 1997. The effect of patchy distribution of *Stipa tenacissima* L. on runoff and erosion. Journal of Arid Environments 36:37-51.

Cuadrat, J. M., M. A. Saz, and S. M. Vicente-Serrano. 2007. Atlas climático de Aragón. Gobierno de Aragón. http://anciles.aragon.es/AtlasClimatico.

Chamizo, S., Y. Cantón, R. Lázaro, and F. Domingo. 2013. The role of biological soil crusts in soil moisture dynamics in two semiarid ecosystems with contrasting soil textures. Journal of Hydrology 489:74-84.

Drezner, T. D. 2007. An analysis of winter temperature and dew point under the canopy of a common Sonoran Desert nurse and the implications for positive plant interactions. Journal of Arid Environments 69:554-568.

Dunkerley, D. L., K. J. Dunkerley, and Brown. 1995. Runoff and runon areas in a patterned chenopod shrubland, arid western New South Wales, Australia: characteristics and origin. Journal of Arid Environments 30:41-55.

Escudero, A., M. J. Albert, J. M. Pita, and F. Pérez-García. 2000. Inhibitory effects of Artemisia herba-alba on the germination of the gypsophyte Helianthemum squamatum. Plant Ecology 148:71-80.

Flores, J., and E. Jurado. 2003. Are nurse-protege interactions more common among plants from arid environments? Journal of Vegetation Science 14:911-916.

Fonteyn, P., and B. Mahall. 1981. An experimental analysis of structure in a desert plant community. The Journal of Ecology:883-896.

Franco, A. C., and P. S. Nobel. 1989. Effect of Nurse Plants on the Microhabitat and Growth of Cacti. Journal of Ecology 77:870-886.

Friedman, J., O. Gideon, and Y. Ziger-Cfir. 1977. Suppression of Annuals by Artemisia Herba-Alba in the Negev Desert of Israel. Journal of Ecology 65:413-426.

Galle, S., M. Ehrmann, and C. Peugeot. 1999. Water balance in a banded vegetation pattern: A case study of tiger bush in western Niger. CATENA 37:197-216.

Gómez-Aparicio, L. 2009. The role of plant interactions in the restoration of degraded ecosystems: a meta-analysis across life-forms and ecosystems. Journal of Ecology 97:1202-1214.

Gomez-Aparicio, L., F. Valladares, R. Zamora, and J. L. Quero. 2005. Response of tree seedlings to the abiotic heterogeneity generated by nurse shrubs: an experimental approach at different scales. Ecography 28:757-768.

Gómez Aparicio, L., R. Gómez Aparicio, J. Zamora, J. Gómez, J. Hódar, E. Castro, and Baraza. 2004. Applying plant facilitation to forest restoration: a meta-analysis of the use of shrubs as nurse plants. Ecological Applications 14:1128-1138.

Greene, R. 1992. Soil physical properties of three geomorphic zones in a semiarid mulga woodland. Soil Research 30:55-69.

Guerrero-Campo, J. 1998. Respuestas de la vegetación y de la morfología de las plantas a la erosión del suelo. PhD thesis, Universidad de Barcelona, Barcelona.

Heanes, D. L. 1984. Determination of total organic-C in soils by an improved chromic acid digestion and spectrophotometric procedure. Communications in Soil Science and Plant Analysis 15:1191-1213.

Holmgren, M., L. Holmgren, J. Gómez Aparicio, F. Quero, and Valladares. 2012. Non-linear effects of drought under shade: reconciling physiological and ecological models in plant communities. Oecologia 169:293-305.

Imeson, A., and H. Prinsen. 2004. Vegetation patterns as biological indicators for identifying runoff and sediment source and sink areas for semi-arid landscapes in Spain. Agriculture, ecosystems & environment 104:333-342.

Jankju, M. 2013. Role of nurse shrubs in restoration of an arid rangeland: Effects of microclimate on grass establishment. Journal of Arid Environments 89:103-109.

Jenerette, G. D., G. A. Barron-Gafford, A. J. Guswa, J. J. McDonnell, and J. Camilo Villegas. 2012. Organization of complexity in water limited ecohydrology. Ecohydrology 5:184-199.

Jurena, P. N., and S. Archer. 2003. Woody plant establishment and spatial heterogeneity in grasslands. Ecology 84:907-919.

Kambatuku, J. R., M. D. Cramer, and D. Ward. 2013. Overlap in soil water sources of savanna woody seedlings and grasses. Ecohydrology 6:464-473.

Köchy, M., and S. D. Wilson. 2000. Competitive effects of shrubs and grasses in prairie. Oikos 91:385-395.

Ludwig, J. A., B. P. Wilcox, D. D. Breshears, D. J. Tongway, and A. C. Imeson. 2005. Vegetation patches and runoff-erosion as interacting ecohydrological processes in semiarid landscapes. Ecology 86:288-297.

Maestre, F. T., S. Bautista, and J. Cortina. 2003. Positive, negative, and net effects in grass-shrub interactions in mediterranean semiarid grasslands. Ecology 84:3186-3197.

Maestre, F. T., S. Bautista, J. Cortina, and J. Bellot. 2001. Potential for using facilitation by grasses to establish shrubs on a semiarid degraded steppe. Ecological Applications 11:1641-1655.

Maestre, F. T., M. A. Bowker, M. D. Puche, M. Belén Hinojosa, I. Martínez, P. García-Palacios, A. P. Castillo, S. Soliveres, A. L. Luzuriaga, and A. M. Sánchez. 2009a. Shrub encroachment can reverse desertification in semi-arid Mediterranean grasslands. Ecology Letters 12:930-941.

Maestre, F. T., R. M. Callaway, F. Valladares, and C. J. Lortie. 2009b. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. Journal of Ecology 97:199-205.

Marascuilo, L. A. 1966. Large-scale multiple comparisons. Psychological Bulletin 69:280-290.

Mayor, Á. G., S. Mayor, J. Bautista, and Bellot. 2009. Factors and interactions controlling infiltration, runoff, and soil loss at the microscale in a patchy Mediterranean semiarid landscape. Earth surface processes and landforms 34:1702-1711.

Michaelides, K., D. Lister, J. Wainwright, and A. J. Parsons. 2009. Vegetation controls on small-scale runoff and erosion dynamics in a degrading dryland environment. Hydrological Processes 23:1617-1630.

Michalet, R. 2007. Highlighting the multiple drivers of change in interactions along stress gradients. New Phytologist 173:3-6.

Miriti, M. N. 2006. Ontogenetic shift from facilitation to competition in a desert shrub. Journal of Ecology 94:973-979.

Moret, D., Y. Pueyo, C. G. Bueno, and C. L. Alados. 2011. Hydro-physical responses of gypseous and non-gypseous soils to livestock grazing in a semi-arid region of NE Spain. Agricultural Water Management. 98:1822-1827.

Moret Fernandez, D., B. Latorre, C. González, and V. Pérez. 2015. A modified hood infiltrometer to estimate the soil hydraulic properties from transient water flow measurements. Journal of Hydrology 530:554-560.

Noy-Meir, I. 1973. Desert ecosystems: environment and producers. Annual Review of Ecology and Systematics 4:25-41.

Perroux, K. M., and I. White. 1988. Designs for disc permeameters. Soil Science Society of America Journal 52:1205-1215.

Phillips, D. L., and J. A. MacMahon. 1987. Competition and spacing patterns in desert shrubs. Journal of Ecology 69:97-115.

Pinheiro, J., D. Bates, S. Debroy, D. Sarkar, and R_Core_Team. 2014. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-117, http://CRAN.R-project.org/package=nlme.

Potts, D. L., R. L. Scott, S. Bayram, and J. Carbonara. 2010. Woody plants modulate the temporal dynamics of soil moisture in a semi-arid mesquite savanna. Ecohydrology 3:20-27.

Poulos, J. M., A. P. Rayburn, and E. W. Schupp. 2014. Simultaneous, independent, and additive effects of shrub facilitation and understory competition on the survival of a native forb (Penstemon palmeri). Plant Ecology 215:417-426.

Pueyo, Y. 2005. Evaluación de los factores ambientales y del uso antrópico como condicionantes de la conservación de la vegetación del sector central de la Depresión del Ebro. Universidad de Zaragoza, Zaragoza.

Pueyo, Y., C. L. Alados, B. García-Ávila, S. Kéfi, M. Maestro, and M. Rietkerk. 2009. Comparing direct abiotic amelioration and facilitation as tools for restoration of semi-arid grassland. Restoration Ecology 17:908-916.

Pueyo, Y., D. Moret-Fernandez, H. Saiz, C. G. Bueno, and C. L. Alados. 2013. Relationships between plant spatial patterns, water infiltration capacity, and plant community composition in semi-arid Mediterranean ecosystems along stress gradients. Ecosystems 16:452-466.

R_Core_Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Reynolds, J. F., P. R. Kemp, K. Ogle, and R. J. Fernández. 2004. Modifying the 'pulse–reserve'paradigm for deserts of North America: precipitation pulses, soil water, and plant responses. Oecologia 141:194-210.

Ritchie, J. T. 1972. Model for predicting evaporation from a row crop with incomplete cover. Water Resources Research 8:1204-1213.

Salido, S., L. R. Valenzuela, J. Altarejos, M. Nogueras, A. Sanchez, and E. Cano. 2004. Composition and infraspecific variability of *Artemisia herba-alba* from southern Spain. Biochemical Systematics and Ecology 32:265-277.

Schenk, H. J., R. M. Callaway, and B. E. Mahall. 1999. Spatial root segregation: Are plants territorial? Advances in Ecological Research Volume 28 28:145-180.

Soliveres, S., L. DeSoto, F. T. Maestre, and J. M. Olano. 2010. Spatio-temporal heterogeneity in abiotic factors modulate multiple ontogenetic shifts between competition and facilitation. Perspectives in plant ecology, evolution and systematics 12:227.

Soriano, A., and O. E. Sala. 1983. Ecological strategies in a Patagonian arid steppe. Vegetatio 56:9+15.

Stoyan, D., and H. Stoyan. 1994. Fractals, random shapes and point fields. Methods of geometrical statistics. Wiley, London, UK.

Valiente-Banuet, A., and E. Ezcurra. 1991. Shade as a Cause of the Association Between the Cactus Neobuxbaumia Tetetzo and the Nurse Plant Mimosa Luisana in the Tehuacan Valley, Mexico. Journal of Ecology 79:961-971.

Vila, M., and J. Sardans. 1999. Plant competition in mediterranean-type vegetation. Journal of Vegetation Science 10:281-294.

Ward, D. 2009. The biology of deserts. Oxford University Press, Oxford, UK.

Wiegand, K., D. Ward, and D. Saltz. 2005. Multi-scale patterns and bush encroachment in an arid savanna with a shallow soil layer. Journal of Vegetation Science 16:311-320.

Wiegand, T., and K. A. Moloney. 2004. Rings, circles and null-models for point pattern analysis in ecology. Oikos 104:209-229.

Wiegand, T., and K. A. Moloney. 2014. Handbook of spatial point-pattern analysis in ecology. CRC Press, Boca Ratón, USA.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer, New York, USA.

Appendix 1

Material and methods of complementary analysis: soil temperature among microsites

To measure soil temperature, in May 2011 we installed temperature sensors (TMCX-HD, Onset, Massachusetts) 5 cm beneath the surface in the bare soil and under the canopies of *A. herba-alba*, *S. vermiculata*, and *L. spartum* adult plants (6 sensors for the plant microsites, and 7 sensors for the bare soil microsite, 25 in total). Soil temperature was recorded every hour from May 25, 2011 to July 5, 2013 and daily maximum and minimum temperatures and daily temperature range were obtained from these data. Daily maximum temperature is directly related to evaporation and transpiration (Valiente-Banuet and Ezcurra 1991, Breshears et al. 1998), and daily minimum temperatures, especially when below freezing, can compromise seedling survival (Drezner 2007). The daily temperature range accounts for the extreme maximum and minimum temperatures. To evaluate the effect of the four microsites at soil temperature, we randomly selected 80 days from May 25, 2011 to July 5, 2013 (20 days per season). We assessed the significance of the differences between microsites in maximum daily soil temperature during summer (Max_Temp), minimum daily soil temperatures during winter (Min_Temp), and daily soil temperature range in all the seasons (Temp_Range) with GLMMs that included the date of each measurement (day) as a random factor. We also checked for temporal autocorrelation in the data by including a first-order autoregressive (AR1) term or an autoregressive-moving-average model (ARMA term). The best final model was selected as described by Zuur et al. (2009).

References

Breshears, D., J. Breshears, C. Nyhan, B. Heil, and Wilcox. 1998. Effects of Woody Plants on Microclimate in a Semiarid Woodland: Soil Temperature and Evaporation in Canopy and Intercanopy Patches. International journal of plant sciences **159**:1010-1017.

Drezner, T. D. 2007. An analysis of winter temperature and dew point under the canopy of a common Sonoran Desert nurse and the implications for positive plant interactions. Journal of Arid Environments **69**:554-568.

Valiente-Banuet, A., and E. Ezcurra. 1991. Shade as a Cause of the Association Between the Cactus Neobuxbaumia Tetetzo and the Nurse Plant Mimosa Luisana in the Tehuacan Valley, Mexico. Journal of Ecology **79**:961-971.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer, New York, USA.

Appendix 2

Table S1. Research questions addressed with the different methodological approaches, variables measured and details about the experimental design and analysis.

Research question	Approach	Variable(s) measured	Contribution to the research question	Experimental design	Response variable(s)	Independent variable(s) and covariables	Microsites	Statitical analysis	Measurements	Figures and tables
1.- Role of shrubs and grasses as nurses of plant establishment (hypothesis 1)	Spatial patterns of seedling establishment	Spatial assotiation among seedlings, juveniles and adults	In-deep analysis of plant-plant assotiations of the most abundant shrubs and grasses along the life-span	8 6x6 m^2 quadrats	Location of seedlings, juveniles and adults (coordinates)	Location of seedlings, juveniles and adults (coordinates)	Shrubs: *A. herba-alba* , *S. vermiculata* Perennial grasses: *L. spartum* , *S. parviflora* , *D. glomerata*	Point pattern analysis: univariate and bivariate pair-correlation functions	autumn 2011, spring 2012, autumn 2012, spring 2013	Table 1, Figure S2
1.- Role of shrubs and grasses as nurses of plant establishment (hypothesis 1)	Spatial patterns of seedling establishemnt	Plant diversity under the canopy of the nurses	Analysis of the stablishment of the whole plant community, including perennial and annual species	8 6x6 m^2 quadrats 15 adult *S. vermiculata* and *A. herba-alba per* quadrat, and 5 adult *L. spartum* individuals	Plant density (ind m^{-2}) and richness (sps m^{-2}) under selected adult plants	Microsite	Shrubs: *A. herba-alba* , *S. vermiculata* Perennial grass: *L. spartum*	Kruskal-Wallis test (unbalanced samples)	spring 2011	-
1.- Role of shrubs and grasses as nurses of plant establishment (hypothesis 1)	Sowing experiment	Seedling emergence, survival and growth survey under different microsites	Experimental approach that supports the observed spatial patterns	200 *S. vermiculata* seeds, 200 *L. spartum* seeds *per* microsite (1200 seeds in total)	Seedling emergence (%) survival (%), height (cm) and number of leaves (-)	Microsite	Shrub: *S. vermiculata* Perennial grasses: *L. spartum* Bare soil	X^2 test (seedling emergence and survival) GLMM (seedling growth)	April 2007 (germination), May 2007, September 2007, March 2008, June 2010, June 2011 (survival and growth)	Table 2, Figure 2, Figure S3
2.-Factors related to the role as nurse plant (hypothesis 2)	Hydrological study	Wetting and drying events effect on soil water availabilty	Spatio-temporal patterns of water availability under shrub and grasses	4 moisture probe *per* microsite (16 probes in total)	Volumetric water content (VWC, -); Increment_VWC (-) and Wetting_slope (-; wetting events) VWC_10days (-; drying events)	Microsite Covariables wetting: Amount_rainfall (mm) and VWC_before (-) Covariables drying: Mean_temp (°C) and Initial_VWC (-)	Shrubs: *A. herba-alba, S. vermiculata* Perennial grasses: *L. spartum* Bare soil	GLMM	VWC: Hourly from January 2012 to July 2013 56 wetting events 18 drying events	Table 3, Figure 3, Figure S4

Research question	Approach	Variable(s) measured	Contribution to the research question	Experimental design	Response variable(s)	Independent variable(s) and covariables	Microsites	Statitical analysis	Measurements	Figures and tables
2.-Factors related to the role as nurse plant (hypothesis 2)	Soil nutrient properties	Soil chemical analyses	Nutrient availability under shrub and grasses	4 samples *per* microsite (16 samples in total)	pH, EC (mS cm-1), OM (%), C (%), N (%) and C/N (-)	Microsite	Shrubs: *A. herba-alba, S. vermiculata* Perennial grasses: *L. spartum* Bare soil	ANOVA	Autumn 2013	Table 4
3.- Factors related to spatio-temporal patterns of water availability (hypothesis 3)	Other microsite properties	Infiltration rate	Are the differences in soil water availability encountered related to different infiltration rates?	~9 infiltration measurements *per* microsite (35 measurements in total)	Water cumulative infiltration, I (mm), at 0.5, 1, 5 and 10 minutes	Microsite	Shrubs: *A. herba-alba, S. vermiculata* Perennial grasses: *L. spartum* Bare soil	ANOVA	Spring 2012	Figure 4a
3.- Factors related to spatio-temporal patterns of water availability (hypothesis 3)	Other microsite properties (non-resource factors)	Solar radiation	Are the differences in soil water availability encountered related to differences in solar radiation?	10 measurements *per* microsite in a sunny and in a cloudy day (80 measurements in total)	Photosynthetic photon flux, PPF (mm mol m^{-2} s^{-1})	Microsite	Shrubs: *A. herba-alba, S. vermiculata* Perennial grasses: *L. spartum* Bare soil	ANOVA	Spring 2012	Figure 4b
3.- Factors related to spatio-temporal patterns of water availability (hypothesis 3)	Other microsite properties (non-resource factors)	Soil temperature	Control of other microsite factors potentially related to water availability	~6 temperature sensors *per* microsite (25 in total)	Max_Temp (ºC), Min_Temp (ºC) and Temp_Range (ºC)	Microsite	Shrubs: *A. herba-alba, S. vermiculata* Perennial grasses: *L. spartum* Bare soil	GLMM	Hourly from May 2011 to July 2013	Table S1, Figure S6

Appendix 3

Table S2. Generalized linear mixed model of (a) maximum daily temperatures in summer (Max_Temp), (b) minimum daily temperatures in winter (Min_Temp), and (c) daily range of temperatures (Temp_Range) relative to bare soil (reference), with the day of measurement (*day)* as a random factor and microsite as the explanatory variable. AR_microsite, *Artemisia herba-alba* microsite; SA_microsite, *Salsola vermiculata* microsite; LY_microsite, *Lygeum spartum* microsite. In (a) and (c), the best model included an autoregressive-moving-average model (ARMA) of temporal autocorrelation. In (b) the best model did not include any term for temporal autocorrelation.

a)

Response variable: Max_Temp			AIC	BIC	logLik
Random effects: ~1|day			2044.055	2076.415	-1014.027
Variable	Value	Std.Error	DF	t-value	p-value
Intercept	36.756	0.812	403	45.282	<**0.001**
AR_microsite	-2.345	0.309	403	-7.585	<**0.001**
SA_microsite	-3.214	0.293	403	-10.971	<**0.001**
LY_microsite	-6.789	0.304	403	-22.352	<**0.001**

b)

Response variable:Min_Temp			AIC	BIC	logLik
Random effects: ~1|day			747.468	772.269	-367.734
Variable	Value	Std.Error	DF	t-value	p-value
Intercept	4.069	0.660	442	6.166	<**0.001**
AR_microsite	1.053	0.059	442	17.948	<**0.001**
SA_microsite	1.524	0.059	442	25.973	<**0.001**
LY_microsite	2.196	0.059	442	37.412	<**0.001**

c)

Response variable: Temp_Range			AIC	BIC	logLik
Random effects: ~1|day			8364.301	8404.243	-4274.151
Variable	Value	Std.Error	DF	t-value	p-value
Intercept	10.449	0.367	1716	28.482	<**0.001**
AR_microsite	-2.624	0.144	1716	-18.210	<**0.001**
SA_microsite	-3.538	0.140	1716	-25.256	<**0.001**
LY_microsite	-5.903	0.143	1716	-41.189	<**0.001**

Appendix 4

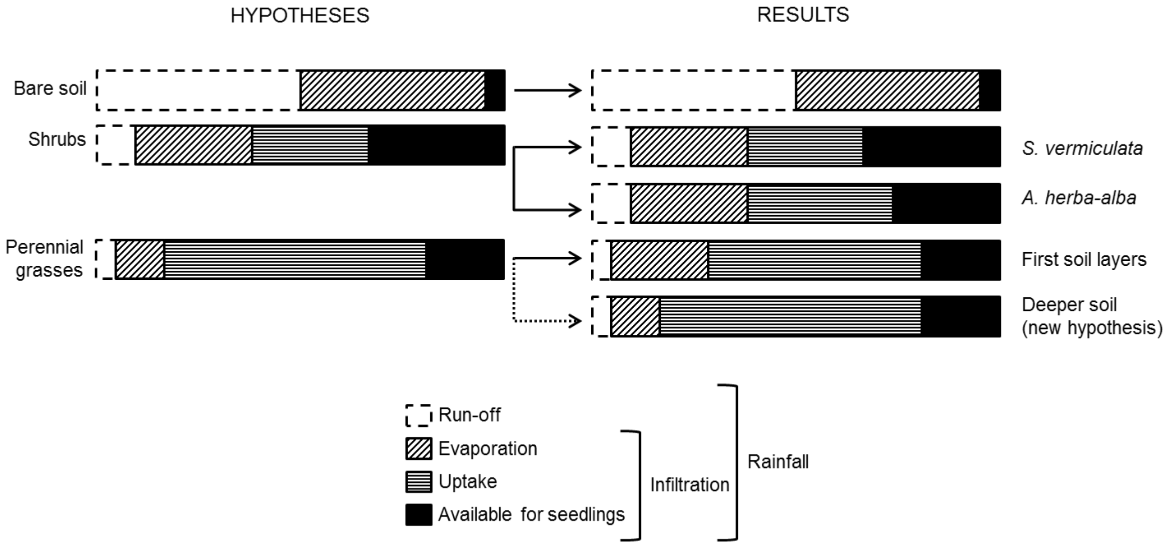


Fig. S1. Left: Conceptual model of the main hypothesis for rainfall partitioning in the three types of microsites in a "source-sink" semiarid ecosystem that is dominated by perennial grasses and shrubs. Dotted rectangles: water loss by run-off, which is greater in bare soil than under shrubs and perennial grasses (Ludwig et al. 2005); rectangles with oblique lines: water loss by evaporation, which is greater in bare soil than under shrubs, and greater under shrubs than perennial grasses, because it depends on shading conditions (i.e. canopy density) (Franco and Nobel 1989, Callaway 2007); rectangles with horizontal lines: water loss by uptake (including transpiration) by neighboring plants, which is non-existent in bare soil, and smaller under shrubs than under perennial grasses (Soriano and Sala 1983, Jurena and Archer 2003); black rectangles: hypothesized water available for seedlings after a rainfall event, which is greatest under shrubs and least on bare soil. Bar wide is based on prediction and literature review, not actual measurements. **Right:** Summary of the main results found in the study. Bar wide is a graphical interpretation of the relative importance of each component found in the study. Dotted arrow indicates a new hypothesis emerged from the study.

References

Callaway, R. M. 2007. Positive interactions and interdependence in plant communities. Springer, Dordrecht, The Netherlands.

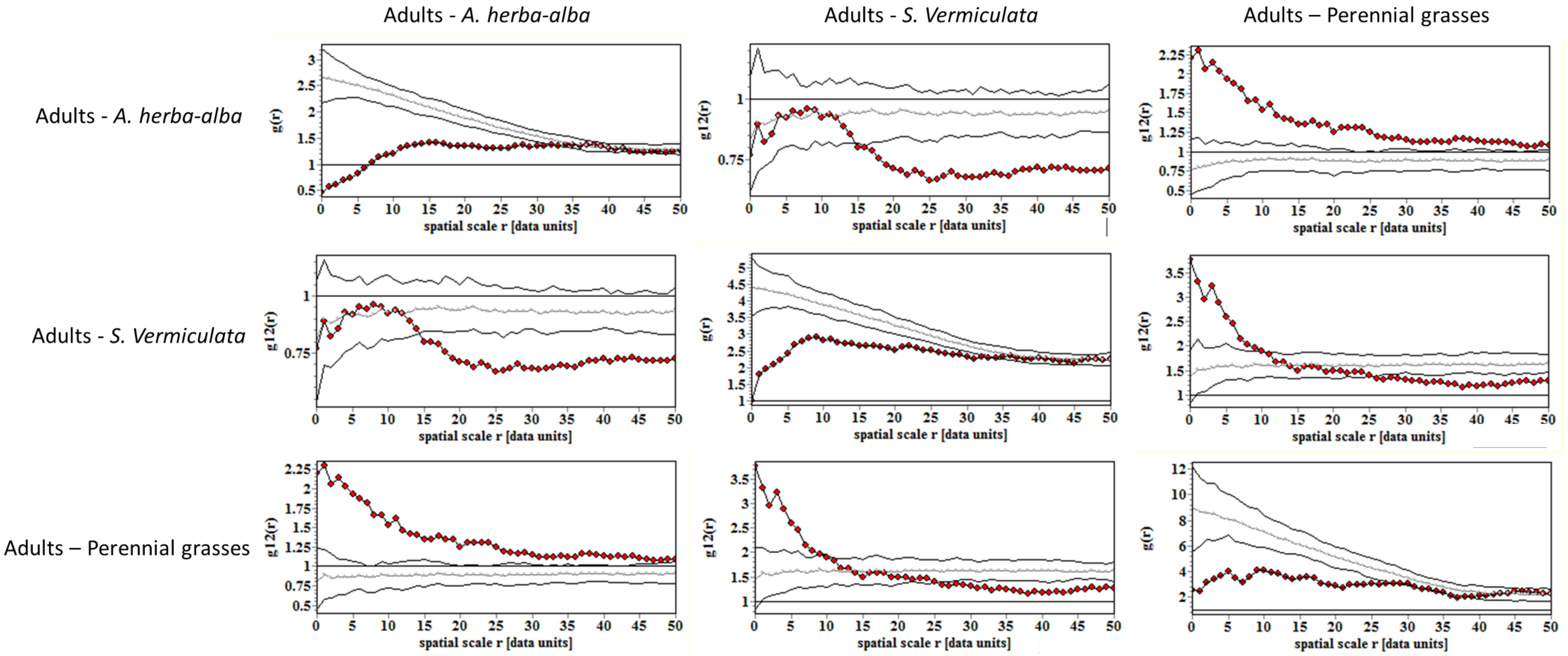
Franco, A. C., and P. S. Nobel. 1989. Effect of Nurse Plants on the Microhabitat and Growth of Cacti. Journal of Ecology **77**:870-886.

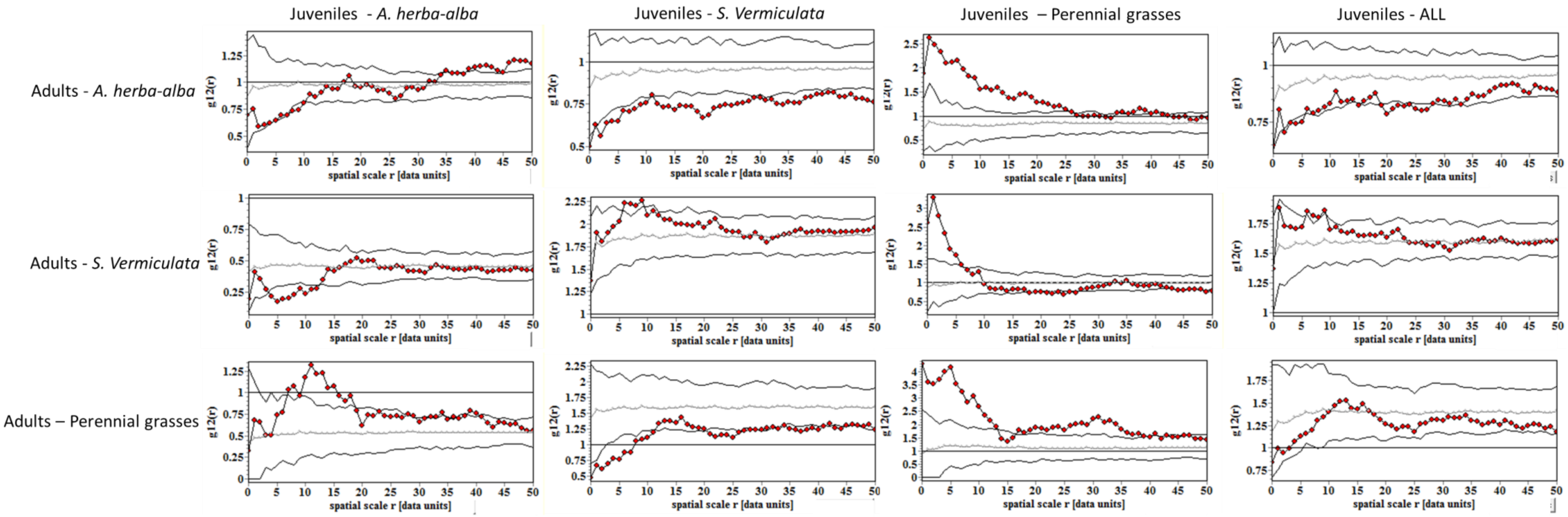
Jurena, P. N., and S. Archer. 2003. Woody plant establishment and spatial heterogeneity in grasslands. Ecology **84**:907-919.

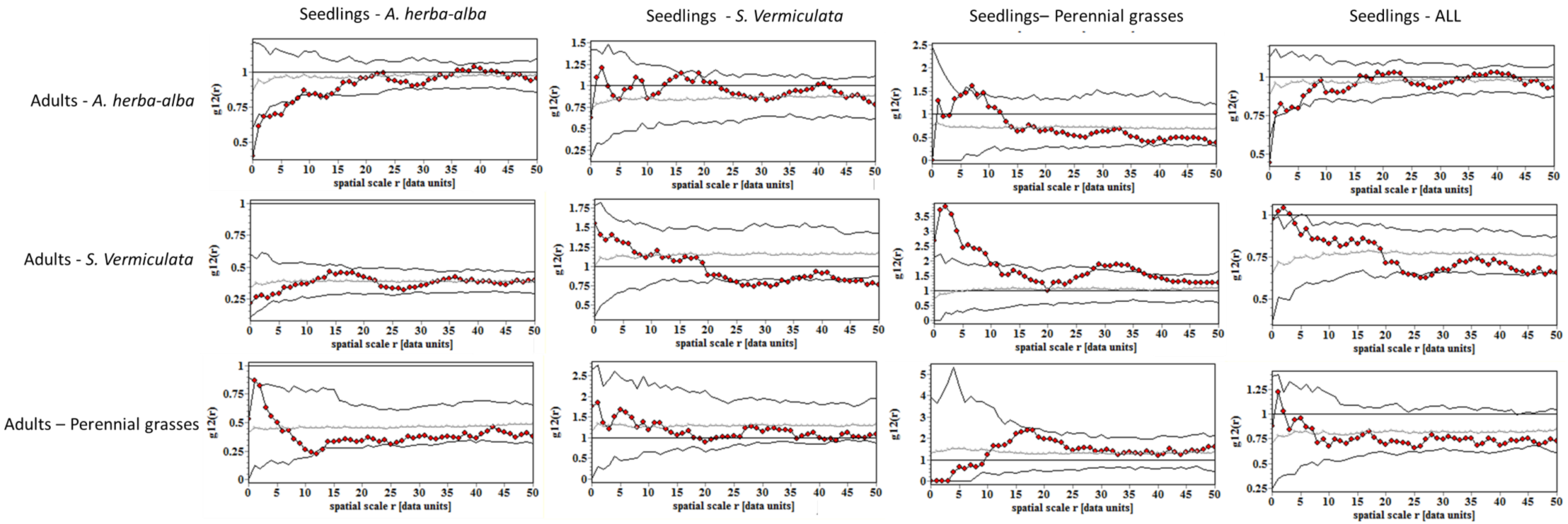
Ludwig, J. A., B. P. Wilcox, D. D. Breshears, D. J. Tongway, and A. C. Imeson. 2005. Vegetation patches and runoff-erosion as interacting ecohydrological processes in semiarid landscapes. Ecology **86**:288-297.

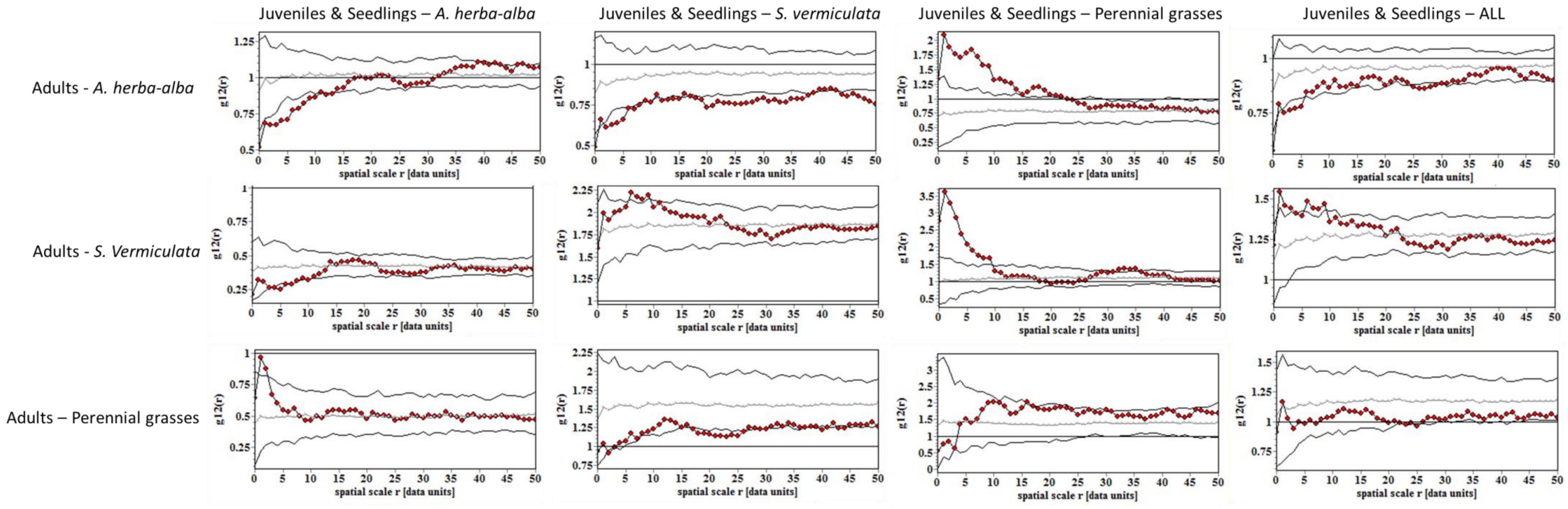
Soriano, A., and O. E. Sala. 1983. Ecological strategies in a Patagonian arid steppe. Vegetatio **56**:9+15.

Appendix 5


Adults - *A. herba-alba*
Adults - *S. Vermiculata*
Adults – Perennial grasses
Adults - *A. herba-alba*
Adults - *S. Vermiculata*
Adults – Perennial grasses
g(r)
g12(r)
spatial scale r [data units]
0
5
10
15
20
25
30
35
40
45
50


Juveniles - *A. herba-alba*
Juveniles - *S. Vermiculata*
Juveniles – Perennial grasses
Juveniles - ALL
Adults - *A. herba-alba*
Adults - *S. Vermiculata*
Adults – Perennial grasses
g12(r)
spatial scale r [data units]
0 5 10 15 20 25 30 35 40 45 50


Seedlings - *A. herba-alba*
Seedlings - *S. Vermiculata*
Seedlings– Perennial grasses
Seedlings - ALL
Adults - *A. herba-alba*
Adults - *S. Vermiculata*
Adults – Perennial grasses
g12(r)
spatial scale r [data units]
0
5
10
15
20
25
30
35
40
45
50


Juveniles & Seedlings – *A. herba-alba*
Juveniles & Seedlings – *S. vermiculata*
Juveniles & Seedlings – Perennial grasses
Juveniles & Seedlings – ALL
Adults - *A. herba-alba*
Adults - *S. Vermiculata*
Adults – Perennial grasses
g12(r)
spatial scale r [data units]

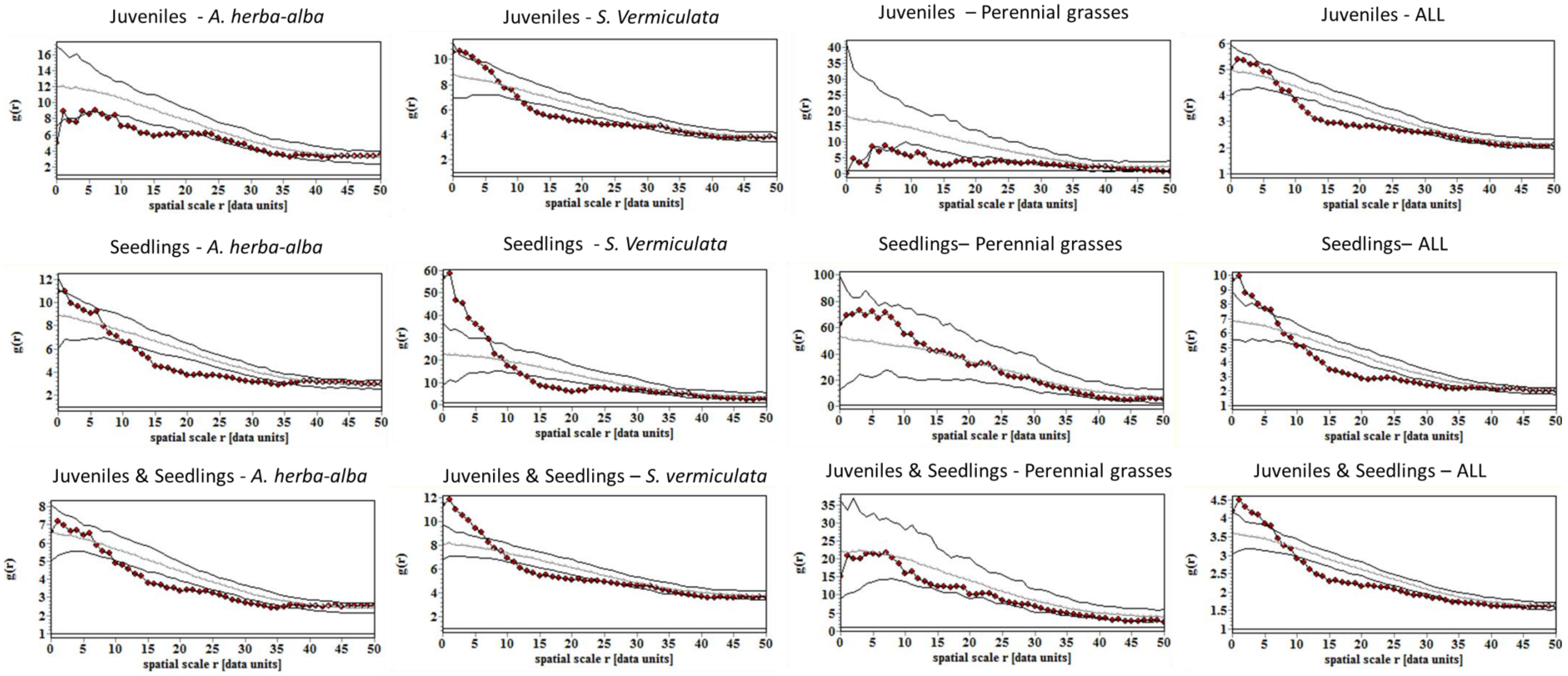

Juveniles - *A. herba-alba*
Juveniles - *S. Vermiculata*
Juveniles – Perennial grasses
Juveniles - ALL
Seedlings - *A. herba-alba*
Seedlings - *S. Vermiculata*
Seedlings– Perennial grasses
Seedlings– ALL
Juveniles & Seedlings - *A. herba-alba*
Juveniles & Seedlings – *S. vermiculata*
Juveniles & Seedlings - Perennial grasses
Juveniles & Seedlings – ALL

Fig. S2. Univariate and bivariate pair-correlation functions between adults, juveniles, and seedlings of *A. herba-alba*, *S. vermiculata,* and perennial grasses. Dots represent observed spatial patterns. Solid lines represent the 50th, 5th, and 95th values for each spatial scale with a heterogeneous null model (intensity function estimated with a kernel width of 20 cm) and a toroidal shift null model that tested the departure from randomness of the univariate and the bivariate point patterns, respectively. When the observed pattern was above the simulation envelopes at a given scale, the pattern is aggregated at that scale; when the observed pattern was below the simulation envelopes, the pattern is segregated; when the observed pattern was inside the simulation envelope, the pattern was random. Table 1 summarized the results from these plots.

Appendix 6

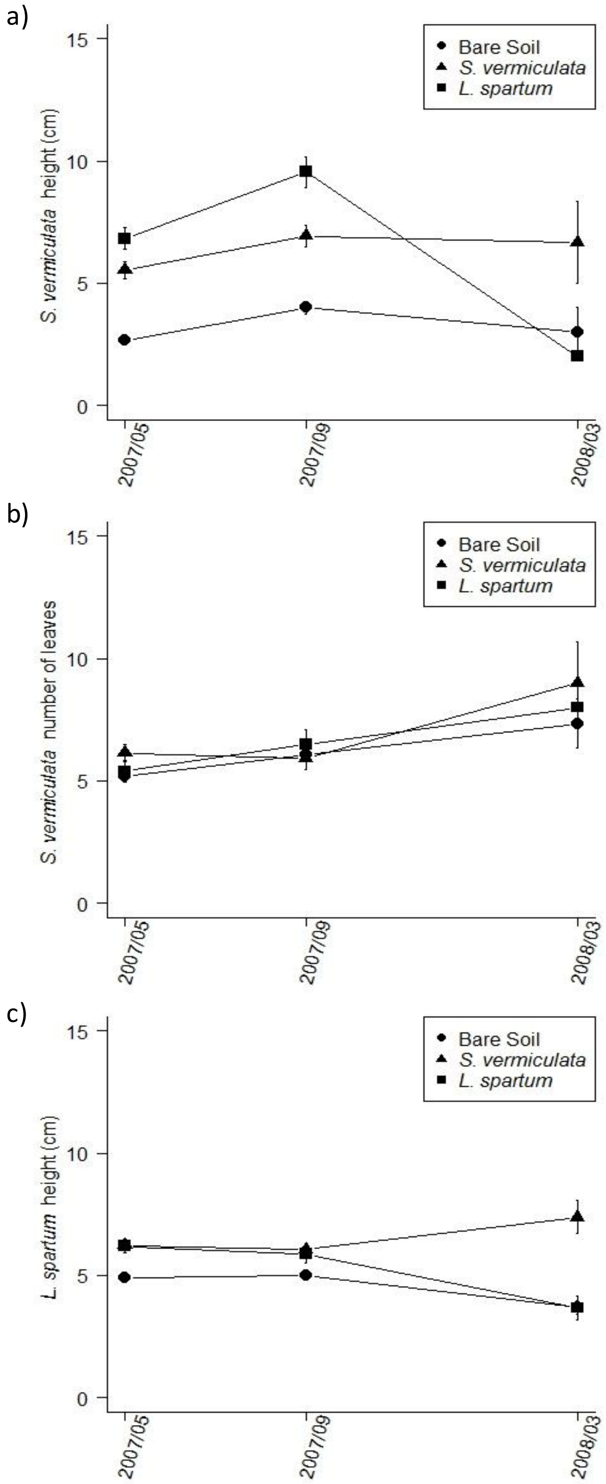


Fig. S3. Means and standard errors of (a) *S. vermiculata* height (cm), (b) *S. vermiculata* leaf number, and (c) *L. spartum* height (cm) for surviving seedlings of the sowing experiment in bare soil (dots), under the canopy of *S. vermiculata* adults (triangles), and under the canopy of *L. spartum* adults (squares).

Appendix 7

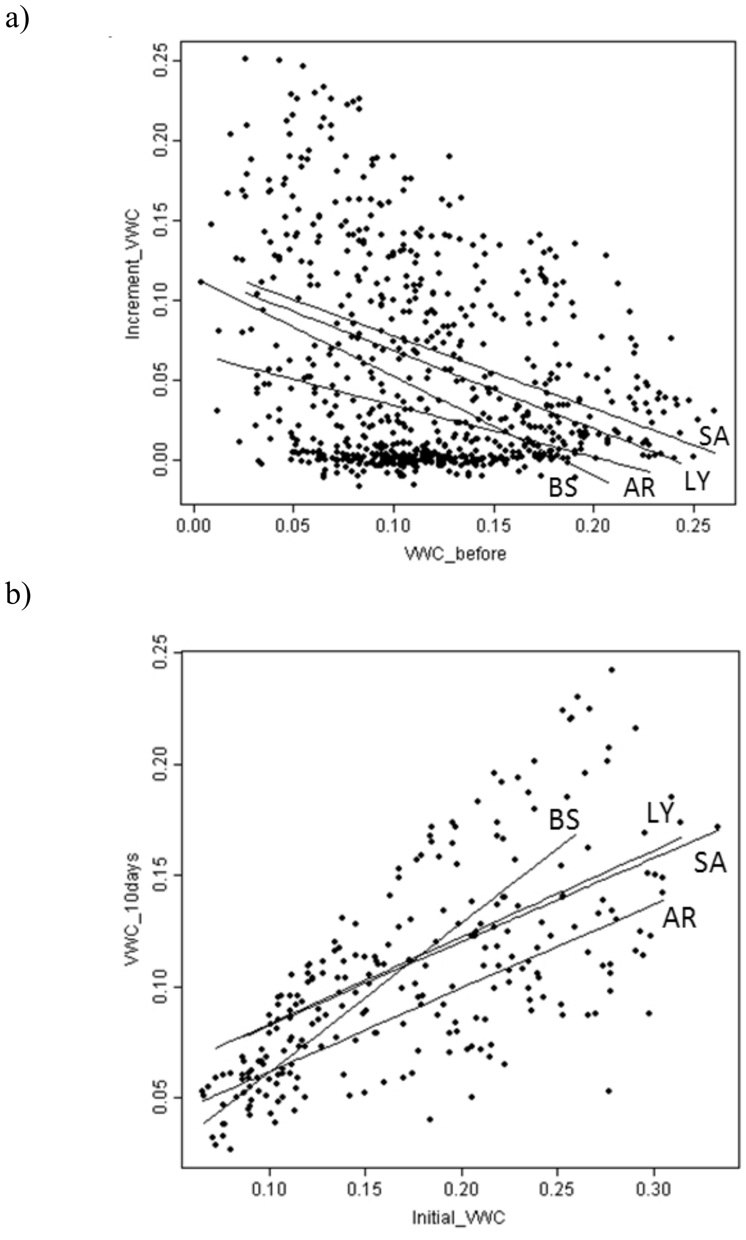


Figure S4. (a) Relationship between the volumetric water content before rainfall events (VWC_before) and the registered increment in VWC after rainfall (Increment_VWC). Black points (n=896 points) represent values per rainfall event on the 16 probes. Lines represent regressions for each microsite (i.e. interaction between VWC_before and the microsite on the Increment_VWC). (b) Relationship between the initial volumetric water content after rainfall (Initial_VWC) and the VWC ten days after rainfall (VWC_10days). Black points (n=288 points) represent values per drying event registered on the 16 probes. Lines represent regressions for each microsite (i.e. interaction between Initial_VWC and the microsite on VWC_10 days). BS, bare soil; AR, *A. herba-alba*; SA, *S. vermiculata*; LY, *L. spartum*.

Appendix 8

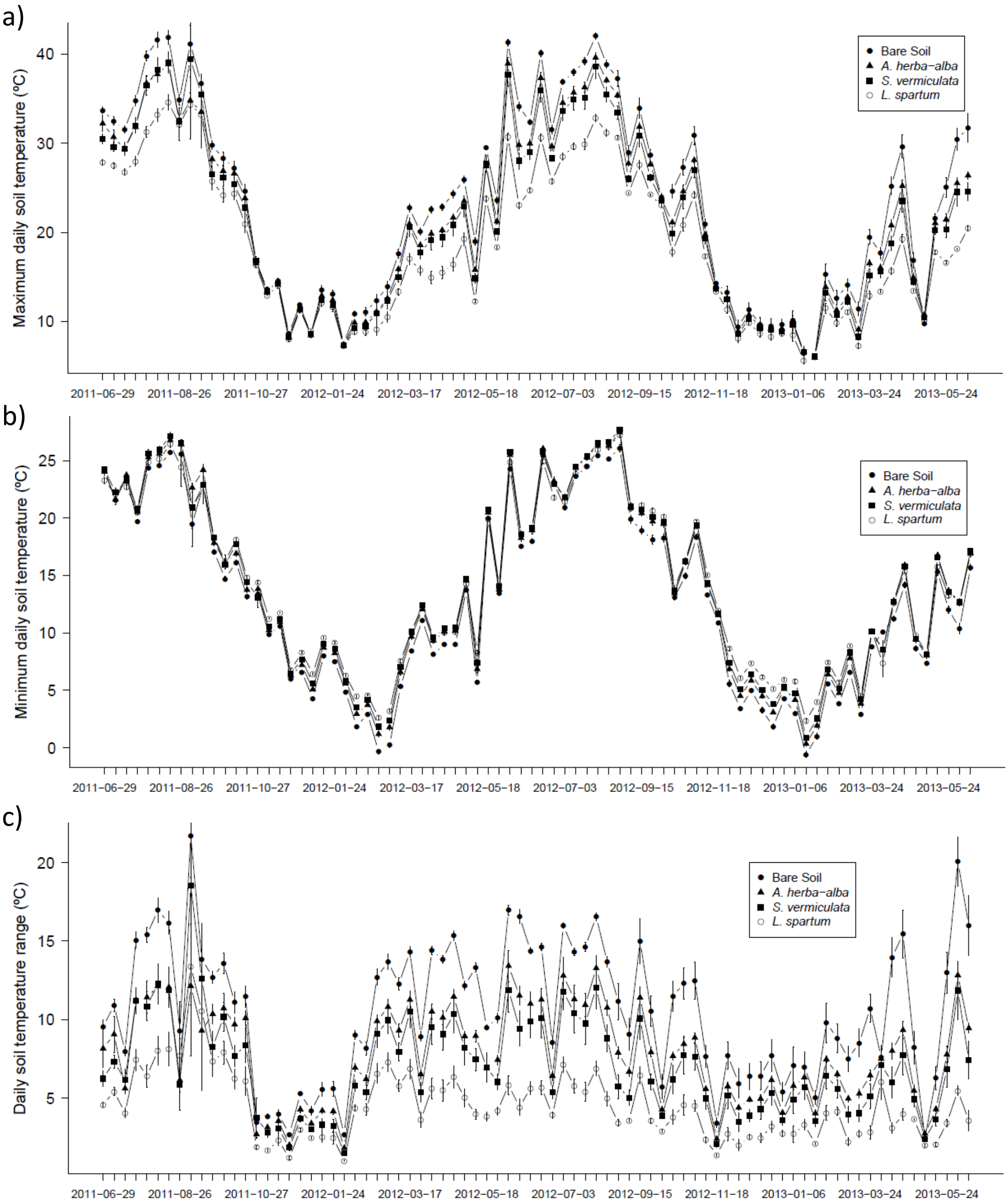


Fig. S5. Means and standard error of (a) maximum daily soil temperature, (b) minimum daily soil temperature, and (c) daily soil temperature range at the four microsites for 80 days randomly selected from May 25, 2011 to July 5, 2013 (n=7 for bare soil microsite, n=6 for the *A. herba-alba*, *S. vermiculata* and *L. spartum* microsites).

Appendix 9

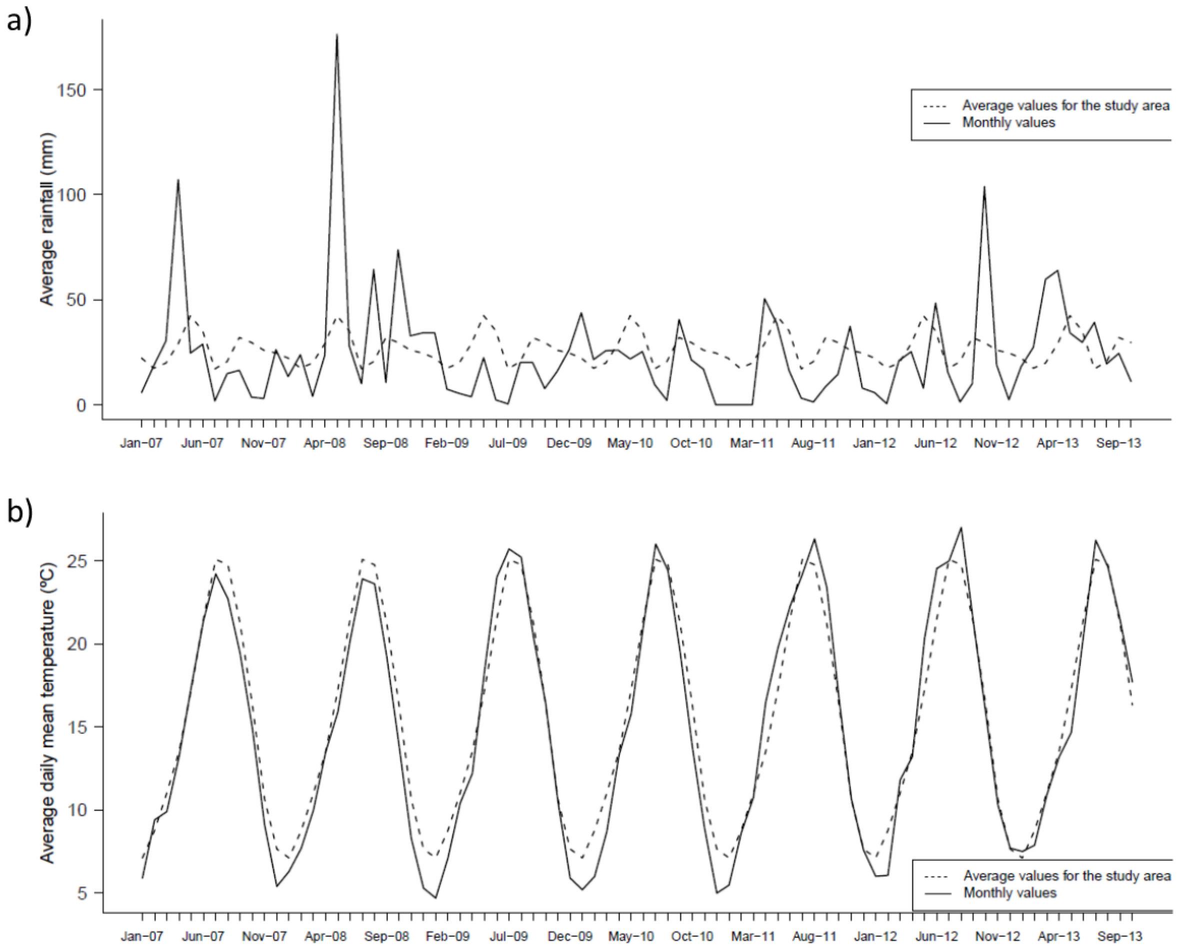


Figure S6. (a) Average rainfall (solid line) at the study area during the time of the field experiments and sampling (2007 to 2013) and (b) average daily mean temperature (solid line). The dashed lines represent average daily mean rainfall and temperature over 30 years (1970-2000) at the nearest meteorological stations. Data sources: (a) and (b) solid lines, 2007 to November 2010: Belchite meteorological station, MAGRAMA, Spanish Government (http://eportal.magrama.gob.es/websiar/SeleccionParametrosMap.aspx?dst=1); (a) and (b) solid lines, December 2010 to May 2012: meteorological station installed in the study area on December 2010; Dashed lines: Digital Climatic Atlas of Aragón (http://anciles.aragon.es/AtlasClimatico/).

Plant-plant interactions as a mechanism structuring plant diversity in a Mediterranean semi-arid ecosystem

Antonio I. Arroyo, Yolanda Pueyo, Hugo Saiz, Concepción L. Alados

Ecology and Evolution (2015), 5, 5305-5317, DOI: 10.1002/ece3.1770

Plant-plant interactions as a mechanism structuring plant diversity in a Mediterranean semi-arid ecosystem

Antonio I. Arroyo[1], Yolanda Pueyo[1], Hugo Saiz[2], Concepción L. Alados[1]

[1] Instituto Pirenaico de Ecología, Consejo Superior de Investigaciones Científicas, Av. Montañana 1005, 50059 Zaragoza, Spain.

[2] UMR 6553 Ecobio. CNRS - Université de Rennes. Avenue du General Leclerc, 35042 Rennes, France.

Abstract

1. Plant-plant interactions are among the fundamental processes that shape structure and functioning of arid and semi-arid plant communities. Despite the large amount of studies that have assessed the relationship between plant-plant interactions (*i.e.* facilitation and competition) and diversity, often researchers forget a third kind of interaction, known as allelopathy.

2. We examined the effect of plant-plant interactions of three dominant species: the perennial grass *Lygeum spartum*, the allelopathic dwarf shrub *Artemisia herba-alba*, and the nurse shrub *Salsola vermiculata,* on plant diversity and species composition in a semi-arid ecosystem in NE Spain. Specifically, we quantified the interaction outcome (IO) based on species co-occurrence, we analyzed diversity by calculation of the individual species-area relationship (ISAR), and compositional changes by calculation of the Chao-Jaccard similarity index.

3. We found that *S. vermiculata* had more positive IO values than *L. spartum*; and *A. herba-alba* had values between them. *Lygeum spartum* and *A. herba-alba* acted as diversity repellers, whereas *S. vermiculata* acted as a diversity accumulator. As aridity increased, *A. herba-alba* transitioned from diversity repeller to neutral and *S. vermiculata* transitioned from neutral to diversity accumulator, while *L. spartum* remained as diversity repeller.

4. *Artemisia herba-alba* had more perennial grass species in its local neighborhood than expected by the null model, suggesting some tolerance of this group to its "chemical

neighbor". Consequently, species that coexist with *A. herba-alba* were very similar among different *A. herba-alba* individuals.

5. *Synthesis*. Our findings highlight the role of the nurse shrub *S. vermiculata* as ecosystem engineer, creating and maintaining patches of diversity, as well as the complex mechanism that an allelopathic plant may have on diversity and species assemblage. Further research is needed to determine the relative importance of allelopathy and competition in the overall interference of allelopathic plants.

Keywords

Allelopathy, aridity, *Artemisia herba-alba*, individual species-area relationship (ISAR), interference, livestock grazing, Middle Ebro Valley, plant-plant interactions.

Introduction

The effect of biotic interactions on the structure and diversity of plant communities has been a central topic in ecology for the last half century. In classical ecological theories, only competitive interactions among plants have been considered to drive community structure and diversity (Grime 1973; Huston 1979). In communities from mid- to high-productivity, a decrease in plant diversity was explained by increased competition and the exclusion of species with lower competitive capacity (Grime 1973). But, in the last years, many studies (Hacker & Gaines 1997; Bruno, Stachowicz & Bertness 2003; Brooker *et al.* 2008; McIntire & Fajardo 2014) have recognized the key role of positive interactions driving diversity in plant communities. Facilitation is especially relevant in harsh environments (Callaway 2007; Soliveres & Maestre 2014), where the presence of nurse plants allows the persistence of stress-intolerant species through expansion of their realized niches (Bruno *et al.* 2003). Therefore, positive interactions are crucial for increasing, maintaining or preventing the loss of species diversity (Hacker & Gaines 1997; Michalet *et al.* 2006; Le Bagousse-Pinguet *et al.* 2014), functional diversity (Schöb, Butterfield & Pugnaire 2012; Gross *et al.* 2013) and phylogenetic diversity (Valiente-Banuet & Verdú 2007; Butterfield *et al.* 2013), not only at the local, but also at the regional and global scales (Cavieres *et al.* 2014).

In arid and semi-arid communities positive and negative interactions among plants occur simultaneously (Holzapfel & Mahall 1999). However, most studies that examine the net effect of plant-plant interactions usually forget that the interference that one plant can exert upon another goes beyond resources uptake (Holmgren, Scheffer & Huston 1997; Holzapfel & Mahall 1999; Tielbörger & Kadmon 2000; Miriti 2006; but see Callaway, Nadkarni & Mahall 1991). Allelopathy, the negative influence that a plant can have on the germination, growth, and survival of other plants due to release of toxic compounds called “allelochemicals” (Muller 1969; Chou & Waller 1983; Rice 1984), is a well-known phenomenon in the fields of invasive plants (Callaway & Ridenour 2004) and agriculture (Chou 1999). But, research on allelopathic effects in natural ecosystems is still scarce, even though many plants may have potential allelopathic activity in the Mediterranean regions

(Thompson 2005). Therefore, allelopathic species should also be considered in studies of plant-plant interactions in natural ecosystems.

Moreover, a recent study suggested that the mechanisms of allelopathic species structuring diversity in plant communities could be more complex than expected. Ehlers *et al.* (2014) found that the allelopathic Mediterranean species, *Thymus vulgaris* L., acts as diversity accumulator because it suppresses a superior competitor. In other words, allelopathy had positive net effects on diversity because of indirect facilitation (Brooker *et al.* 2008), in this mesic, Mediterranean, and species-rich community. It remains unknown whether allelopathic species have similar effects on diversity in more arid areas, where competition for space is less important. In addition, in communities where allelopathic plants coexist with dozens of species, it has been observed that some plants may have resistance to allelopathic compounds (Vivanco *et al.* 2004; Grøndahl & Ehlers 2008; Thorpe *et al.* 2009). The extent of this resistance remains unclear, this is, whether plants have developed tolerance to *"chemical neighbors"* because of co-evolutionary adaptations or whether species that appear in the same community than an allelopathic plant simply avoid establishment nearby. Consequently, allelopathic species may have previously overlooked effects on the composition and diversity of plant communities.

The net outcome of plant-plant interactions changes as environmental stress changes (Bertness & Callaway 1994). At the global scale, exist a shift towards a more positive outcome in plant-plant interactions as stress level increases (He, Bertness & Altieri 2013; Cavieres *et al.* 2014), although some research has noted a decline of positive interactions under extreme stress (Maestre, Valladares & Reynolds 2005; Smit *et al.* 2007; Michalet *et al.* 2014). In nature, several stressors do not occur separately, but they occur simultaneously and potentially interact. Although a growing body of studies have examined the effects of the interplay of several stress factors (*e.g.* aridity and grazing) on biotic interactions (Maalouf *et al.* 2012; Mod, le Roux & Luoto 2014; Verwijmeren *et al.* 2014), its effects on diversity, through regulation of biotic interactions, still remains unclear (Le Bagousse-Pinguet *et al.* 2014). On the other hand, production of allelochemicals is greater in stressful environments in which there are harsh biotic and abiotic conditions such as water deficit, extreme temperatures or physical damage from herbivores (Tang *et al.* 1995; Reigosa, Sánchez-Moreiras & González 1999; Pedrol, González & Reigosa 2006). Moreover, in stressed environments, plants may be more susceptible to allelochemicals (Pedrol *et al.* 2006; Reigosa *et al.* 1999) because they are already under stress. Therefore, when considering allelopathic species, the balance between facilitation and interference might change with increasing stress, leading to reduction or even prevention of positive net effects on diversity.

In this research, we aimed to evaluate the role of plant-plant interactions structuring diversity and species composition in a semi-arid ecosystem NE Spain. Specifically, we assessed interaction outcome, diversity and changes in species composition in the local neighborhood of the perennial grass *Lygeum spartum* L., the dwarf shrub *Artemisia herba-alba* Asso., and the shrub *Salsola vermiculata* L. under different conditions of stress level (aridity and grazing). *Artemisia herba-alba* and *S. vermiculata* are two species with similar physiognomy and plant traits. Both are long-lived shrubs, with moderately dense canopy and deep roots, which are well-known traits to host high plant diversity underneath in semi-arid ecosystems (Callaway 2007; Parsons & Abrahams 2009; Pugnaire 2010). Therefore, they could potentially have the same facilitative effects on diversity. However, while *S. vermiculata* is considered an effective nurse plant, there are dozens of works in literature demonstrating the allelopathic nature of *A. herba-alba*, dealing with ecological, physiological, biochemical and medical approaches (Friedman, Orshan & Ziger-Cfir 1977; Escudero *et al.* 2000; Mohamed *et al.* 2010). On the other hand, *L. spartum* has very different plant traits from shrubs (dense and shallow rooting zone and very dense canopy) that confer to this species a high competitive ability for water and resources (Jurena & Archer 2003).

We expected mostly negative interactions between the allelopathic (*A. herba-alba*) and competitive (*L. spartum*) plants and the other species present in this plant community, and mostly positive interactions between the nurse plant (*S. vermiculata*) and these other species. We hypothesized that species with mostly negative interaction would act as diversity repellers (*i.e.* would have a local neighborhood less diverse than expected) and that species with mostly positive interactions would act as diversity accumulators (*i.e.* would have a local neighborhood more diverse than expected) (*sensu* Wiegand *et al.* 2007).

In particular, we expected that in drylands, where competition for space is not a dominant process, the allelopathic species would have a negative effect on diversity, in contrast to results found in mesic Mediterranean areas (Ehlers, Charpentier & Grøndahl 2014). Also, we hypothesized that if allelopathic compounds have determinant effects on germination and survival of other species present in community, only species adapted to allelochemicals will be able to coexist with the allelopathic species. Thus, we expected low compositional changes of species nearby the allelopathic species. Based on previous global observations, we expected a shift towards facilitation (or reduced competition) with increasing aridity. Also, we expected a similar shift with the presence of grazing pressure due to associational resistance (Olff & Ritchie 1998). Finally, we expected that allelopathy would partially or totally suppress the potential facilitative effects of the allelopathic species with increasing environmental stress (aridity and grazing).

Material and methods

Study area

This study was conducted in the Middle Ebro Valley (NE Spain; Fig. 1). This region is one of the most arid areas in Spain, with an average annual temperature of 15ºC and average annual precipitation of 353 mm year^{-1} (at 250 m.a.s.l., Zaragoza station, n = 50 years). The landscape mainly consists of flat-bottomed valleys and low hills. Dry croplands and extensive sheep (*Rasa aragonesa*) production are the principal human activities (Pueyo 2005). The grass-shrub steppe community on non-cultivated lands includes shrubs (*S. vermiculata*, *A. herba-alba*, and *Suaeda vera* J.F.G.mel. among others), perennial grasses (*L. spartum*, *Brachypodium retusum* (Pers.) P.Beauv., *Dactylis glomerata* L., and *Stipa parviflora* Desf. among others) and many annual and ephemeral herbs.

Field surveys

We chose non-cultivated lands in two zones. One zone is among the most arid regions in the Middle Ebro Valley ("El Planeron de Belchite" Ornithological Reserve, 41º22'24''N, 00º37'55''O) and has an average annual temperature of 15.4ºC and average annual precipitation of 319 mm year^{-1}; the other zone (Castejon de Monegros municipality, 41º41'36''N, 00º09'49''O) is less arid and has an average annual temperature of 14.7ºC and average annual precipitation of 377 mm year^{-1} (Fig. 1; Table 1). The two zones have similar lithology, topography and grazing regime (<0.7 head ha^{-1} year^{-1}) (Pueyo *et al.* 2013). At each zone we selected a grazed location and an ungrazed location for establishment of four study sites: LAUn (least arid, ungrazed), LAG (least arid, grazed), MAUn (most arid, ungrazed) and MAG (most arid, grazed) (Fig. 1; Table 1). LAG and MAG were continuously grazed until the date when sampling was carried out. In these communities, grazing tends to favor shrubs, while perennial grasses would be more dominant in the absence of grazing (Puigdefábregas & Mendizabal 1998). In the summer of 2010, we sampled six 250 m transects at each study site (n=24) using the point-intercept method (Goodall 1952). Along each transect, we recorded the location of all species found each 20 cm. No distinction was made for ontogenetic stages.

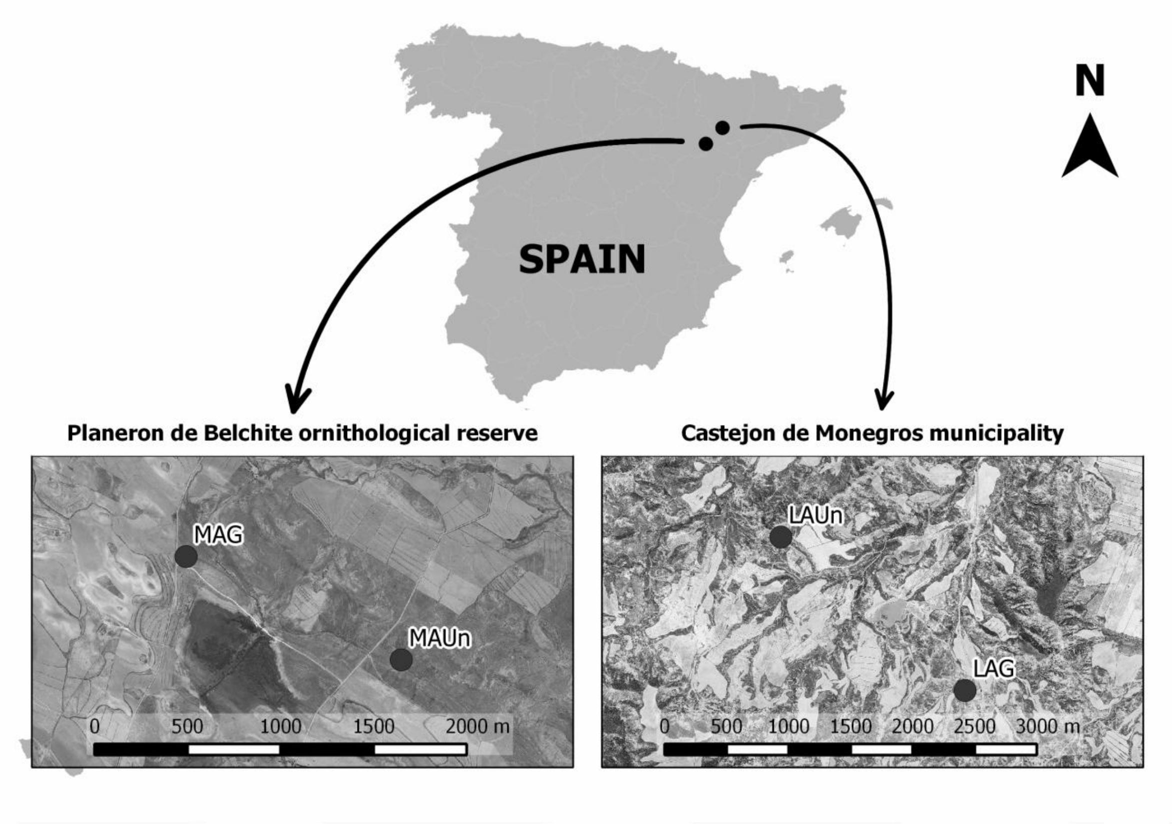


Fig. 1. Locations of the four study sites in the Middle Ebro Valley of Spain. The distance between Planeron de Belchite ornithological reserve and Castejon de Monegros municipality is 53 km.

Table 1. Location, grazing regime, climate (annual rainfall, mean annual temperature and aridity index), relative abundance (percent of species abundance respect to total species abundance, mean ± standard error) of target species (*L. spartum*, *A. herba-alba* and *S. vermiculata*), and species abundance of different plant types (mean ± standard error) at each study site. See Pueyo *et al.* (2013) for more details about grazing management in the study sites. Annual rainfall and mean annual temperature data obtained from Digital Climatic Atlas of Aragón (http://anciles.aragon.es/AtlasClimatico/).

							Plant type abundance (%)			
Study site	Location	Grazing	Annual rainfall (mm)	Mean annual temperature (ºC)	Aridity index (ºC mm^{-1})	Relative abundance of target species (%)	Annuals	Perennial grasses	Dwarf shrubs	Shrubs
LAUn	Less arid	No	377	14.7	3.90	40.32±1.29	41.93±1.43	20.30±0.70	24.61±0.73	13.16±1.55
LAG	Less arid	Yes	377	14.7	3.90	47.20±3.46	52.51±1.65	18.11±0.81	21.97±2.09	7.40±0.91
MAUn	Most arid	No	319	15.4	4.83	54.64±1.41	63.34±0.78	13.36±1.02	18.51±0.63	4.79±0.66
MAG	Most arid	Yes	319	15.4	4.83	38.05±2.32	60.50±2.94	15.79±1.78	19.25±1.31	4.46±0.79

Target species

We selected three perennial species that were present in all study sites to test our hypotheses: the competitive perennial grass *L. spartum*, the dwarf shrub and well-known allelopathic *A. herba-alba* (Friedman *et al.* 1977; Escudero *et al.* 2000; Mohamed *et al.* 2010) and the nurse shrub *S. vermiculata*. Together, these three target species accounted for a mean relative abundance of 45.35% ± 1.72%, ranging from a minimum of 38.05% ± 2.32% in MAG to a maximum of 54.64% ± 1.41% in MAUn (Table 1).

Interaction outcome of target species

To determine whether each target species had mostly positive or negative interactions, we analyzed the spatial association of all possible pairs of species composed by target species and other species in the transect.

We assessed spatial association by comparing the number of co-occurrences, C_r, found for a given pair of species with the expected number of co-occurrences, C_e, that the pair of species would have based on their abundances. A co-occurrence of a pair of species was considered when, in a transect, both species appeared together at the same point (Saiz & Alados 2012). Later, as co-occurrences are count data, we compared C_r and C_e using a

Poisson distribution with the λ parameter fitted to C_e. When C_r was significantly greater than C_e for a pair of species, this means that they co-occurred more times than expected by chance, and therefore, we assumed a positive spatial association. On the other hand, when C_r was significantly less than C_e for a pair of species, this means that they co-occurred less often than expected by chance, and we assumed a negative association. Non-significant differences between C_r and C_e indicate a random spatial association. Although co-occurrence of species can be driven not only by plant-plant interactions, but also by, for example, similar habitat requirements, co-occurrence is generally accepted as an indicator of plant interactions in drylands (Saiz & Alados 2012; Soliveres *et al.* 2014). Thus, we interpreted positive and negative associations as proxies for positive and negative interactions. Then, we computed the number of positive and negative associations (*i.e.* positive and negative interactions) for each target species, and the interaction outcome (IO) of each target species based on its number of positive and negative interactions with the other plants in the community:

$$\text{Interaction Outcome (IO)} = \frac{\text{Positive interactions} - \text{Negative interactions}}{\text{Total interactions}}$$

For each target species, an IO value of 1 indicates positive interactions with all species along the transect, an IO value of -1 indicates negative interactions with all species, and an IO value of 0 indicates the same number of positive and negative interactions. Differences in IO among target species and sites were analyzed with two-way ANOVA. Tukey´s *post hoc* honest significant difference (HSD) tests were used to detect differences for pairs of target species and sites. Assessment of spatial associations and statistical analyses were performed with R (R Core Team 2013).

Diversity patterns near target species

We analyzed the diversity in the local neighborhood of individuals of the three target species. For this purpose, we followed the ISAR (individual species-area relationships) method proposed by Wiegand *et al.* (2007). The $\text{ISAR}_{(d)}$ can be defined as the expected number of species within a distance *d* of a given individual of the target species *t*,

$$\mathrm{ISAR}_{(d)} = \sum_{j=1}^{S} \left[1 - P_{t,j}(0, d)\right]$$

where $P_{t,j}(0,d)$ is the probability that species j was not present within distance d of individuals of target species t. Thus, the $\mathrm{ISAR}_{(d)}$ value will be the sum of $1 - P_{t,j}(0,d)$ for all species j present in the transect (Wiegand *et al.* 2007). $\mathrm{ISAR}_{(d)}$ was calculated along 1500 m transect (six 250 m transects) at each study site to a maximal distance of 4 m ($d = 4$), which is considered sufficient for detection of plant-plant interactions (Rayburn & Wiegand 2012). The ISAR method considers plant-plant interactions at several scales, disentangling the spatial-dependency of the interactions. A predominance of positive interactions would lead to diversity accumulation in the local neighborhood of individuals of the target species; conversely, a predominance of negative interactions would lead to diversity repulsion. On the other hand, a neutral balance of positive and negative interactions or the presence of only weak interactions with other species would lead to a local neighborhood as diverse as expected.

We calculated a confidence envelope using a Monte Carlo test with 199 heterogeneous Poisson null model simulations (Wiegand & Moloney 2004) to determine whether for a given distance d, the $\mathrm{ISAR}_{(d)}$ of a target species was significantly greater or less than expected by chance (Wiegand *et al.* 2007; Rayburn & Wiegand 2012). Each heterogeneous Poisson null model simulation replaces individuals of the target species randomly within the maximal distance in which plant-plant interactions are expected to occur. Hence, for distances greater than 4 m ($d = 4$), the spatial distribution of target species individuals was maintained, and for distances less than 4 m, the spatial structure was removed. If the $\mathrm{ISAR}_{(d)}$ was greater than the fifth largest value (or less than the fifth lowest value) from simulations, then individuals of that target species were surrounded at distance d by more (or fewer) species than expected by the null model, with $\alpha \approx 0.05$. If the $\mathrm{ISAR}_{(d)}$ was within the confidence envelope, then individuals of that target species were surrounded by the same number of species than expected by the null model. We assessed the significance of the relationship between interaction outcome (IO) of target species and ISAR values at local neighborhood ($d = 20$ cm) with a linear model.

The local neighborhood of individuals of a target species could be as diverse as expected by the null model even when such target species interacts significantly with a few species or plant types. To assess this effect, we classified plant species into four types based on life form: annual, perennial grass, shrub and small shrub. Then, we computed ISAR$_{(d)}$ for each combination of target species and plant type. Thus, at each study site, we performed ISAR analyses by considering the entire diversity (excluding j when $j = t$) and by considering different plant types separately.

ISAR analyses and comparisons with null models were performed with MATLAB R2010b. The linear relationship for IO and ISAR$_{(d\ =\ 20)}$ was determined with R (R Core Team 2013).

Compositional changes of species associated with target species

In order to assess compositional changes of species spatially associated with target species, we employed the Chao-Jaccard similarity index (Chao *et al.* 2005). For each target species, we calculated this index for all possible pairs of transects of the same study site, using data of co-occurrence between pair of species (number of times that target species and other species appeared at the same point in a transect). Chao-Jaccard index is based on the probability that two randomly chosen individuals that co-occur with the target species (one from each of two transects) both belong to any of the species shared by the two transects (Chao *et al.* 2005) (*i.e.* species that co-occur with the target species in both transects). This index considers the similarity of the species list that co-occurs with a given target species, and the similarity of the relative abundances of co-occurring species. Thus, a high similarity among transects indicates that nearly the same species co-occur with a target species and approximately the same frequencies. The Chao-Jaccard similarity index was calculated using the vegan package in R (Oksanen *et al.* 2013). We compared Chao-Jaccard similarity index among target species at each study site by use of one-way ANOVA. Tukey´s *post hoc* HSD test was employed to detect differences among pairs of target species. These statistical analyses were performed with R (R Core Team 2013).

Results

Outcome of plant-plant interactions of target species

Positive and negative interactions were found for all three target species (*L. spartum*, *S. vermiculata* and *A. herba-alba*) (Fig. 2a). In particular, these target species had significantly positive and negative interactions with approximately 20% of the plant species in the community (Fig. 2a). Overall, the three target species had positive IO values, but there were significant differences among the different species ($F_{2,54} = 5.48$, $p = 0.007$). Tukey´s *post hoc* HSD test showed that *S. vermiculata* had a significantly more positive IO value than *L. spartum*, and that *A. herba-alba* had an intermediate IO value between them (Fig. 2b).

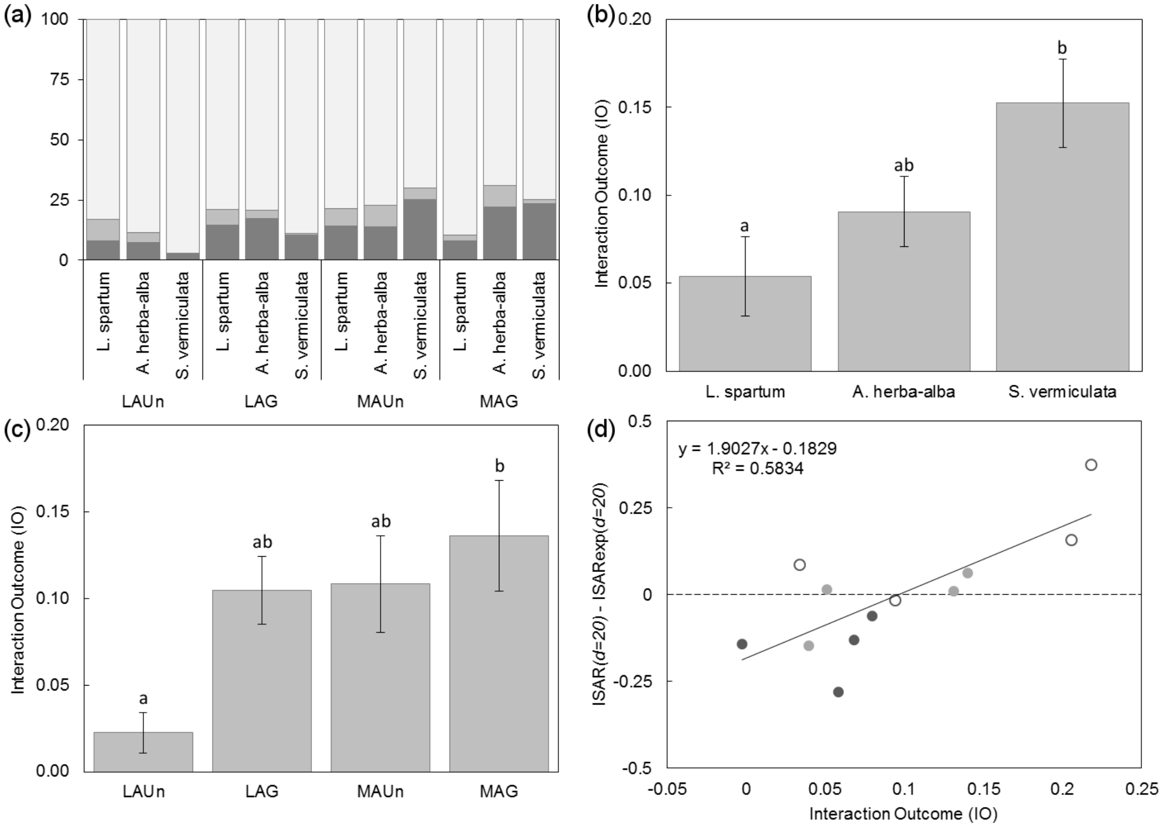


Fig. 2. a) Percentage of positive (dark grey), negative (medium grey) and neutral (light grey) interactions of target species at each study site. b) Mean interaction outcome (IO) values (bars) and standard errors (error bars) of *L. spartum* (n = 21), *A. herba-alba* (n = 24) and *S. vermiculata* (n = 21). Different letters indicate significant differences between target species. c) Mean IO values (bars) and standard errors (error bars) at LAUn (n = 16), LAG (n = 18), MAUn (n = 18), and MAG (n = 18). Different letters indicate significant differences. d) Relationship between IO and species diversity within 20 cm ($ISAR_{(d=20)}$ - $ISAR_{exp(d=20)}$) of target species individuals at different study sites ($R^2 = 0.583$, $p = 0.004$). *L. spartum*: dark grey dots, *A. herba-alba*: light grey dots, *S. vermiculata*: open circles.

The IO differed significantly among the study sites ($F_{3,54}$ = 3.65, p = 0.018). In particular, Tukey´s *post hoc* HSD test showed that the IO was significantly more positive at MAG than at LAUn, and that the IO had intermediate values at LAG and MAUn, (Fig. 2c). On the other hand, the interaction between target species and study site was non-significant ($F_{6,54}$ = 1.38, p = 0.238), indicating that the differences found in IO among the target species were similar at all study sites.

Diversity patterns of nearby target species

The IO of target species and the diversity near individuals of target species had a significantly positive relationship ($F_{1,10}$ = 13.93, p = 0.004; Fig. 2d). In other words, a predominance of positive interactions was associated with diversity accumulation and a less positive balance was associated with diversity repulsion.

ISAR analysis showed that the perennial grass *L. spartum* acted as diversity repeller in all study sites at distances less than 100 cm (Figs 3a-d); there were significant and positive departures of the ISAR curve in the less arid study sites at greater distances (Figs 3a and 3b). *Lygeum spartum* mainly repelled other perennial grasses (Table 2; see Fig. S1 in Supporting Information), and it had greater diversity of nearby annuals and dwarf shrubs species than expected by the null model (Table 2; Fig. S1).

The allelopathic species *A. herba-alba* acted as diversity repeller in the less arid study sites (LAUn and LAG; Figs 3e and 3f); there were significant departures of the ISAR curve at distances less than 50 cm in LAUn (Fig. 3e) and at distances close to 0 cm in LAG (Fig. 3f). On the other hand, *A. herba-alba* individuals had a local neighborhood as diverse as expected by the null model in the most arid study sites (MAUn and MAG; Figs 3g and 3h). Calculation of ISAR for different plant types showed that *A. herba-alba* repelled other shrubs and dwarf shrubs species (Table 2; Fig. S2) and, although facilitative effects on the whole plant diversity were not found, this species acted as diversity accumulator of perennial grasses in all study sites, as it had more species of perennial grasses in the local neighborhood of its individuals than expected by the null model (Table 2; Fig. S2).

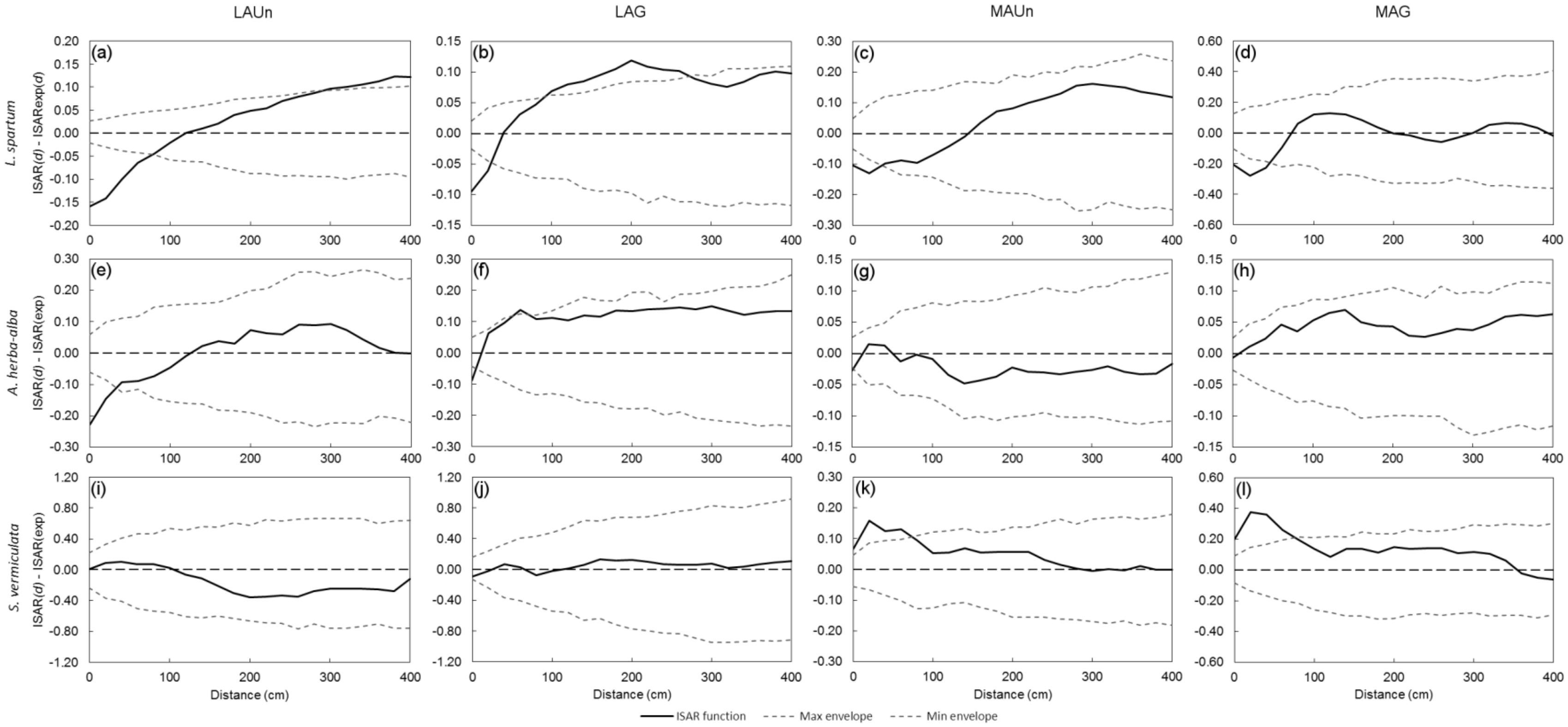


Fig. 3. Individual species-area relationship (ISAR) curves of target species at each study site. Solid lines are ISAR values (ISAR$_{(d)}$ - ISAR$_{\exp(d)}$, where ISAR$_{\exp(d)}$ is the average of 199 heterogeneous Poisson null model simulations) and dotted grey lines are the 95% confidence envelope (fifth largest and the fifth smallest values from the simulations) for each spatial scale. When ISAR was above the confidence envelope, the local neighborhood is more diverse than expected by the null model (diversity accumulation). When ISAR was below the confidence envelope, the local neighborhood is less diverse than expected by the null model (diversity repulsion). When ISAR was within the confidence envelope, the local neighborhood was as diverse as expected by the null model.

The shrub *S. vermiculata* was neutral in the less arid study sites (LAUn and LAG; Figs 3i and 3j), but acted as a diversity accumulator in the most arid sites (MAUn and MAG; Figs 3k and 3l) at distances of 0 to 80 cm. This effect was mostly for annuals and perennial grass species (Table 2; Fig. S3).

Table 2. Summary of ISAR curves for *L. spartum*, *A. herba-alba* and *S. vemiculata* based on plant type at each study site (see Figs S1, S2, and S3). +, presence of diversity accumulation at some distance; -, presence of diversity repulsion at some distance; 0, no departure from the confidence envelope at any distance. Symbols separated by commas indicate different behaviors at different scales; ++ and -- indicate large departures from confidence envelope; symbols within parentheses indicate marginal departures from confidence envelope.

	L. spartum			
	LAUn	LAG	MAUn	MAG
Annuals	(-), + +	+ +	0	0
Perennial grasses	- -, (+)	- -	- -	-
Dwarf shrubs	(-), +	(-), + +	- -	- -
Shrubs	- -	- -	(-)	0
	A. herba-alba			
	LAUn	LAG	MAUn	MAG
Annuals	(-)	0	0	(+)
Perennial grasses	(-), + +	+	+ +	+
Dwarf shrubs	-	-	0	- -
Shrubs	- -	-	- -	-
	S. vermiculata			
	LAUn	LAG	MAUn	MAG
Annuals	(-)	0	+ +	+ +
Perennial grasses	0	0	+	(+)
Dwarf shrubs	0	0	-	(-)
Shrubs	0	0		

Compositional changes of species associated with target species

There were significant differences in the Chao-Jaccard similarity index among target species at all study sites ($F_{2,30} = 18.88$, $p < 0.001$ for LAUn; $F_{2,42} = 55.57$, $p < 0.001$ for LAG; $F_{2,37} = 10.96$, $p < 0.001$ for MAUn; $F_{2,33} = 17.21$, $p < 0.001$ for MAG; Fig. 4). Tukey´s *post hoc* HSD test showed that *L. spartum* had significantly lower similarity than *A. herba-alba* and *S. vermiculata* in the most arid study sites (MAUn and MAG), but there were no significant differences in the Chao-Jaccard similarity index between *A. herba-alba* and *S. vermiculata* in those study sites (Fig. 4). On the other hand, in the less arid study sites (LAUn and LAG), the diversity associated with *S. vermiculata* had a significantly lower similarity between individuals of this species than diversity associated to *L. spartum* and *A. herba-alba*. There were no differences in the Chao-Jaccard similarity index between *L. spartum* and *A. herba-alba* at LAUn, but *L. spartum* had a significantly lower similarity than *A. herba-alba* at LAG (Fig. 4).

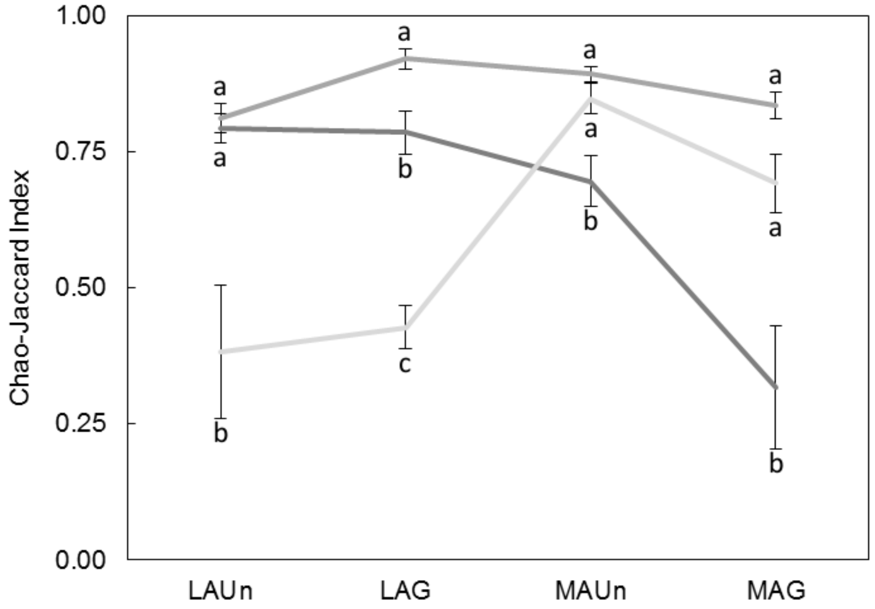


Fig. 4. Means (line) and standard errors (error bars) of the Chao-Jaccard similarity index of *L. spartum* ($n_{LAUn} = 15$, $n_{LAG} = 15$, $n_{MAUn} = 10$, $n_{MAG} = 6$; dark grey), *A. herba-alba* ($n_{LAUn} = 15$, $n_{LAG} = 15$, $n_{MAUn} = 15$, $n_{MAG} = 15$; medium grey) and *S. vermiculata* ($n_{LAUn} = 3$, $n_{LAG} = 15$, $n_{MAUn} = 15$, $n_{MAG} = 15$; light grey), where n_{site} is the number of transects comparisons per study site. The number of comparisons differ because only transects in which the target species were present were analyzed. Different letters indicate significant differences between target species.

Discussion

The purpose of this study was to assess the effects of plant-plant interactions, and its modulation along contrasting conditions of aridity and grazing pressure, of three dominant species on plant diversity and species composition in a semi-arid ecosystem. Our results indicated that *S. vermiculata* (nurse plant) had in its local neighborhood more species than expected by the null model, while *L. spartum* (perennial grass) and *A. herba-alba* (allelopathic plant) had fewer species than expected by the null model. Overall, perennial grasses tolerated allelopathic compounds of *A. herba-alba*. Moreover, the composition of species that appeared near *A. herba-alba* was very similar among transects. Together these results suggested that allelopathic activity of *A. herba-alba* may act as a biotic filter in species assemblage. The role of *S. vermiculata* and *A. herba-alba* on diversity changed as stress level increased, but *L. spartum* exhibited no such change. Aridity rather than grazing seemed to be the main driver of those changes.

The nurse shrub *S. vermiculata* had a significantly more positive net interaction outcome than the perennial grass *L. spartum*, while the allelopathic dwarf shrub had a net interaction outcome between them. In addition, individuals of *S. vermiculata* had more species around them than expected only by chance. In general, shrubs are considered good acting as nurse plants (Gómez-Aparicio 2009) in a wide variety of ecosystems. The greater number of species and plant density under the canopy of *S. vermiculata* than under *A. herba-alba* and *L. spartum* could be due to its nursing effect during plant establishment. *Salsola vemiculata* greatly improves the abiotic conditions under its canopy, such as water infiltration and nutrient enrichment, and thus improves the survival and growth of seedlings. More specifically, *S. vermiculata* facilitated annuals and also perennial grasses. Numerous studies have documented positive interactions between shrubs and annuals in drylands throughout the world (Wilby & Shachak 2004; Holzapfel *et al.* 2006; López, Larrea-Alcázar & Teresa 2009).

The perennial grass *L. spartum* repelled diversity of all plant types at short distances. Perennial grasses have a dense root system in the upper soil layers (Jackson *et al.* 1996) which exerts a strong belowground competition (Armas & Pugnaire 2011), mainly in water uptake. It has been argued that competition for resources such as water is especially important during the establishment of seedlings (Jankju 2013). A higher efficiency of *L. spartum* in water uptake would increase the mortality of nearby seedlings, thus explaining its strong role as diversity repeller. The shrub *S. vermiculata* facilitated perennial grasses, but *L. spartum* did not facilitate shrubs. This finding is in line with studies that identified an ontogenetic shift in the relationship between shrubs and grasses; shrubs facilitate grasses at early stages of development, and established grasses finally replace and impede establishment of new shrubs (Armas & Pugnaire 2005).

Surprisingly, in the less arid study sites *L. spartum* had more species of annuals and dwarf shrubs at medium and large distances than expected by the null model. We suggest two possible alternative explanations for this observation. First, the most of species of these plant types may appear at the edge of *L. spartum* individuals because competitive exclusion is less intense than within *L. spartum* clumps and stress is not as high as in open bare soil (Armas & Pugnaire 2011; Pescador *et al.* 2014). Second, it seems that the aggregated spatial pattern at large scales may reflect a heterogeneous spatial pattern in the environment (Wiegand & Moloney 2014). In other words, perennial grasses, annuals and dwarf shrubs may have similar environmental requirements heterogeneously distributed along transects.

Ehlers *et al.* (2014) provided evidence that allelopathic plants can have potential positive effects on plant diversity in natural ecosystems. However, the allelopathic species *A. herba-alba* had no positive net effects on diversity. It may be that in plant communities of arid and semi-arid environments, where facilitation is a dominant process (Soliveres & Maestre 2014), allelopathy may constitute an evolutionary mechanism to avoid becoming nurse species (van der Putten 2009), and thus, reduce the negative impact that beneficiaries may cause on nurses fitness because of resource competition (Holzapfel & Mahall 1999; Schöb *et al.* 2014). Nevertheless, we found higher diversity of perennial grasses than expected in the local neighborhood of *A. herba-alba*. Interestingly, facilitation of perennial grasses by *A. herba-alba* occurred at all study sites and levels of stress. Previous research indicated that some species that coexist with allelopathic species may exhibit a tolerance to its “chemical neighbor” (Grøndahl & Ehlers 2008) due to co-evolution. In agreement, *A. herba-alba* generally had higher similarity in its understory plant composition than the competitive (*L. spartum*) and the nurse (*S. vermiculata*) species. This means that abundance and identity of species associated with *A. herba-alba* were more similar among *A. herba-alba* than the abundance and identity of species associated with *L. spartum* and *S. vermiculata* among themselves. It is possible that, beyond diversity repulsion, the allelopathic activity of *A. herba-alba* acts as an environmental filter that reduces the species pool that can occur nearby, because only species adapted to its allelochemicals (*i.e.*

perennial grasses) can coexists with *A. herba-alba*. Thus, we could observe two plant strategies to coexist with *A. herba-alba* in the same community: tolerance to allelopathic compounds, as in the case of perennial grasses, and avoidance of direct coexistence under the *A. herba-alba* canopy, in the case of other plant types. These two markedly different strategies –tolerance and avoidance- have different underlying mechanisms, and there is a clear need to be further examined to better understand plant interactions and predict community dynamics during conservation and restoration practices.

Despite *A. herba-alba* and *L. spartum* were diversity repellers, all three target species had a positive net interaction outcome. These apparently contradictory results could be explained by one particularity in the method used to assess species association at the pairwise level (Saiz & Alados 2012). For those pair of species in which the abundance of one of the species was very low, we were not able to detect significant negative spatial associations. In particular, we were not able to detect a negative association when, in a transect, the number of expected co-occurrences, C_e, was lower than approximately C_e=2.9, because the minimum possible number of co-occurrences, C_r, for the pair of species (C_r=0, species do not co-occur in the field) is included within the 95% confidence interval (Saiz & Alados 2012), and a neutral association was assigned in those cases. Therefore, as we could only detect positive or neutral associations between target species and rare species, the net IO of target species could be biased towards positive values. This limitation could explain why *L. spartum* and *A. herba-alba*, which were expected to have negative IO values (*i.e.* a predominance of negative interactions), exhibited a positive net IO (*i.e.* a predominance of positive interactions). Nevertheless, these two species had smaller IO values than *S. vermiculata*, suggesting that, actually, *A. herba-alba* and *L. spartum* were less facilitative species.

In line with predictions of the *Stress-Gradient Hypothesis* (SGH), we found more positive IO values with increasing stress level (Bertness & Callaway 1994; He *et al.* 2013). Interestingly, significant differences in the IO occurred only between the reference site (LAUn) and the site with the greatest stress (MAG). This suggests that, although each stressor alone has little effect, the combined effects of multiple stressors drive the net interaction outcome (Le Bagousse-Pinguet *et al.* 2014). This reinforces the view that the severity of different environmental stressors (*e.g.* aridity in drylands) must be considered when interpreting the different effects that a species has on diversity (Mod *et al.* 2014).

In accordance with previous works that found amelioration of abiotic stress was more important than grazing protection (Gómez-Aparicio 2008; Howard, Eldridge & Soliveres 2012), our results indicated that aridity rather than livestock grazing was the main factor modulating the role of the target species on diversity. In our study, higher aridity caused both an increase in facilitation (from neutral to diversity accumulator) and a decrease in interference (from diversity repeller to neutral) in the local neighborhood of the nurse shrub *S. vermiculata* and the allelopathic dwarf shrub *A. herba-alba* respectively. As expected we found that allelopathy of *A. herba-alba* seems to counterbalance its potential facilitative effects on diversity (*i.e.* species accumulation) nearby its individuals (Jankju 2013) at most arid sites. Thus, when allelopathic species are involved, the balance between interference and facilitation may depend of the number of species able to tolerate allelopathic compounds, because negative effects on intolerant species can be counteracted by positive effects on tolerant species. On the other hand, aridity is an important driver of interactions when woody nurses are involved, but this does not hold for perennial grasses (Soliveres *et al.* 2014). Our finding that aridity did not modulate the negative effect of *L. spartum* on diversity agrees with this finding. Together, these results suggest species-specific traits may influence biotic interactions more than or as much as environmental stress (Maalouf *et al.* 2012; Mod *et al.* 2014; Soliveres *et al.* 2014).

There are contrasting results in the literature regarding the effect of grazing pressure. Some research indicates that greater grazing pressure leads to positive plant-plant interactions due to grazing protection (Graff, Aguiar & Chaneton 2007; Smit *et al.* 2007), especially when there are other stressor such as water scarcity (Anthelme & Michalet 2009; Soliveres *et al.* 2012). However, other research of areas with low productivity indicated that the effect of grazing driving positive interactions is less important than other factors such as environmental conditions (Smit, Rietkerk & Wassen 2009; Howard *et al.* 2012). Our results are more in line with these later studies, because we found that livestock grazing alone was not enough to change the role of the target species on diversity. We suggest two possible explanations for our results. First, livestock grazing pressure in the study area was at a sustainable level (Pueyo 2005) and may be too low to have any effects on biotic interactions and diversity. Second, our ungrazed study sites were not totally free of grazing by wild animals such as rabbits (*personal observation*), and this would reduce differences between ungrazed and grazed sites.

In conclusion, our results point out the major role that biotic interactions of dominant species have in shaping the structure of a plant community (le Roux *et al.* 2014). Specifically, given the strong implications of vegetation patches in the function of arid and semi-arid ecosystems (Aguiar & Sala 1999), our results highlight the importance of nurse shrubs as ecosystem engineers, as they created and maintained vegetation patches with high diversity in our study area. On the other hand, the allelopathic species had mainly a negative effect on diversity, contrary to the situation in mesic Mediterranean ecosystems. Interestingly, other species appeared to develop two different strategies to coexist with the allelopathic species *A. herba-alba*: tolerance, as exemplified by perennial grasses, and avoidance, as exemplified by the other plant types. Usually, studies involving allelopathic species mainly focus on assessing, in greenhouse experiments, the negative impact of isolated compounds or extracts of fresh material on seed germination and plant growth (Escudero *et al.* 2000; Gómez-Aparicio & Canham 2008; Tilaki *et al.* 2013). Such studies often employ model species that do not coexist with the allelopathic plant in nature (*e.g. Lactuca sativa,* lettuce; Escudero *et al.* 2000; Jankju 2013). Hence, the ecological consequences of allelopathic species structuring plant diversity in natural communities are poorly understood (Chou 1999). Our results provide valuable information about the role of an allelopathic dwarf shrub on plant diversity and species assemblage at local scale in natural ecosystems, although these conclusions should be taken with caution as they are based on a single allelopathic species. Further field experiments will be necessary to determine the relative importance of allelopathy and competition in the overall interference of allelopathic plants (Nilsson 1994; Inderjit & Callaway 2003). Also, further research should test the generality of these findings on allelopathic species of other semi-arid communities, and the variability in chemical composition among individuals and genotypes.

Acknowledgements

This work was funded by the Spanish Ministry of Education, Culture and Sport (FPU grant AP-2012-4126 to A.I.A), and by the project CGL-2012-37508 (Spanish Ministry of Science and Innovation). We especially acknowledge the invaluable help of many fieldworkers with data collection.

Supplementary material

Supplementary Figures S1, S2 and S3 may be found in the online version of this article.

References

Aguiar, M.R. & Sala, O.E. (1999) Patch structure, dynamics and implications for the functioning of arid ecosystems. *Trends in Ecology & Evolution*, **14**, 273–277.

Anthelme, F. & Michalet, R. (2009) Grass-to-tree facilitation in an arid grazed environment (Air Mountains, Sahara). *Basic and Applied Ecology*, **10**, 437–446.

Armas, C. & Pugnaire, F.I. (2005) Plant interactions govern population dynamics in a semi-arid plant community. *Journal of Ecology*, **93**, 978–989.

Armas, C. & Pugnaire, F.I. (2011) Belowground zone of influence in a tussock grass species. *Acta Oecologica*, **37**, 284–289.

Le Bagousse-Pinguet, Y., Xiao, S., Brooker, R.W., Gross, N., Liancourt, P., Straile, D. & Michalet, R. (2014) Facilitation displaces hotspots of diversity and allows communities to persist in heavily stressed and disturbed environments. *Journal of Vegetation Science*, **25**, 66–76.

Bertness, M.D. & Callaway, R. (1994) Positive interactions in communities. *Trends Ecol Evol*, **9**, 191–3.

Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.L., Cavieres, L.A., Kunstler, G., Liancourt, P., Tielbörger, K., Travis, J.M.J., Anthelme, F., Armas, C., Coll, L., Corcket, E., Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F., Quiroz, C.L., Saccone, P., Schiffers, K., Seifan, M., Touzard, B. & Michalet, R. (2008) Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology*, **96**, 18–34.

Bruno, J.F., Stachowicz, J.J. & Bertness, M.D. (2003) Inclusion of facilitation into ecological theory. *Trends in Ecology & Evolution*, **18**, 119–125.

Butterfield, B.J., Cavieres, L.A., Callaway, R.M., Cook, B.J., Kikvidze, Z., Lortie, C.J., Michalet, R., Pugnaire, F.I., Schöb, C., Xiao, S., Zaitchek, B., Anthelme, F., Björk, R.G., Dickinson, K., Gavilán, R., Kanka, R., Maalouf, J.-P., Noroozi, J., Parajuli, R., Phoenix, G.K., Reid, A., Ridenour, W., Rixen, C., Wipf, S., Zhao, L. & Brooker, R.W. (2013) Alpine cushion plants inhibit the loss of phylogenetic diversity in severe environments. *Ecology Letters*, **16**, 478–486.

Callaway, R.M. (2007) *Positive Interactions and Interdependence in Plant Communities*. Springer Netherlands, Dordrecht.

Callaway, R.M., Nadkarni, N.M. & Mahall, B.E. (1991) Facilitation and Interference of Quercus Douglasii on Understory Productivity in Central California. *Ecology*, **72**, 1484–1499.

Callaway, R.M. & Ridenour, W.M. (2004) Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment*, **2**, 436–443.

Cavieres, L.A., Brooker, R.W., Butterfield, B.J., Cook, B.J., Kikvidze, Z., Lortie, C.J., Michalet, R., Pugnaire, F.I., Schöb, C., Xiao, S., Anthelme, F., Björk, R.G., Dickinson, K.J.M., Cranston, B.H., Gavilán, R., Gutiérrez-Girón, A., Kanka, R., Maalouf, J.-P., Mark, A.F., Noroozi, J., Parajuli, R., Phoenix, G.K., Reid, A.M., Ridenour, W.M., Rixen, C., Wipf, S., Zhao, L., Escudero, A., Zaitchik, B.F., Lingua, E., Aschehoug, E.T. & Callaway, R.M. (2014) Facilitative plant interactions and climate simultaneously drive alpine plant diversity. *Ecology Letters*, **17**, 193–202.

Chao, A., Chazdon, R.L., Colwell, R.K. & Shen, T.-J. (2005) A new statistical approach for assessing similarity of species composition with incidence and abundance data. *Ecology Letters*, **8**, 148–159.

Chou, C.-H. (1999) Roles of Allelopathy in Plant Biodiversity and Sustainable Agriculture. *Critical Reviews in Plant Sciences*, **18**, 609–636.

Chou, C.H. & Waller, G.R. (1983) *Allechemicals and Pheromones*. Institute of Botany, Academia Sinica Monograph Series No. 5, Taipei, Taiwan.

Ehlers, B.K., Charpentier, A. & Grøndahl, E. (2014) An allelopathic plant facilitates species richness in the Mediterranean garrigue. *Journal of Ecology*, **102**, 176–185.

Escudero, A., Albert, M.J., Pita, J.M. & Pérez-García, F. (2000) Inhibitory effects of Artemisia herba-alba on the germination of the gypsophyte Helianthemum squamatum. *Plant Ecology*, **148**, 71–80.

Friedman, J., Orshan, G. & Ziger-Cfir, Y. (1977) Suppression of Annuals by Artemisia Herba-Alba in the Negev Desert of Israel. *Journal of Ecology*, **65**, 413–426.

Gómez-Aparicio, L. (2008) Spatial patterns of recruitment in Mediterranean plant species: linking the fate of seeds, seedlings and saplings in heterogeneous landscapes at different scales. *Journal of Ecology*, **96**, 1128–1140.

Gómez-Aparicio, L. (2009) The role of plant interactions in the restoration of degraded ecosystems: a meta-analysis across life-forms and ecosystems. *Journal of Ecology*, **97**, 1202–1214.

Gómez-Aparicio, L. & Canham, C.D. (2008) Neighbourhood analyses of the allelopathic effects of the invasive tree Ailanthus altissima in temperate forests. *Journal of Ecology*, **96**, 447–458.

Goodall, D.W. (1952) Some considerations in the use of poit quadrats for the analysis of vegetation. *Australian Journal of Scientific Research Series B-Biological Sciences*, **5**, 1–41.

Graff, P., Aguiar, M.R. & Chaneton, E.J. (2007) Shifts in Positive and Negative Plant Interactions along a Grazing Intensity Gradient. *Ecology*, **88**, 188–199.

Grime, J.P. (1973) Competitive Exclusion in Herbaceous Vegetation. *Nature*, **242**, 344–347.

Grøndahl, E. & Ehlers, B.K. (2008) Local adaptation to biotic factors: reciprocal transplants of four species associated with aromatic Thymus pulegioides and T. serpyllum. *Journal of Ecology*, **96**, 981–992.

Gross, N., Börger, L., Soriano-Morales, S.I., Le Bagousse-Pinguet, Y., Quero, J.L., García-Gómez, M., Valencia-Gómez, E. & Maestre, F.T. (2013) Uncovering multiscale effects of aridity and biotic interactions on the functional structure of Mediterranean shrublands. *Journal of Ecology*, **101**, 637–649.

Hacker, S.D. & Gaines, S.D. (1997) Some implications of direct positive interactions for community species diversity. *Ecology*, **78**, 1990–2003.

He, Q., Bertness, M.D. & Altieri, A.H. (2013) Global shifts towards positive species interactions with increasing environmental stress. *Ecol Lett*, **16**, 695–706.

Holmgren, M., Scheffer, M. & Huston, M.A. (1997) The interplay of facilitation and competition in plant communities. *Ecology*, **78**, 1966–1975.

Holzapfel, C. & Mahall, B.E. (1999) Bidirectional Facilitation and Interference between Shrubs and Annuals in the Mojave Desert. *Ecology*, **80**, 1747–1761.

Holzapfel, C., Tielbörger, K., Parag, H.A., Kigel, J. & Sternberg, M. (2006) Annual plant–shrub interactions along an aridity gradient. *Basic and Applied Ecology*, **7**, 268–279.

Howard, K.S.C., Eldridge, D.J. & Soliveres, S. (2012) Positive effects of shrubs on plant species diversity do not change along a gradient in grazing pressure in an arid shrubland. *Basic and Applied Ecology*, **13**, 159–168.

Huston, M. (1979) A General Hypothesis of Species Diversity. *The American Naturalist*, **113**, 81–101.

Inderjit & Callaway, R.M. (2003) Experimental designs for the study of allelopathy. *Plant and Soil*, **256**, 1–11.

Jackson, R.B., Canadell, J., Ehleringer, J.R., Mooney, H.A., Sala, O.E. & Schulze, E.D. (1996) A global analysis of root distributions for terrestrial biomes. *Oecologia*, **108**, 389–411.

Jankju, M. (2013) Role of nurse shrubs in restoration of an arid rangeland: Effects of microclimate on grass establishment. *Journal of Arid Environments*, **89**, 103–109.

Jurena, P.N. & Archer, S. (2003) Woody plant establishment and spatial heterogeneity in grasslands. *Ecology*, **84**, 907–919.

López, R.P., Larrea-Alcázar, D.M. & Teresa, O. (2009) Positive effects of shrubs on herbaceous species richness across several spatial scales: evidence from the semiarid Andean subtropics. *Journal of Vegetation Science*, **20**, 728–734.

Maalouf, J.-P., Bagousse-Pinguet, Y.L., Marchand, L., Touzard, B. & Michalet, R. (2012) The interplay of stress and mowing disturbance for the intensity and importance of plant interactions in dry calcareous grasslands. *Annals of Botany*, **110**, 821–828.

Maestre, F.T., Valladares, F. & Reynolds, J.F. (2005) Is the change of plant-plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. *Journal of Ecology*, **93**, 748–757.

McIntire, E.J.B. & Fajardo, A. (2014) Facilitation as a ubiquitous driver of biodiversity. *New Phytologist*, **201**, 403–416.

Michalet, R., Le Bagousse-Pinguet, Y., Maalouf, J.-P. & Lortie, C.J. (2014) Two alternatives to the stress-gradient hypothesis at the edge of life: the collapse of facilitation and the switch from facilitation to competition. *Journal of Vegetation Science*, **25**, 609–613.

Michalet, R., Brooker, R.W., Cavieres, L.A., Kikvidze, Z., Lortie, C.J., Pugnaire, F.I., Valiente-Banuet, A. & Callaway, R.M. (2006) Do biotic interactions shape both sides of the humped-back model of species richness in plant communities? *Ecology Letters*, **9**, 767–773.

Miriti, M.N. (2006) Ontogenetic shift from facilitation to competition in a desert shrub. *Journal of Ecology*, **94**, 973–979.

Mod, H.K., le Roux, P.C. & Luoto, M. (2014) Outcomes of biotic interactions are dependent on multiple environmental variables. *Journal of Vegetation Science*, **25**, 1024–1032.

Mohamed, A., El-Sayed, M.A., Hegazy, M.E., Helaly, S.E., Esmail, A.M. & Mohamed, N.S. (2010) Chemical Constituents and Biological Activities of Artemisia herba-alba. *Records of Natural Products*, **4**, 1–25.

Muller, C.H. (1969) Allelopathy as a factor in ecological process. *Vegetatio*, **18**, 348–357.

Nilsson, M.-C. (1994) Separation of allelopathy and resource competition by the boreal dwarf shrub Empetrum hermaphroditum Hagerup. *Oecologia*, **98**, 1–7.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O´Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H. & Wagner, H. (2013) vegan: Community Ecology Package. *R package version 2.0-10*.

Olff, H. & Ritchie, M.E. (1998) Effects of herbivores on grassland plant diversity. *Trends in Ecology & Evolution*, **13**, 261–265.

Parsons, A.J. & Abrahams, A.D. (eds). (2009) *Geomorphology of Desert Environments*. Springer Netherlands, Dordrecht.

Pedrol, N., González, L. & Reigosa, M.J. (2006) Allelopathy and abiotic stress. *Allelopathy* (eds M.J. Reigosa, N. Pedrol & L. González), pp. 171–209. Springer Netherlands.

Pescador, D.S., Chacon-Labella, J., de la Cruz, M. & Escudero, A. (2014) Maintaining distances with the engineer: patterns of coexistence in plant communities beyond the patch-bare dichotomy. *New Phytologist*, **204**, 140–148.

Pueyo, Y. (2005) *Evaluación de Los Factores Ambientales Y Del Uso Antrópico Como Condicionantes de La Conservación de La Vegetación Del Sector Central de La Depresión Del Ebro*. PhD Thesis, Departamento de Agricultura y Economía Agraria, Universidad de Zaragoza, Zaragoza.

Pueyo, Y., Moret-Fernández, D., Saiz, H., Bueno, C.G. & Alados, C.L. (2013) Relationships Between Plant Spatial Patterns, Water Infiltration Capacity, and Plant Community Composition in Semi-arid Mediterranean Ecosystems Along Stress Gradients. *Ecosystems*, **16**, 452–466.

Pugnaire, F. (ed). (2010) *Positive Plant Interactions and Community Dynamics*. CRC Press.

Puigdefábregas, J. & Mendizabal, T. (1998) Perspectives on desertification: Western Mediterranean. *Journal of Arid Environments*, **39**, 209–224.

Van der Putten, W.H. (2009) A multitrophic perspective on functioning and evolution of facilitation in plant communities. *Journal of Ecology*, **97**, 1131–1138.

Rayburn, A.P. & Wiegand, T. (2012) Individual species-area relationships and spatial patterns of species diversity in a Great Basin, semi-arid shrubland. *Ecography*, **35**, 341–347.

R Core Team. (2013) R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing, Vienna, Austria,*, **URL http://www.R-project.org/.**

Reigosa, M.J., Sánchez-Moreiras, A. & González, L. (1999) Ecophysiological Approach in Allelopathy. *Critical Reviews in Plant Sciences*, **18**, 577–608.

Rice, E.L. (1984) *Allelopathy*. Academic Press.

Le Roux, P.C., Pellissier, L., Wisz, M.S. & Luoto, M. (2014) Incorporating dominant species as proxies for biotic interactions strengthens plant community models. *Journal of Ecology*, **102**, 767–775.

Saiz, H. & Alados, C.L. (2012) Changes in Semi-Arid Plant Species Associations along a Livestock Grazing Gradient. *PLoS ONE*, **7**, e40551.

Schöb, C., Butterfield, B.J. & Pugnaire, F.I. (2012) Foundation species influence trait-based community assembly. *New Phytologist*, **196**, 824–834.

Schöb, C., Prieto, I., Armas, C. & Pugnaire, F.I. (2014) Consequences of facilitation: one plant's benefit is another plant's cost. *Functional Ecology*, **28**, 500–508.

Smit, C., Rietkerk, M. & Wassen, M.J. (2009) Inclusion of biotic stress (consumer pressure) alters predictions from the stress gradient hypothesis. *Journal of Ecology*, **97**, 1215–1219.

Smit, C., Vandenberghe, C., Ouden, J. den & Müller-Schärer, H. (2007) Nurse plants, tree saplings and grazing pressure: changes in facilitation along a biotic environmental gradient. *Oecologia*, **152**, 265–273.

Soliveres, S., Eldridge, D.J., Hemmings, F. & Maestre, F.T. (2012) Nurse plant effects on plant species richness in drylands: The role of grazing, rainfall and species specificity. *Perspectives in Plant Ecology Evolution and Systematics*, **14**, 402–410.

Soliveres, S. & Maestre, F.T. (2014) Plant–plant interactions, environmental gradients and plant diversity: A global synthesis of community-level studies. *Perspectives in Plant Ecology, Evolution and Systematics*, **16**, 154–163.

Soliveres, S., Maestre, F.T., Bowker, M.A., Torices, R., Quero, J.L., García-Gómez, M., Cabrera, O., Cea, A.P., Coaguila, D., Eldridge, D.J., Espinosa, C.I., Hemmings, F., Monerris, J.J., Tighe, M., Delgado-Baquerizo, M., Escolar, C., García-Palacios, P., Gozalo, B., Ochoa, V., Blones, J., Derak, M., Ghiloufi, W., Gutiérrez, J.R., Hernández, R.M. & Noumi, Z. (2014) Functional traits determine plant co-occurrence more than environment or evolutionary relatedness in global drylands. *Perspectives in Plant Ecology, Evolution and Systematics*, **16**, 164–173.

Tang, C.S., Cai, W.F., Kohl, K. & Nishimoto, R.K. (1995) Plant stress and allelopathy. *Allelopathy: Organisms, Processes, and Applications*, Acs Symposium Series (eds A. Inderjit, K.M.M. Dakshini & F.A. Einhellig), pp. 142–157. Amer Chemical Soc, Washington.

Thompson, J.D. (2005) *Plant Evolution in the Mediterranean*. Oxford University Press, Oxford [etc.].

Thorpe, A.S., Thelen, G.C., Diaconu, A. & Callaway, R.M. (2009) Root exudate is allelopathic in invaded community but not in native community: field evidence for the novel weapons hypothesis. *Journal of Ecology*, **97**, 641–645.

Tielbörger, K. & Kadmon, R. (2000) Temporal environmental variation tips the balance between facilitation and interference in desert plants. *Ecology*, **81**, 1544–1553.

Tilaki, G.A.D., Gholami, F., Behtari, B. & Bezdi, K.G. (2013) Chemical composition and allelopathic effect of the essential oil of Artemisia herba-alba Asso. on seed germination and early seedling growth of legumes and grasses species. *Legume Research*, **36**, 33–40.

Valiente-Banuet, A. & Verdú, M. (2007) Facilitation can increase the phylogenetic diversity of plant communities. *Ecology Letters*, **10**, 1029–1036.

Verwijmeren, M., Rietkerk, M., Bautista, S., Mayor, A.G., Wassen, M.J. & Smit, C. (2014) Drought and grazing combined: Contrasting shifts in plant interactions at species pair and community level. *Journal of Arid Environments*, **111**, 53–60.

Vivanco, J.M., Bais, H.P., Stermitz, F.R., Thelen, G.C. & Callaway, R.M. (2004) Biogeographical variation in community response to root allelochemistry: novel weapons and exotic invasion. *Ecology Letters*, **7**, 285–292.

Wiegand, T., Gunatilleke, C.V., Gunatilleke, I.A. & Huth, A. (2007) How individual species structure diversity in tropical forests. *Proc Natl Acad Sci U S A*, **104**, 19029–33.

Wiegand, T. & Moloney, K.A. (2004) Rings, circles, and null-models for point pattern analysis in ecology. *Oikos*, **104**, 209–229.

Wiegand, T. & Moloney, K.A. (2014) *Handbook of Spatial Point-Pattern Analysis in Ecology*. CRC Press.

Wilby, A. & Shachak, M. (2004) Shrubs, granivores and annual plant community stability in an arid ecosystem. *Oikos*, **106**, 209–216.

Appendix 1

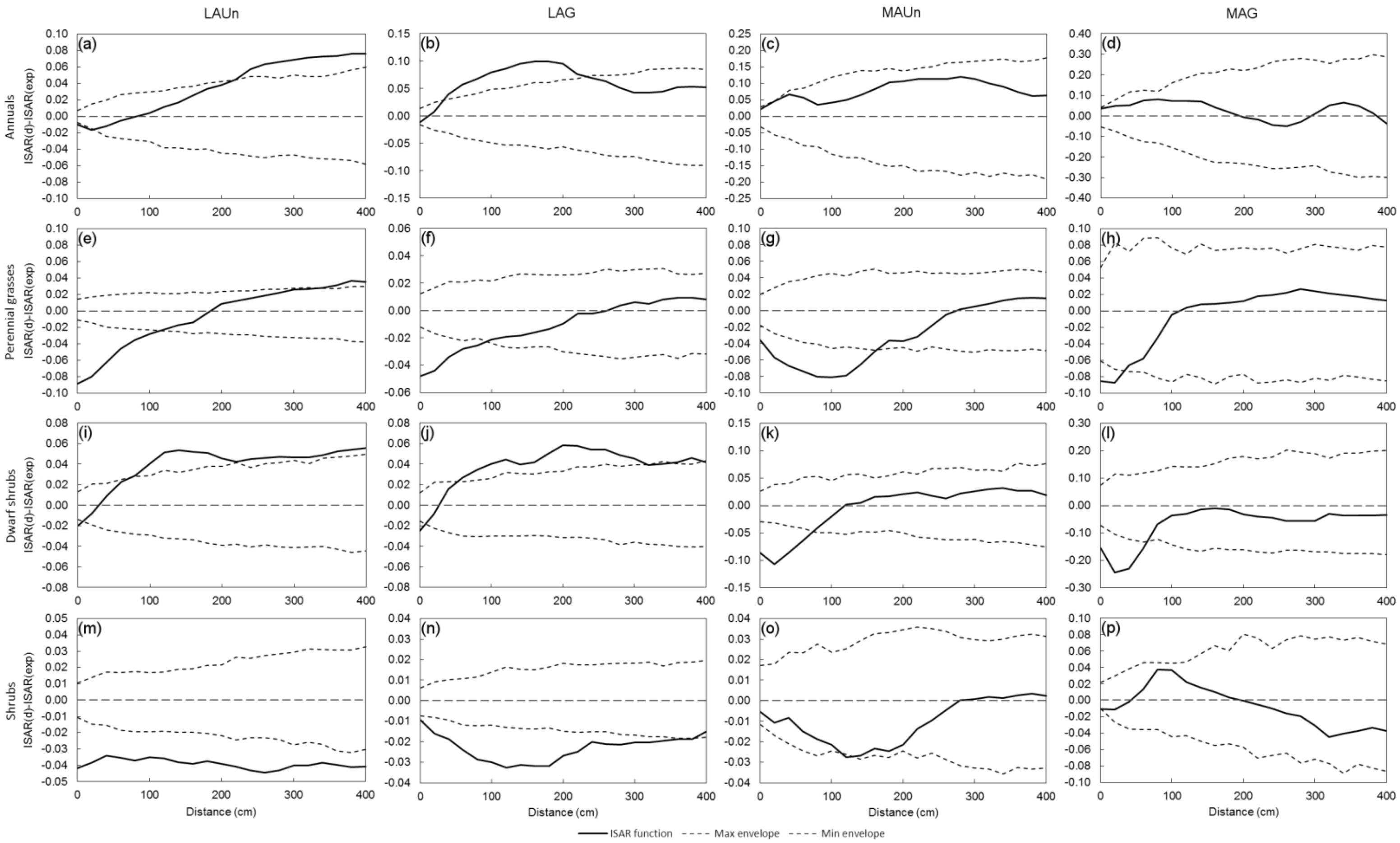


Fig. S1. Individual species-area relationship (ISAR) curves of *L. spartum* according to plant type at each study site. Here and below: these curves can be interpreted as described in the legend of Fig. 3; Table 2 provides summaries of all curves.

Appendix 2

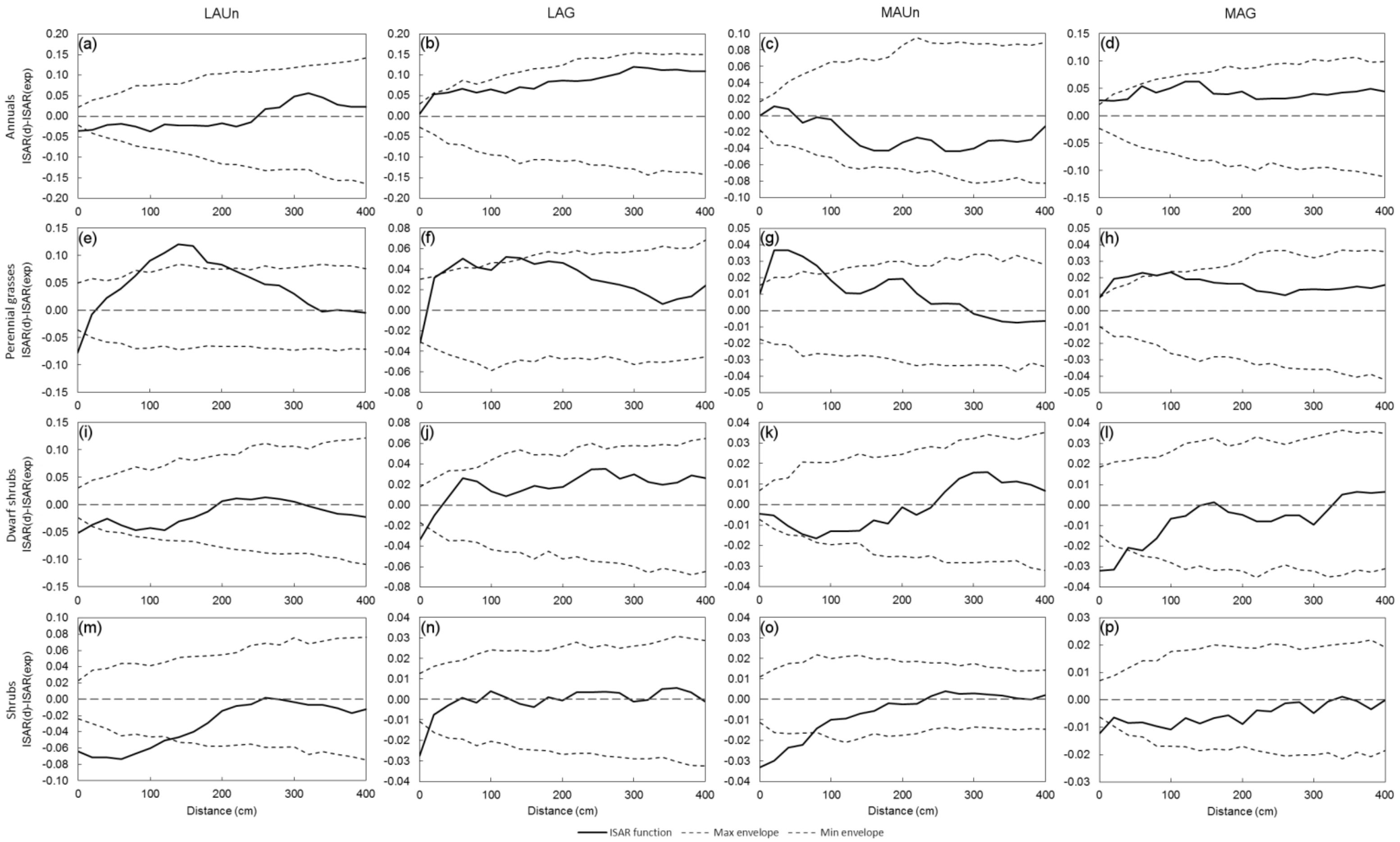


Fig. S2. Individual species-area relationship (ISAR) curves of *A. herba-alba* according to plant type at each study site.

Appendix 3

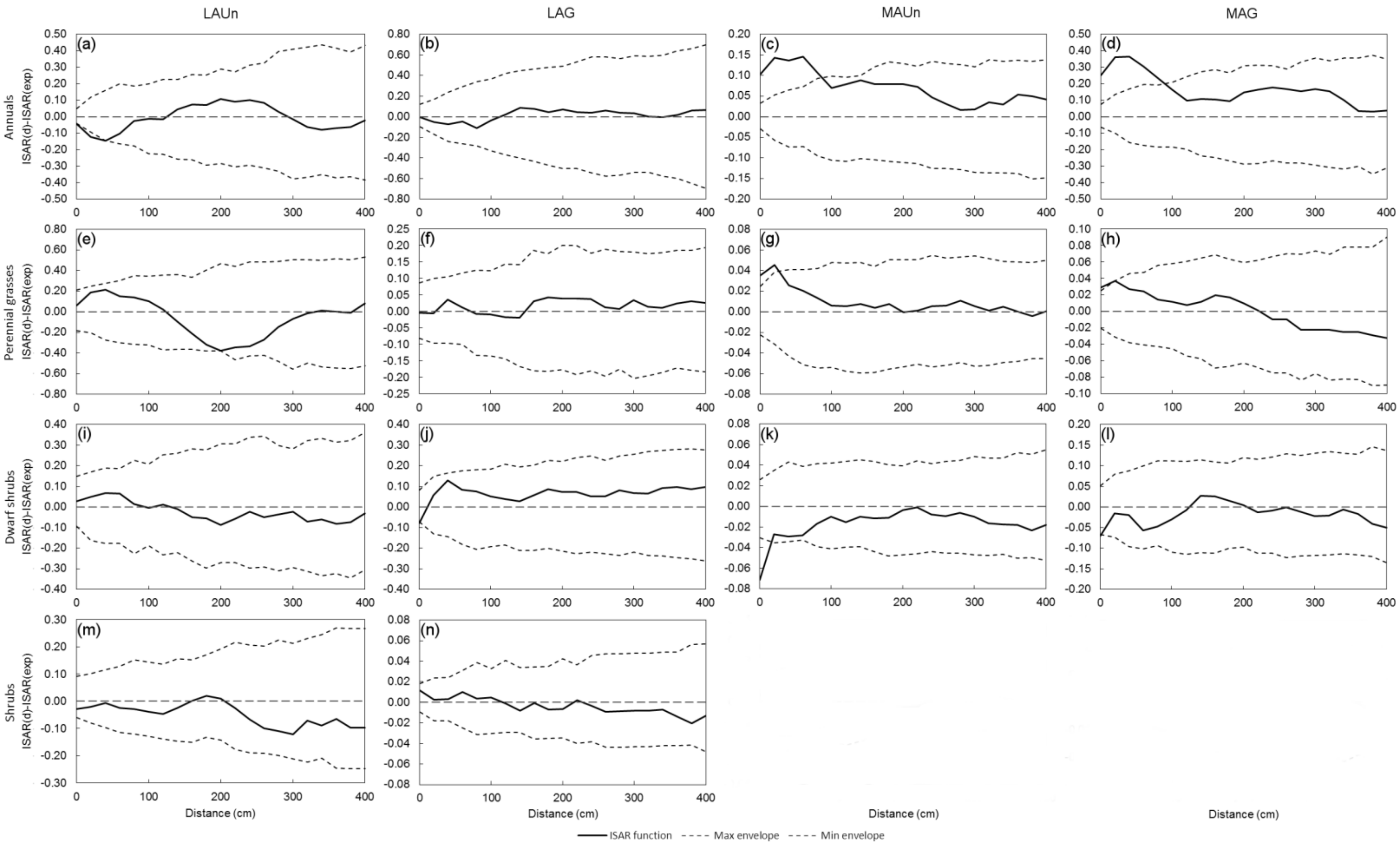


Fig. S3. Individual species-area relationship (ISAR) curves of *S. vermiculata* according to plant type at each study site.

Effects of the allelopathic plant *Artemisia herba-alba* Asso on the soil seed bank of a semi-arid plant community

Antonio I. Arroyo, Yolanda Pueyo, Ramón Reiné, Mª Luz Giner,
Concepción L. Alados

Journal of Plant Ecology (2016), DOI: 10.1093/jpe/rtw120

Effects of the allelopathic plant Artemisia herba-alba Asso on the soil seed bank of a semi-arid plant community

Antonio I. Arroyo[1], Yolanda Pueyo[1], Ramón Reiné[2], Mª Luz Giner[1], Concepción L. Alados[1]

[1] Instituto Pirenaico de Ecología, Consejo Superior de Investigaciones Científicas, Av. Montañana 1005, 50059 Zaragoza, Spain.

[2] Escuela Politécnica Superior, Universidad de Zaragoza, Ctra. Cuarte s/n. 22071 Huesca, Spain.

Abstract

Aims: Seed bank strongly influences composition and structure of aboveground vegetation. Little attention has been paid to the role of allelopathy driving soil seed bank dynamics, even though allelochemicals released by allelopathic plants might determine whether a species can or cannot germinate from the soil seed bank and, therefore, to co-exist in the neighborhood of the allelopathic species. Hence, the effects of allelopathic plants on community organization through the effects of allelochemicals on soil seed bank remain largely unknown. In this study, we assessed spatial heterogeneities in soil seed bank caused by the presence of allelopathic plants, and evaluated allelopathic effects on seed bank germination and seedling mortality.

Methods: We examined the effects of the allelopathic shrub *Artemisia herba-alba* Asso on the spatial structure (in terms of species richness and seed density) of the soil seed bank of a semi-arid ecosystem in NE Spain. Specifically, we evaluated seed bank richness and density at three microsites: under the canopy of *A. herba-alba* individuals, under the canopy of *Salsola vermiculata* L. (a non-allelopathic shrub) individuals, and in bare soil. In addition, we assessed the effects of aqueous extract of *A. herba-alba* on soil seed bank germination (seedling emergence and emergence timing) and seedling mortality.

Important findings: We found that seed bank richness and density were higher under shrub canopy than they were in bare soil. *Artemisia herba-alba* and *S. vermiculata* microsites had similar seed bank richness. However, seed bank density was higher under *A. herba-alba* because of the high abundance of *A. herba-alba* seeds. Aqueous extract reduced seedling emergence from the seed bank by 50%, and affected mortality of some emerged seedlings. On the other hand, aqueous extract did not influence the emergence timing of seedlings. We conclude that, although the presence of allelopathic plants does not cause relevant changes in seed bank structure relative to similar non-allelopathic shrubs, their presence can actually result in a reduction of seedling emergence from the seed bank, which leads to low plant species richness and density nearby. To our knowledge, this is the first study to examine the effect of an allelopathic plant on the structure and germination in an entire soil seed bank.

Keywords

Allelopathic shrub, aqueous extract, Middle Ebro Valley, seed bank inhibition, seed bank density, seedling emergence.

Introduction

Allelopathy is defined as the influence that a plant exerts over other plants through production and release into the environment of secondary metabolites known as allelochemicals (Rice 1984; Mallik 2008). It is a well-studied process in the field of agriculture (Narwal *et al.* 2000; Cheng and Cheng 2015) as a natural alternative to herbicides and pesticides in the control of weeds and insect pests (Cheng and Cheng 2015). In addition, allelopathic mechanisms are proposed often to explain the success of non-native plant species in invaded communities (Callaway and Ridenour 2004; Thorpe *et al.* 2009; Yuan *et al.* 2013). However, much less attention has been paid to the role of allelopathy in natural ecosystems (Inderjit *et al.* 2011; Meiners *et al.* 2012), despite its potential impacts in ecosystem functioning (Muller 1969; Wardle *et al.* 1998; Inderjit *et al.* 2011). In Mediterranean-like regions, many herbaceous, aromatic shrubs and trees species exhibit allelopathy (Thompson 2005; Scognamiglio *et al.* 2013). Apart from being a widespread feature of plants in those habitats, production of allelochemicals or its phytotoxicity are enhanced under stressful conditions (Pedrol *et al.* 2006; Rivoal *et al.* 2011; Zhang *et al.* 2014). Furthermore, in those stressful environments, where facilitative interactions predominate (Soliveres and Maestre 2014), allelopathy might arise as an evolutionary mechanism for avoiding such interactions (van der Putten 2009) and, therefore, lessen direct competition for resources with surrounding vegetation (Gant and Clebsch 1975). Thus, allelopathy might be particularly relevant driving the dynamics of arid and semi-arid plant communities.

The highly unpredictable climate of drylands makes soil seed bank a crucial factor that strongly influences the persistence and co-existence of some species in the community (Kemp 1989; Chesson *et al.* 2004; Long *et al.* 2015) and, ultimately, the composition of aboveground vegetation (Caballero *et al.* 2005, 2008b; Martinez-Duro *et al.* 2012). In those environments, vegetation cover is clumped into patches that alternate with inter-patches areas of bare soil. Patches of vegetation act as seed sources and sinks (Caballero *et al.* 2008a). Consequently, the soil seed bank follows a similar patchy pattern, and the number of species and density of seeds under vegetation patches is higher than it is in bare soil (Aguiar and Sala 1997; Guo *et al.* 1998; Caballero *et al.* 2008a; Stark *et al.* 2008; Giladi *et* *al.* 2013). Thus, it is expected that seedling emergence occurs mostly in vegetation patches (Aguiar and Sala 1997). But the chemical microenvironment constitutes a determinant factor that promotes or inhibits seed germination (Long *et al.* 2015). The presence of allelochemicals can determine whether a species can (tolerant) or cannot (intolerant) co-exist in the neighborhood of the allelopathic species (Ehlers *et al.* 2014; Linhart *et al.* 2015) which affects the spatial pattern of diversity and species composition (Arroyo *et al.* 2015). However, the consequences that the presence of allelopathic plants have on community organization through the effects of allelochemicals on soil seed bank remain poorly understood (Fabbro *et al.* 2013; Renne *et al.* 2014).

Assessing the allelopathic activity of allelochemicals often relies on identifying their influence on seed germination and seedling performance (Gniazdowska and Bogatek 2005; Scognamiglio *et al.* 2013). It is well known that allelochemicals typically inhibit seed germination (Escudero *et al.* 2000; Vivanco *et al.* 2004; Fernandez *et al.* 2013), delay germination (Fernandez *et al.* 2013) and increase seedling mortality (Linhart *et al.* 2015). Mechanisms underlying those effects are multiple and they are associated with a disruption of normal cell metabolism, rather than with a cell damage (Gniazdowska and Bogatek 2005). Positive effects of allelochemicals have been reported too (Tsubo *et al.* 2012; Fernandez *et al.* 2013), although they are less common. Many of those studies on allelopathy, however, have been based on greenhouse experiments that included one or two test species only (Escudero *et al.* 2000; Fernandez *et al.* 2006), which in some cases, were model species (*e.g. Lactuca sativa* L., lettuce) (Escudero *et al.* 2000; Scognamiglio *et al.* 2013; Zhang *et al.* 2014). Model species are not useful for demonstrating the significance of allelopathy in natural communities because they do not co-exists with the allelopathic species (Inderjit and Callaway 2003). Furthermore, the use of a single test species does not allow for the assessment of species-specific responses to allelopathy (Zhang *et al.* 2014; Linhart *et al.* 2015). Soil seed bank might constitute a valuable alternative to test for the effects of allelopathy on several species that co-exists with the allelopathic one in natural plant communities and, thus, provide meaningful information about the role of allelopathy in seed germination in natural environments.

In this study we investigated the effects of the allelopathic species *Artemisia herba-alba* Asso on the dynamics of a semi-arid plant community through the effects on soil seed bank. Specifically, we assessed (i) spatial heterogeneities in soil seed bank caused by the presence of allelopathic plants and (ii) the allelopathic effects on seed bank germination and seedling mortality. *Artemisia herba-alba* (desert wormwood) is an aromatic dwarf shrub that is very common in many arid and semi-arid regions of the Mediterranean basin. Like many other species in the genus *Artemisia* (Asteraceae), its allelopathic nature is well documented (Escudero *et al.* 2000; Mohamed *et al.* 2010; Abad *et al.* 2012; Tilaoui *et al.* 2015). We hypothesized that (1) vegetation patches (of either allelopathic or non-allelopathic plants) will act as sources and sinks of seeds, which increases species richness and density of seeds in the seed bank compared to bare soil areas. In addition, we expected to find differences between allelopathic and non-allelopathic plant species in seed bank structure. Although patches created by *A. herba-alba* might act as seed sinks, they would be seed sources to a lesser extent than would be patches created by a similar non-allelopathic shrub (*Salsola vermiculata* L.; Arroyo *et al.* unpublished data), because of the low plant diversity underneath (Arroyo *et al.* 2015). Therefore, we hypothesized that (2) species richness and density of the soil seed bank will be lower under *A. herba-alba* than it will be under *S. vermiculata*. Alternatively, dispersal and seed trapping might allow *A. herba-alba* to have a similar seed bank to that of *S. vermiculata*, but still, we hypothesized that (3) the germination of many seed in the seed bank will be inhibited or delayed, and seedling mortality will increase because of the allelochemicals produced by *A. herba-alba*, leading to the observed low plant diversity underneath its canopy (Arroyo *et al.* 2015). To test the first hypothesis we compared the structure (in terms of species richness and density of seeds) of viable seed bank of soil samples collected from bare soil areas and vegetation patches. To test the second hypothesis we compared the structure of viable seed bank of samples collected from under *A. herba-alba* and *S. vermiculata* individuals. To test the third hypothesis, we measured seed bank germination (seedling emergence and emergence timing) and seedling mortality of samples that were treated with aqueous extract of *A. herba-alba,* which simulated the presence of allelopathic plants.

Material and methods

Study area

This study was conducted in the middle Ebro Valley (NE Spain), specifically, in "El Planerón de Belchite" ornithological reserve (41º22´28´´N, 00º38´24´´ W; Fig. 1A). This is one of the most arid areas of the Iberian Peninsula, with an average annual precipitation of 319 mm year^{-1}. The average temperature ranges from 7.21 ºC in January to 25.04 ºC in July (data obtained from Digital Climatic Atlas of Aragón; http://anciles.aragon.es/AtlasClimatico/). Topography is characterized by a flat-bottomed valley surrounded by low hills. Soils are very rich in clay and are slightly saline. The main human activity in the study area involved a traditional agro-pastoral land use (Pueyo 2005). Natural plant communities on non-cultivated lands are dominated by shrubs (*e.g. A. herba-alba*, *S. vermiculata* and *Suaeda vera* J.F.G.mel.) and perennial grasses (*e.g. Dactylis glomerata* L. subsp. *hispanica* (Roth) Nyman, *Lygeum spartum* L. and *Stipa parviflora* Desf.). Soil is sparsely covered by lichens (*e.g. Diploschistes diacapsis*, *Squamarina lentigera* and *Fulgensia* sp.).

Seed bank sampling and experimental design

In January 2014, 160 soil samples were collected from three microsites. Specifically, 20 *A. herba*-alba individuals and 20 *S. vermiculata* individuals were selected randomly (Fig. 1A). Then, two soil samples were collected from under the canopy of each individual, in the direction of the main winds (NW) (n=80; microsites ART and SAL for *A. herba-alba* and *S. vermiculata* respectively; Fig. 1B). Other two soil samples were collected in open bare soil, 30 cm from the canopy edge of each individual (n=80; microsite BS; Fig. 1B). Those three microsites were the most abundant in the natural plant communities of the study area, and accounted for about 60% of the cover. Selected individuals of A. *herba-alba* and *S. vermiculata* had a very similar physiognomy. Indeed, the two species did not differ in their mean height ($F_{1,38} = 3.08$, $p = 0.09$; Fig. S1), although *S. vermiculata* had a longer radius ($F_{1,38} = 7.94$, $p < 0.01$; Fig. S1). Soil cores were 3.5 cm in diameter and 10 cm deep, which is sufficient for sampling the entire seed bank in drylands (Guo *et al.* 1998). Soil samples were kept in hermetic plastic bags and stored in a cold chamber at 4 ºC for three months.

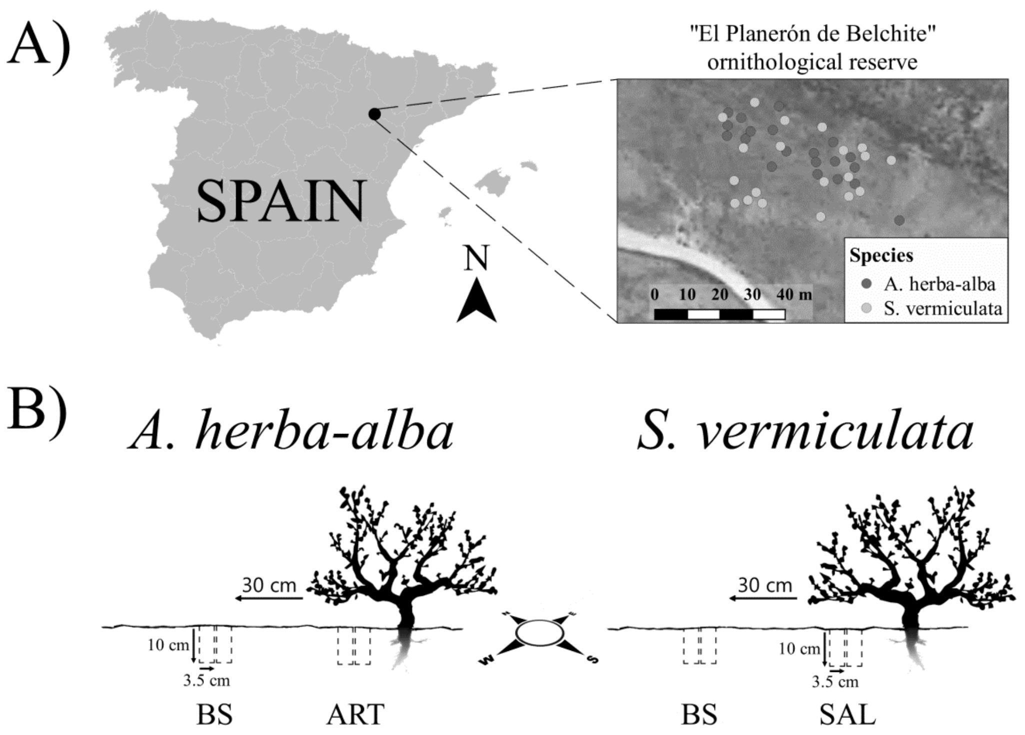


Fig. 1 A) Location of the study area and selected individuals. B) Schematic of the seed bank sampling design.

We followed the seedling emergence method (Heerdt *et al.* 1996) to assess the viable soil seed bank. First, soil samples were soaked about ten minutes in a sodium bicarbonate ($NaHCO_3$) solution to cause disaggregation of clays. Then, soil samples were washed over a 4 mm mesh sieve to remove the coarse fraction of the soil and washed again over a 0.25 mm mesh sieve to obtain concentrated samples rich in seeds. Final samples were placed in 23x9x7 cm aluminum pots filled up with substrate (a mixture of white peat, 70%, and soil of pine forest, 30%), and were set to germinate in a greenhouse for four months, under a controlled temperature regime (25ºC at day and 15ºC at night). A 0.25 mm nylon mesh was placed between samples and substrate to prevent germination from the substrate. Pots were irrigated with fresh water regularly. To induce germination of dormant seeds, 1 g l^{-1} gibberellic acid (AC_3) was added to irrigation water during the final month (Ikuma and Thimann 1960). Seedling emergence was monitored during 30 days after the end of the experiment, but further germination did not occur. Pot positions were changed randomly to prevent any potential site effect.

To simulate seed bank germination in the presence of an allelopathic plant, one of the two samples collected from each individual of two species, and one of the two samples collected from each bare soil (treatment block; n=80), were irrigated with a 5 g l^{-1} dilution of aqueous extract of *A. herba-alba*, which replaced a normal watering once every two weeks. The concentration was equivalent to add approximately 0.6 g of *A. herba-alba* per pot, which can be considered similar to the amounts found in nature (Orr *et al.* 2005). Aqueous extract was obtained by soaking aerial parts (fresh shoots and leaves) of different *A. herba-alba* individuals in distilled water (100 g l^{-1}), for 24 h at room temperature and in total darkness (Escudero *et al.* 2000; Orr *et al.* 2005; Fernandez *et al.* 2013). Fresh material was collected in spring 2014 from a natural population of the study area. The resultant extract was filtered, stored in small bottles (100 ml) and frozen until it was used. Allelopathic activity of aqueous extract of *S. vermiculata* was discarded in a preliminary experiment (Arroyo *et al.* unpublished data).

Seedling emergence was tracked weekly. In particular, species richness and seed density were quantified as the number of species and the number of seedlings, respectively, that emerged from each sample. Emergence timing was quantified as the difference, in days, between the time at which the sample was set to germinate and the time at which the seedling was first observed. Seedling mortality was quantified as the ratio between the number of dead seedlings and the number that emerged from each sample.

Data analyses

Differences in seed bank richness and density among microsites (BS, ART and SAL) were analyzed with generalized linear models (GLMs). We employed Poisson distribution of errors because our count data did not meet the assumptions of normality, even after various data transformations. In the analysis of seed density, a negative binomial GLM was performed to deal with data overdispersion. Tukey´s *post hoc* honest significant difference (HSD) tests were used to detect differences between pairs of microsites. Assessments of seed bank structure were based on the pots that were not treated with aqueous extract.

The effect of aqueous extract of *A. herba-alba* on seedling emergence was analyzed with generalized linear mixed models (GLMMs). Specifically, we assessed the significance of differences in species richness and seedling abundance between treated and non-treated pots with separate GLMMs with Poison distribution of errors. Treatment was set as a fixed factor while microsite and identity of each plant individual or bare soil were included as random factors because soil samples collected from the same individual or bare soil were closer to each other than they were to the other samples. Best model selection was based on Akaike´s Information Criterion (AIC) (Zuur *et al.* 2009). If two or more models had the same AIC (*i.e.* a difference < 2 AIC points) the most parsimonious model was selected.

Differences in emergence timing and seedling mortality of the most abundant species in the seed bank between treated and non-treated pots were evaluated performing separate Species x Treatment GLMMs. A Poison distribution of errors was specified for germination time, and a binomial distribution of errors was specified for seedling mortality. Pot was included as random factor in each model. An observation-level random effect was included to account for overdispersion in emergence timing (Harrison 2014).

All statistical analyses were performed with R software (R Core Team 2015). GLMMs were performed using the *glmer* function in the *lme4* package (Bates *et al.* 2015).

Results

Seed bank structure

423 seedlings from 20 different species emerged from the seed bank samples in non-treated pots (n=80; Table 1). Mean seed bank density was 5499 seeds m^{-2} (Table 1). Approximately 85% of seeds in the seed bank samples were from one of the four most abundant species (*A. herba-alba*, *D. glomerata, Frankenia pulverulenta* L. and *Plantago afra* L.). *Artemisia herba-alba* was the most representative species of seed bank with more than 65% of seeds (Table 1).

Soil seed bank samples collected from the ART and SAL microsites (shrubs) had significantly more species than did those collected from the BS microsite (bare soil; Fig. 2A; Table S1). In addition, seed density was approximately seven and two times higher in seed bank samples collected from microsites ART and SAL, respectively, than it was in samples collected from bare soil (Fig. 2B; Table S1).

Table 1. Seed bank density per species and microsite (based only on counts from non-treated pots; n=80) and seedling emergence per species and treatment.

	Seed bank density (seeds m^{-2})			Seedling emergence (seedlings m^{-2})	
Species	BS	ART	SAL	Non-treated	Treated
Anacyclus clavatus (Desf.) Pers.	26	0	0	13	0
Artemisia herba-alba Asso	780	12271	832	3666	1638
Astragalus sesameus L.	0	156	260	104	13
Brachypodium retusum (Pers.) P.Beauv.	0	0	0	0	52
Brachypodium distachyon (L.) P.Beauv.	78	52	156	91	52
Bromus sp.	26	52	52	39	39
Unidentified sp. 1	26	0	0	13	0
Dactylis glomerata L. subsp. *hispanica* (Roth) Nyman	78	260	208	156	130
Filago pyramidata L.	0	52	156	52	13
Frankenia pulverulenta L.	208	156	1040	403	208
Juncus sp. 1	52	0	52	39	65
Juncus sp. 2	52	104	0	52	65
Unidentified sp. 2	104	104	52	91	104
Plantago afra L.	208	572	1352	585	143
Plantago albicans L.	0	52	0	13	13
Plantago coronopus L.	26	0	0	13	0
Salsola kali L.	78	0	0	39	0
Sonchus tenerrimus L.	26	104	52	52	26
Spergularia diandra (Guss.) Boiss.	52	0	0	26	13
Suaeda vera J.F.Gmel.	26	0	0	13	0
Trigonella polyceratia L.	0	156	0	39	39
Total	1846	14091	4212	5499	2613

Species richness did not differ significantly between seed banks of ART and SAL microsites (Fig. 2A; Table S1). However, seed density was significantly higher in the seed bank samples from ART microsite than it was in the samples from SAL microsite (Fig. 2B; Table S1). Nevertheless, a new analysis that excluded the abundance of *A. herba-alba* seeds revealed non-significant differences in seed density between seed banks of ART and SAL microsites (Fig. 2C; Table S1). Thus, *A. herba-alba* and *S. vermiculata* had a similar seed bank structure in terms of species richness and seed density, with the exception of the abundance of *A. herba-alba* seeds.

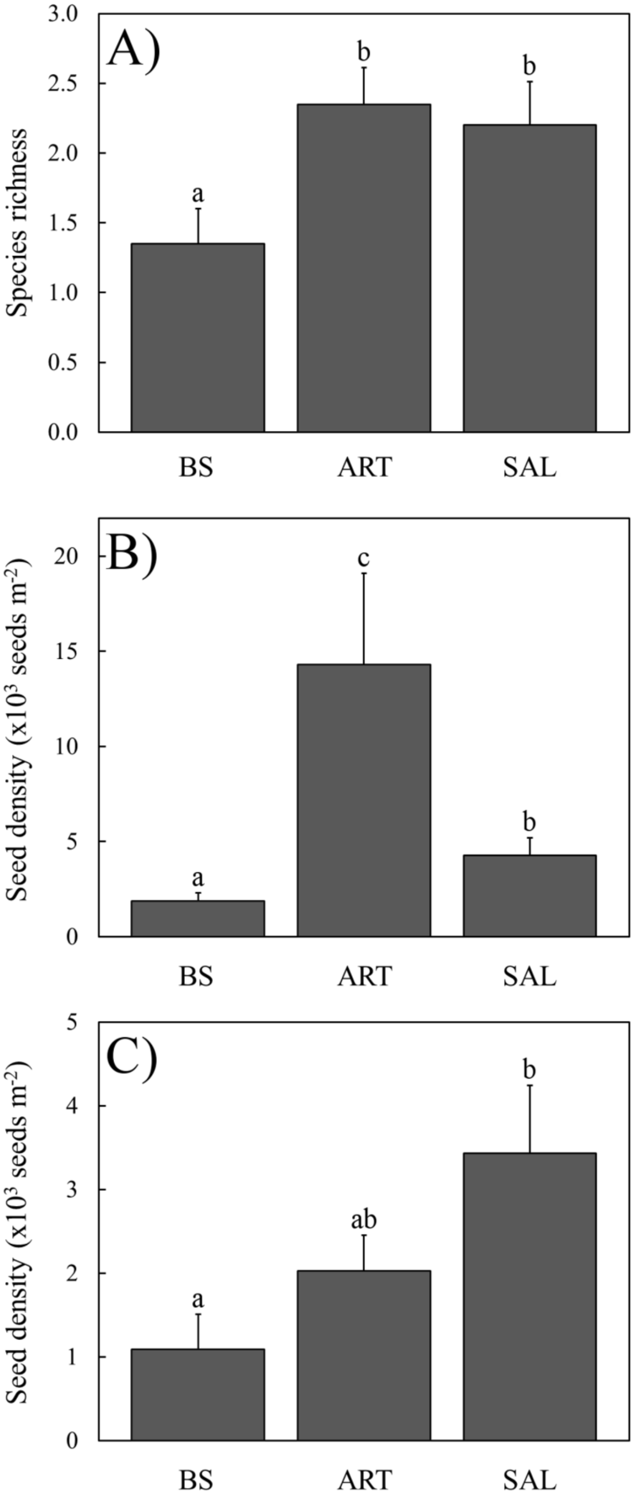


Fig. 2 A) Species richness (mean ± SE) and B) density of seeds per square meter (mean ± SE) of seed bank samples collected from bare soil (BS) and shrub (ART and SAL) microsites. C) Density of seeds per square meter (mean ± SE) of seed bank samples, excluding *A. herba-alba* seeds. Different letters indicate significant differences. Data is based on counts of non-treated pots. Statistical results are in Table S1.

Effects of aqueous extract of *A. herba-alba*

Aqueous extract of *A. herba-alba* had an inhibitory effect on seedling emergence. Specifically, seed bank samples from pots that were treated with aqueous extract had significantly lower species richness than did those from non-treated pots (Fig. 3A; Table S2). Furthermore, number of emerged seedlings in seed bank samples that were irrigated with aqueous extract was 50% lower than it was from seed bank samples in non-treated pots (Fig. 3B; Table S2).

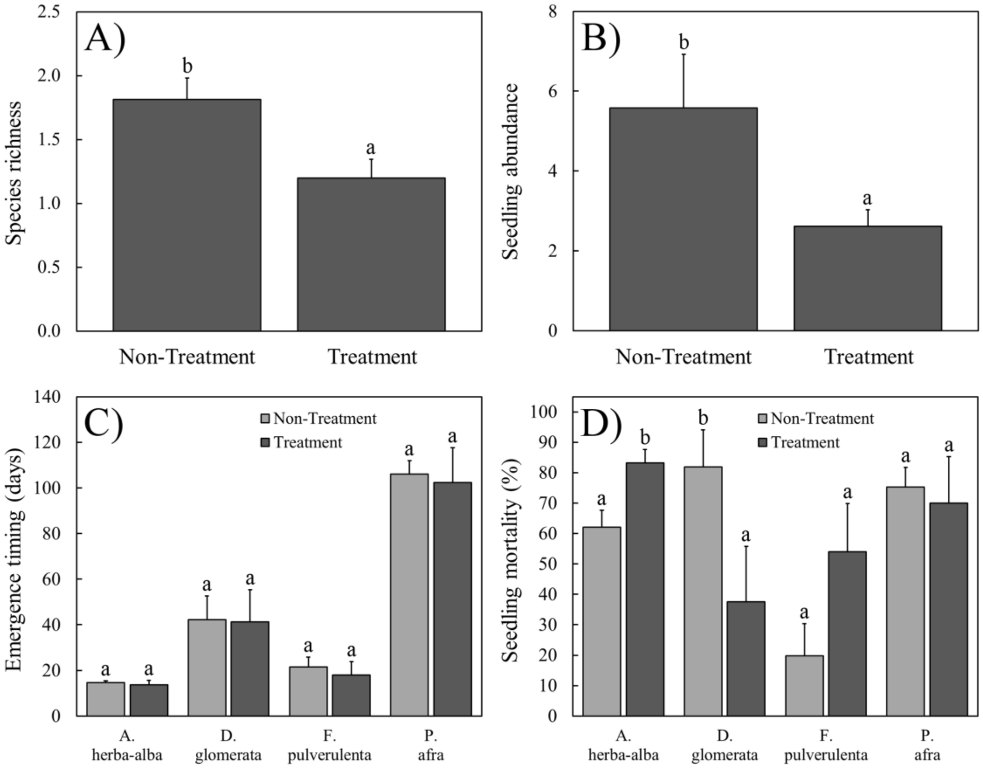


Fig. 3 A) Species richness (mean ± SE) and B) abundance of emerged seedlings (mean ± SE) of treated and non-treated pots. C) Emergence timing (mean ± SE) and D) mortality (percentage ± SE) of seedlings of the four most abundant species (*A. herba-alba, D. glomerata, F. pulveruenta* and *P. afra*) in treated and non-treated pots. Different letters indicate significant differences. Statistical results are in Tables S2 and S3.

Emergence timing of seedlings did not differ significantly between treated and non-treated pots for any of the four most abundant species (*A. herba-alba*, *D. glomerata, F. pulverulenta* and *P. afra*) present in seed bank samples (Fig. 3C; Table S3). On the contrary, treatment with aqueous extract of *A. herba-alba* affected significantly seedling mortality of some of those species. Specifically, aqueous extract significantly increased the mortality of *A. herba-alba* seedlings (Fig. 3D; Table S3) and reduced the mortality of *D. glomerata* seedlings (Fig. 3D; Table S3). The mortality of *F. pulverulenta* and *P. afra* seedlings did not differ significantly between treated and non-treated pots (Fig. 3D, Table S3).

Discussion

In semi-arid ecosystems, there is a positive feedback between vegetation patches and soil seed bank. Vegetation patches favor seeds accumulation (Caballero *et al.* 2008a; Liu *et al.* 2012), which leads to a dense and diverse seed bank beneath (Giladi *et al.* 2013). On the other hand, microclimate conditions are more benign and resources availability is higher in vegetation patches than they are on bare soil (Callaway 2007). Therefore, seedling recruitment and establishment is enhanced in vegetation patches (Aguiar and Sala 1997), which undoubtedly contributes to the persistence of the patchy structure of vegetation in semi-arid plant communities (Aguiar and Sala 1997). Allelopathic activity of *A. herba-alba* could hinder the positive feedback between vegetation patches and soil seed bank. Indeed, in a previous study it was found that while *S. vermiculata* acts as an effective nurse plant, which creates and maintains diversity in vegetation patches, *A. herba-alba* remains fairly isolated (Arroyo *et al.* 2015). The presence of allelochemicals could reduce seed production in susceptible plant species (Yarnia 2012) and, therefore, their contribution to the soil seed bank. Here, we showed that, although the structure of the soil seed bank at the *A. herba-alba* microsite was analogous to that of the similar non-allelopathic shrub *S. vermiculata* (with the exception of the abundance of *A. herba-alba* seeds), the presence of allelochemicals strongly reduced seedling emergence from the seed bank and increased premature mortality of some species, which might explain vegetation patterns in nature (Friedman *et al.* 1977).

In line with previous findings in other semi-arid plant communities, we found that seed bank richness and density were higher under vegetation patches than they were in bare soil areas, thus, shaping a spatially heterogeneous soil seed bank (Kemp 1989; Aguiar and Sala 1997; Guo *et al.* 1998; Giladi *et al.* 2013). Those spatial heterogeneities are produced not only by the high seed capture rate in vegetation patches compared to the low capture and retention of seeds in bare soil (Liu *et al.* 2012; Giladi *et al.* 2013), but also because vegetation patches act as source of seeds (Caballero *et al.* 2008a). *Salsola vermiculata* individuals have higher plant diversity nearby than do individuals of *A. herba-alba* in this semi-arid plant community (Arroyo *et al.* 2015). Therefore, it could be expected that patches of *S. vermiculata* act, to some extent, as a source of higher number of species to the seed bank than would patches of *A. herba-alba*, resulting in a richer seed bank. However, contrary to our hypothesis, seed bank richness was similar at the allelopathic and the nurse species microsites. This might have resulted from effective seed dispersal (Saatkamp *et al.* 2014) that prevented any potential differences between the two microsites.

On the other hand, seed bank density of the soil samples collected from under *A. herba-alba* individuals was higher than seed bank density of those collected from under *S. vermiculata* individuals, even though selected *S. vermicualta* individuals had a longer radius than did *A. herba-alba* individuals. Actually, the vertical structure (*i.e.* height) of patches, rather than width, modulates the role of vegetation patches as seeds sink (Caballero *et al.* 2008a). In our study, *A. herba-alba* and *S. vermiculata* did not differ in height, which suggests that these species should have similar seed capture rates. Indeed, *A. herba-alba* and *S. vermiculata* had similar seed bank densities, as differences in seed bank density between the ART and SAL microsites were just caused by the extraordinary abundance of *A. herba-alba* seeds at the ART microsite. The most plausible explanation for that finding is two-fold. Firstly, the flowering period of *A. herba-alba* spans from October to December, which is close to time at which the soil samples were collected (January), while secondary dispersal by wind or animals might take longer. Secondly, seeds of *A. herba-alba* are round and relatively heavy (~11 mg per seed), features which do not guarantee a high wind dispersal potential (Tackenberg *et al.* 2003; Liu *et al.* 2012) and, consequently, seeds remain close to the mother plant.

Our results showed that one of the main impacts of the presence of the allelopathic plants was the inhibition of germination from the seed bank. In general, allelochemicals produced by *A. herba-alba* reduced seedling emergence by up to 50%. These findings are in line with other studies that reported inhibitory effects of aqueous extract of *A. herba-alba* (Friedman *et al.* 1977; Escudero *et al.* 2000). The magnitude of this inhibition appeared to differ among the species in the seed bank. Germination of some species appeared to be strongly inhibited (*e.g. A. herba-alba*; Table 1), but others appeared to be unaffected by the allelochemicals. Surprisingly, allelochemicals of *A. herba-alba* apparently stimulated germination of *Brachypodium retusum*. Actually, some allelochemicals, or a mixture of them, can act as promoters of germination (Kawaguchi *et al.* 1997; Fernandez *et al.* 2013). However, the presence of this species only in the treated pots might have been due to the high heterogeneity of the soil seed bank, even at a fine scale (3.5 cm). Similarly, we found contrasting effects of allelochemicals of *A. herba-alba* on seedling mortality. Seedling mortality of some species (*i.e. A. herba-alba*) was increased in presence of allelochemicals. On the contrary seedling mortality of other species was not affected by allelochemicals, and, in the particular case of seedlings of *D. glomerata*, mortality was reduced. Previously, it has been suggested that at least some species of perennial grasses that co-exists with the allelopathic species *A. herba-alba* appear to tolerate its allelochemicals (Arroyo *et al.* 2015). Our finding points out that some grass species, such as *D. glomerata*, might take advantage of C input, that involves allelochemicals of *A. herba-alba*, to grow better (but see Viard-Crétat *et al.* 2012). Collectively, those results underscore the species-specific nature of allelopathy (Herranz *et al.* 2006; Linhart *et al.* 2015).

In semi-arid ecosystems, emergence timing can be considered as a key factor that determines the fate of the seedling after the summer drought. The later the seedling emerges the lower its probability of survival under adverse conditions (Escudero *et al.* 1999; Castro 2006). In that sense, allelopathy can hamper seedling survival in the following summer delaying germination from the seed bank (Herranz *et al.* 2006; Fernandez *et al.* 2013). In our study, however, the allelochemicals produced by *A. herba-alba* did not have that effect. Therefore, although that process might be important in other plant communities, the low diversity near *A. herba-alba* individuals in our semi-arid plant community (Arroyo *et al.* 2015) cannot be attributed to this allelopathic effect.

Soil seed bank is characterized by a high temporal heterogeneity in semi-arid ecosystems (Caballero *et al.* 2005; Facelli *et al.* 2005). Seed bank density varies seasonally, and is lowest after the emergence peak in spring and highest after seeds dispersal (Caballero *et al.* 2005). Future research should incorporate and explore the interplay between allelopathic plants and the temporal heterogeneity of seed banks. Additional evidence of allelopathy could be obtained by comparing the seed bank structure of an allelopathic and a similar non-allelopathic plant in contrasting seasons. If there is a clear allelopathic effect that inhibits seedling emergence from the soil seed bank, then seed density and, perhaps, richness, would be higher beneath allelopathic plant species than it would be beneath similar non-allelopathic plants just after seedling emergence, while there would be no differences after seeds dispersal. Inhibition of seedling emergence might lead to an accumulation of seeds beneath allelopathic plants year after year. Going further, long term inhibition can result in a loss of the viability of susceptible seeds because of the oxidative stress caused by allelochemicals (Long *et al.* 2015; Harun *et al.* 2015) and, eventually, seeds might die. That only could be tested using alternative methods for estimating soil seed bank (Abella *et al.* 2013), considering not only viable fraction of the seed bank, but also non-viable seeds. Further research should investigate the effects of other potential allelopathic organisms, such as lichens, on soil seed bank structure and germination.

In conclusion, our results support the heterogeneous spatial structure of soil seed banks in semi-arid plant communities (Aguiar and Sala 1997; Guo *et al.* 1998). We did not find relevant differences in seed bank structure (in terms of species richness and seed density) caused by the presence of allelopathic plant species relative to similar non-allelopathic plants. However, we found allelopathic effects on seedling emergence from the seed bank and seedling performance. Our results point out that diversity repulsion that occurs near *A. herba-alba* individuals (Arroyo *et al.* 2015) can be attributed to its allelopathic effect in inhibiting seedling emergence from the seed bank and in hindering seedling establishment. Those results improve our understanding of the role of allelopathy in plant community organization in a semi-arid ecosystem through its effects on the soil seed bank. In addition, our study brings to light the potential of the soil seed bank as a useful way of testing for allelopathic effects in an ecological-like context. Although further studies are needed to fully understand how the presence of allelopathic plants affects temporal variability of soil seed bank. To our knowledge, this is the first study to examine the effect of an allelopathic plant on an entire seed bank.

Acknowledgements

This work was supported by the Spanish Ministry of Education, Culture and Sport (AP-2012-4126 grant to A.I.A., FPU Program) and by the Spanish Ministry of Economy and Competitiveness and FEDER (CGL-2012-37508 project). We especially thank to A. Foronda for the help with the experiment maintenance tasks.

Supplementary material

Supplementary Tables S1, S2, S3 and Figure S1 may be found in the online version of this article.

References

Abad MJ, Bedoya LM, Apaza L, and Bermejo P (2012) The Artemisia L. genus: a review of bioactive essential oils. *Molecules* **17**:2542–66.

Abella SR, Chiquoine LP, and Vanier CH (2013) Characterizing soil seed banks and relationships to plant communities. *Plant Ecology* **214**:703–15.

Aguiar MR and Sala OE (1997) Seed distribution constrains the dynamics of the Patagonian steppe. *Ecology* **78**:93–100.

Arroyo AI, Pueyo Y, Saiz H, and Alados CL (2015) Plant-plant interactions as a mechanism structuring plant diversity in a Mediterranean semi-arid ecosystem. *Ecology and Evolution* **5**:5305–17.

Bates D, Maechler M, Bolker B, and Walker S (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**:1–48.

Caballero I, Olano JM, Escudero A, and Loidi J (2008a) Seed bank spatial structure in semi-arid environments: beyond the patch-bare area dichotomy. *Plant Ecology* **195**:215–23.

Caballero I, Olano JM, Loidi J, and Escudero A (2008b) A model for small-scale seed bank and standing vegetation connection along time. *Oikos* **117**:1788–95.

Caballero I, Olano JM, Luzuriaga A l., and Escudero A (2005) Spatial coherence between seasonal seed banks in a semi-arid gypsum community: density changes but structure does not. *Seed Science Research* **15**:153–160.

Callaway RM (2007) Positive interactions and interdependence in plant communities. Dordrecht: Springer Netherlands.

Callaway RM and Ridenour WM (2004) Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment* **2**:436–43.

Castro J (2006) Short delay in timing of emergence determines establishment success in Pinus sylvestris across microhabitats. *Annals of Botany* **98**:1233–40.

Cheng F and Cheng Z (2015) Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Frontiers in Plant Science* **6**.

Chesson P, Gebauer RLE, Schwinning S, Huntly N, Wiegand K, Ernest MSK, Sher A, Novoplansky A, and Weltzin JF (2004) Resource pulses, species interactions, and diversity maintenance in arid and semi-arid environments. *Oecologia* **141**:236–53.

Ehlers BK, Charpentier A, and Grøndahl E (2014) An allelopathic plant facilitates species richness in the Mediterranean garrigue. *Journal of Ecology* **102**:176–85.

Escudero A, Albert MJ, Pita JM, and Pérez-García F (2000) Inhibitory effects of Artemisia herba-alba on the germination of the gypsophyte Helianthemum squamatum. *Plant Ecology* **148**:71–80.

Escudero A, Somolinos RC, Olano JM, and Rubio A (1999) Factors controlling the establishment of Helianthemum squamatum, an endemic gypsophile of semi-arid Spain. *Journal of Ecology* **87**:290–302.

Fabbro CD, Güsewell S, and Prati D (2013) Allelopathic effects of three plant invaders on germination of native species: a field study. *Biological Invasions* **16**:1035–42.

Facelli JM, Chesson P, and Barnes N (2005) Differences in seed biology of annual plants in arid lands: a key ingredient of the storage effect. *Ecology* **86**:2998–3006.

Fernandez C, Lelong B, Vila B, Mévy JP, Robles C, Greff S, Dupouyet S, and Bousquet-Mélou A (2006) Potential allelopathic effect of Pinus halepensis in the secondary succession: an experimental approach. *Chemoecology* **16**:97–105.

Fernandez C, Santonja M, Gros R, Monnier Y, Chomel M, Baldy V, and Bousquet-Mélou A (2013) Allelochemicals of Pinus halepensis as drivers of biodiversity in Mediterranean open mosaic habitats during the colonization stage of secondary succession. *Journal of Chemical Ecology* **39**:298–311.

Friedman J, Orshan G, and Ziger-Cfir Y (1977) Suppression of annuals by Artemisia herba-alba in the Negev desert of Israel. *Journal of Ecology* **65**:413–26.

Gant RE and Clebsch EEC (1975) Allelopathic influences of Sassafras albidum in olf field succession in Tennessee. *Ecology* **56**:604–615

Giladi I, Segoli M, and Ungar ED (2013) Shrubs and herbaceous seed flow in a semi-arid landscape: dual functioning of shrubs as trap and barrier. *Journal of Ecology* **101**:97–106.

Gniazdowska A and Bogatek R (2005) Allelopathic interactions between plants. Multi site action of allelochemicals. *Acta Physiologiae Plantarum* **27**:395–407.

Guo Q, Rundel PW, and Goodall DW (1998) Horizontal and vertical distribution of desert seed banks: patterns, causes, and implications. *Journal of Arid Environments* **38**:465–78.

Harrison XA (2014) Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ* **2**:e616.

Harun MAYA, Johnson J, and Robinson RW (2015) The contribution of volatilization and exudation to the allelopathic phytotoxicity of invasive Chrysanthemoides monilifera subsp. monilifera (boneseed). *Biological Invasions* **17**:3609–24.

Heerdt GNJT, Verweij GL, Bekker RM, and Bakker JP (1996) An improved method for seed-bank analysis: seedling emergence after removing the soil by sieving. *Functional Ecology* **10**:144–51.

Herranz JM, Ferrandis P, Copete MA, Duro EM, and Zalacaín A (2006) Effect of allelopathic compounds produced by Cistus ladanifer on germination of 20 Mediterranean taxa. *Plant Ecology* **184**:259–72.

Ikuma H and Thimann KV (1960) Action of gibberellic acid on lettuce seed germination. *Plant Physiology* **35**:557–66.

Inderjit and Callaway RM (2003) Experimental designs for the study of allelopathy. *Plant and Soil* **256**:1–11.

Inderjit, Wardle DA, Karban R, and Callaway RM (2011) The ecosystem and evolutionary contexts of allelopathy. *Trends in Ecology and Evolution* **26**:655–62.

Kawaguchi S, Yoneyama K, Yokota T, Takeuchi Y, Ogasawara M, and Konnai M (1997) Effects of aqueous extract of rice plants (Oryza sativa L.) on seed germination and radicle elongation of Monochoria vaginalis var. plantaginea. *Plant Growth Regulation* **23**:183–9.

Kemp PR (1989) Seed banks and vegetation processes in deserts. In Leck MA, Parker VT, Simpson RL (eds), *Ecology of Soil Seed Banks*, San Diego: Academic Press, 257–281.

Linhart YB, Gauthier P, Keefover-Ring K, and Thompson JD (2015) Variable phytotoxic effects of Thymus vulgaris (Lamiaceae) terpenes on associated species. *International Journal of Plant Sciences* **176**:20–30.

Liu Z, Zhu J, and Deng X (2012) Arrival vs. retention of seeds in bare patches in the semi-arid desertified grassland of Inner Mongolia, northeastern China. *Ecological Engineering* **49**:153–9.

Long RL, Gorecki MJ, Renton M, Scott JK, Colville L, Goggin DE, Commander LE, Westcott DA, Cherry H, and Finch-Savage WE.(2015) The ecophysiology of seed persistence: a mechanistic view of the journey to germination or demise. *Biological Reviews* **90**:31–59.

Mallik AU (2008) Allelopathy: advances, challenges and opportunities. In Zeng RS, Mallik AU, Luo SM (eds), *Allelopathy in Sustainable Agriculture and Forestry*, New York, NY: Springer New York, 25–38.

Martinez-Duro E, Luzuriaga AL, Ferrandis P, Escudero A, and Herranz JM (2012) Does aboveground vegetation composition resemble soil seed bank during succession in specialized vegetation on gypsum soil? *Ecological Research* **27**:43–51.

Meiners SJ, Kong CH, Ladwig LM, Pisula NL, and Lang KA (2012) Developing an ecological context for allelopathy. *Plant Ecology* **213**:1861–7.

Mohamed AE-HH, El-Sayed MA, Hegazy ME, Helady SE, Esmail AM, and Mohamed NS (2010) Chemical constituents and biological activities of Artemisia herba-alba. *Records of Natural Products* **4**:1–25.

Muller CH (1969) Allelopathy as a factor in ecological process. *Vegetatio* **18**:348–57.

Narwal SS, Hoagland RE, Dilday RH, and Reigosa MJ (2000) Allelopathy in ecological agriculture and forestry. Dordrecht: Springer Netherlands.

Orr SP, Rudgers JA, and Clay K (2005) Invasive plants can inhibit native tree seedlings: testing potential allelopathic mechanisms. *Plant Ecology* **181**:153–65.

Pedrol N, González L, and Reigosa MJ (2006) Allelopathy and abiotic stress. In Reigosa MJ, Pedrol N, González L (eds), *Allelopathy*, Dordrecht: Springer Netherlands, 171–209.

Pueyo Y. 2005. Evaluación de los factores ambientales y del uso antrópico como condicionantes de la conservación de la vegetación del sector central de la depresión del Ebro. *PhD thesis*, Universidad de Zaragoza, Spain.

van der Putten WH (2009) A multitrophic perspective on functioning and evolution of facilitation in plant communities. *Journal of Ecology* **97**:1131–8.

R Core Team (2015) R: A Language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Renne J, Sinn BT, Shook GW, Sedlacko DM, Dull JR, Villarreal D, and Hierro JL (2014) Eavesdropping in plants: delayed germination via biochemical recognition. *Journal of Ecology* **102**:86–94.

Rice EL (1984) Allelopathy (Second Edition). San Diego: Academic Press.

Rivoal A, Fernandez C, Greff S, Montes N, and Vila B (2011) Does competition stress decrease allelopathic potential? *Biochemical Systematics and Ecology* **39**:401–7.

Saatkamp A, Poschlod P, and Venable DL (2014) The functional role of soil seed banks in natural communities. In Gallagher RS (ed), *Seeds: the ecology of regeneration in plant communities*, Wallingford: CABI.

Scognamiglio M, D'Abrosca B, Esposito A, Pacifico S, Monaco P, and Fiorentino A (2013) Plant growth inhibitors: allelopathic role or phytotoxic effects? Focus on Mediterranean biomes. *Phytochemistry Reviews* **12**:803–30.

Soliveres S and Maestre FT (2014) Plant–plant interactions, environmental gradients and plant diversity: A global synthesis of community-level studies. *Perspectives in Plant Ecology, Evolution and Systematics* **16**:154–63.

Stark KE, Arsenault A, and Bradfield GE (2008) Variation in soil seed bank species composition of a dry coniferous forest: spatial scale and sampling considerations. *Plant Ecology* **197**:173–81.

Tackenberg O, Poschlod P, and Bonn S (2003) Assessment of wind dispersal potential in plant species. *Ecological Monographs* **73**:191–205.

Thompson JD (2005) Plant evolution in the Mediterranean. Oxford: Oxford University Press.

Thorpe AS, Thelen GC, Diaconu A, and Callaway RM (2009) Root exudate is allelopathic in invaded community but not in native community: field evidence for the novel weapons hypothesis. *Journal of Ecology* **97**:641–5.

Tilaoui M, Ait Mouse H, Jaafari A, and Zyad A (2015) Comparative phytochemical analysis of essential oils from different biological parts of Artemisia herba alba and their cytotoxic effect on cancer cells. *PLoS ONE* **10**:e0131799.

Tsubo M, Nishihara E, Nakamatsu K, Cheng Y, and Shinoda M (2012) Plant volatiles inhibit restoration of plant species communities in dry grassland. *Basic Applied Ecology* **13**:76–84.

Viard-Crétat F, Baptist F, Secher-Fromell H, and Gallet C (2012) The allelopathic effects of Festuca paniculata depend on competition in subalpine grasslands. *Plant Ecology* **213**:1963–73.

Vivanco JM, Bais HP, Stermitz FR, Thelen GC, and Callaway RM (2004) Biogeographical variation in community response to root allelochemistry: novel weapons and exotic invasion. *Ecology Letters* **7**:285–92.

Wardle DA, Nilsson M-C, Gallet C, and Zackrisson O (1998) An ecosystem-level perspective of allelopathy. *Biological Reviews* **73**:305–19.

Yarnia M (2012) Germination, growth and seed bank of red-root pigweed (Amaranthus retroflexus) as influenced by allelopathic extract and residues of alfalfa. *Research on Crops* **13**:1057–63.

Yuan Y, Wang B, Zhang S, Tang J, Tu C, Hu S, Young JWH, and Chen X (2013) Enhanced allelopathy and competitive ability of invasive plant Solidago canadensis in its introduced range. *Journal of Plant Ecology* **6**:253–63.

Zhang Q, Zhang Y, Peng S, and Zobel K (2014) Climate warming may facilitate invasion of the exotic shrub Lantana camara. *PLoS ONE* **9**:e105500.

Zuur AF, Ieno EN, Walker N, Saveliev AA, and Smith GM (2009) Mixed effects models and extensions in ecology with R. New York, NY: Springer New York.

Appendix 1

Table S1. Results of GLM analyses for the effects of microsites (BS, ART and SAL) on seed bank richness and density. Significance of the change in deviance was tested by comparing models with and without microsite effect, assuming a χ^2 distribution. Significant effects ($p < 0.05$) are highlighted in bold.

	Species richness				Seed density				Seed density (without *A. herba-alba* seeds)			
	Res. Deviance	df	χ^2	p	Res. Deviance	df	χ^2	p	Res. Deviance	df	χ^2	p
Null	103.73				134.35				96.97			
Microsite	94.08	2	9.65	**<0.01**	84.29	2	50.06	**<0.001**	82.55	2	14.42	**<0.001**

Appendix 2

Table S2. Results of GLMM analyses for treatment effect on seedling emergence from the seed bank. Significance of the change in deviance was tested by comparing models with and without treatment effect, assuming a χ^2 distribution. Significant effects ($p < 0.05$) are highlighted in bold.

	Species richness				Seedling abundance			
	Res. Deviance	df	χ^2	p	Res. Deviance	df	χ^2	p
Null	497.07				857.05			
Treatment	487.04	1	10.03	**<0.05**	772.64	1	84.42	**<0.001**

Appendix 3

Table S3. Results of GLMM analyses for treatment effect on emergence timing and seedling mortality of the four most abundant species in the seed bank. Significance of the change in deviance was tested by comparing models with and without treatment effect, assuming a χ^2 distribution. Significant effects ($p < 0.05$) are highlighted in bold.

Variable	*A. herba-alba*				*D. glomerata*				*F. pulverulenta*				*P. afra*			
	Res. Deviance	df	χ^2	p	Res. Deviance	df	χ^2	p	Res. Deviance	df	χ^2	p	Res. Deviance	df	χ^2	p
Emergence timing																
Null	2799.6				196.67				354.87				603.17			
Treatment	2796.4	1	3.21	0.07	196.65	1	0.02	0.88	353.74	1	1.13	0.29	603.01	1	0.16	0.69
Seedling mortality																
Null	514.92				27.32				53.75				77.69			
Treatment	502.67	1	12.25	**<0.001**	15.27	1	12.04	**<0.001**	50.30	1	3.45	0.06	77.68	1	0.01	0.92

Appendix 4

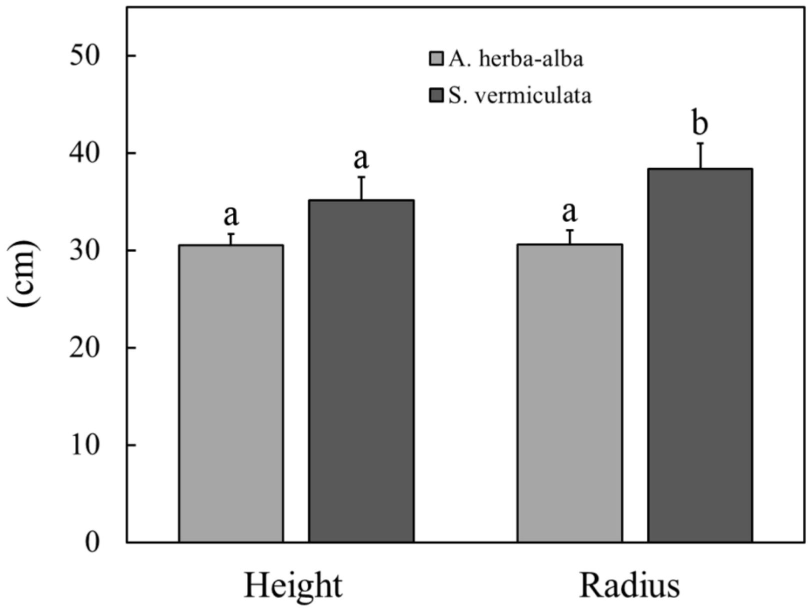


Fig. S1 Height and radius (mean ± SE) of selected individuals of *A. herba-alba* and *S. vermiculata*. Significance of differences was assessed by the use of one-way ANOVA. Different letters indicate significant differences.

Capítulo 4 / Chapter 4

Autotoxic and allelopathic effects of volatile and water soluble chemicals of *Artemisia herba-alba* Asso

Antonio I. Arroyo, Yolanda Pueyo, François Pellissier, Annie Millery, Javier Ramos, Concepción L. Alados

Frontiers in Plant Science (*in rev.*)

Autotoxic and allelopathic effects of volatile and water soluble chemicals of *Artemisia herba-alba* Asso

Antonio I. Arroyo[1], Yolanda Pueyo[1], François Pellissier[2], Annie Millery[2], Javier Ramos[3], Concepción L. Alados[1]

[1] Instituto Pirenaico de Ecología, Consejo Superior de Investigaciones Científicas, Av. Montañana 1005, 50059 Zaragoza, Spain.

[2] Université Savoie Mont Blanc, CNRS, LECA, Chambéry, France.

[3] Estación Experimental Aula Dei, Consejo Superior de Investigaciones Científicas, Av. Montañana 1005, 50059 Zaragoza, Spain.

Abstract

Chemically mediated plant-plant interactions (*i.e.*, allelopathy and autotoxicity) have the potential to shape the composition and structure of plant communities. The notion that allelopathic plants of drylands produce mostly volatile chemicals is widespread; yet, because conditions in those environments are stressful, plants produce a wide variety of secondary metabolites. Many of those are water soluble (*e.g.*, most phenolic compounds). However, the role of water soluble chemicals in semiarid plant communities has received limited attention. In this study, we aimed to investigate the allelopathic and autotoxic effects of the volatile and the water soluble chemicals released by the shrub *Artemisia herba-alba* Asso. We conducted a number of germination and early seedling growth bioassays on species that co-exist with the allelopathic species in natural communities (*Salsola vermiculata* L., *Lygeum spartum* L., *Pinus halepensis* Mill. and *A. herba-alba* itself). In addition, we assessed the effects of a mixture of phenols that were identified in the aqueous extract of A. herba-alba on the germination of such species. We found that effects of the volatile and water soluble chemicals were species-specific. Specifically, volatile chemicals inhibited the germination of *P. halepensis* seeds, promoted the growth of *P. halepensis* seedlings and reduced the root biomass of *S. vermiculata* seedlings, while water soluble chemicals promoted the germination of *P. halepensis* seeds and the growth of *L. spartum* and *P. halepensis* seedlings. On the other hand, both volatile and water soluble compounds inhibited the germination of *A. herba-alba* seeds, highlighting its strong autotoxic nature. Phenolic compounds were abundant in *A. herba-alba* aqueous extract. However, we did not find an allelopathic effect of phenols identified (catechol, protocatechuic and vanillic acids), although they had a moderate autotoxic effect. Our results suggest that both the volatile and water soluble chemicals can be involved in chemical interactions among plants in semiarid environments. In general, those compounds had a negative effect on seed germination in susceptible species, and a positive effect on the early seedling growth of tolerant species. Nevertheless, further research is required to evaluate the relative contribution to allelopathy and autotoxicity of compounds that belong to different chemical classes acting jointly.

Keywords

Aqueous extract, bioassay, germination, high performance liquid chromatography (HPLC), seedling growth, phenolic compounds, semiarid environment.

Introduction

Many plants species interfere with neighboring plants beyond direct competition for resources, through the production and release into the environment of secondary metabolites (allelochemicals) by leachates, litter decomposition, root exudates and volatilization (Meiners, 2014; Rice, 1984). Allelochemicals can affect other plants directly by reducing seed germination, growth and survival (Rice, 1984) and indirectly by altering soil microbial communities and their effects on soil biogeochemical processes, *e.g.*, nitrification (Castaldi et al., 2009; Wang et al., 2012). However, allelochemicals are often non-specific compounds and allelopathy may also occur within the same species, which is known as autotoxicity (Ruan et al., 2011). Thus, autotoxicity occurs when allelochemicals released by a plant interfere with germination, growth and survival of the same plant species (Friedman and Waller, 1985; Singh et al., 1999). As such, plant-plant interactions mediated by allelochemicals (*i.e.*, allelopathy and autotoxicity) have the potential to shape the composition and structure of the aboveground vegetation (Alías et al., 2006; Fernandez et al., 2013; Herranz et al., 2006).

Phenolic compounds are one of the best-known classes of plant allelochemicals (Muscolo and Sidari, 2010). Phenols comprise several groups including simple phenols, phenolic acids, coumarins, tannins and some flavonoids (Li et al., 2010; Muscolo and Sidari, 2010). Mainly, they are introduced into the environment through plant litter decomposition and, to a lesser extent, as leachates of plant parts (Hättenschwiler and Vitousek, 2000). Once the phenols are released, they interact with nutrient cycling and can limit the availability of certain nutrients (Hättenschwiler and Vitousek, 2000; Inderjit and Mallik, 1997). Biological effects of phenolic compounds in target plants range from changes in cell membrane permeability to effects on plant photosynthesis, which affect the normal growth and development of the entire plant (for an extensive review, see Li et al., 2010).

Many species are known to be potentially allelopathic in semiarid environments (Araniti et al., 2012; Friedjung et al., 2013; Vilà and Sardans, 1999). Indeed, harsh abiotic factors such as high temperatures, intense solar radiation and water deficit often enhance the production, accumulation and phytotoxicity of allelochemicals (Akula and Ravishankar, 2011; Chen et al., 2012). Furthermore, in those communities, the joint action of multiple stresses might increase the plant´s susceptibility to the effects of allelochemicals (Pedrol et al., 2006). It has been argued that allelopathy may be the result of selection against being facilitative plants (van der Putten, 2009). Similarly, autotoxicity can play a relevant role in avoiding the impacts of intraspecific competition for scarce resources (*e.g.*, water) under harsh environmental conditions (Armas and Pugnaire, 2011). However, autotoxicity has been rarely explored despite its potential adaptive value in those systems.

It has been claimed that plants in arid areas release mostly volatile allelochemicals (*e.g.*, terpenoids) while water soluble allelochemicals (*e.g.*, phenols) are more common in cool temperate areas (Chou, 1999; Moral and Cates, 1971; Reigosa et al., 1999). In semiarid environments, however, plants are especially rich in phenols because drought-tolerant plants produce and accumulate those compounds to overcome the oxidative damage caused by hydric stress (Bautista et al., 2016; Varela et al., 2016). In addition, even though water scarcity might act as a physical limitation to diffusion of water soluble chemicals, in these systems, there is a substantial water redistribution from the bare soil to vegetation patches and other mechanisms (*e.g.*, water input from fog drip; Callaway, 2007; Cerdà, 1997; Noy-Meir, 1973; Pueyo et al., 2016) that may increase the water soluble allelochemicals available to vegetation patches. Consequently, the role of water soluble chemicals, and in particular phenols, on allelopathy and autotoxicity in semiarid environments might have been overlooked.

The objective of this study was to investigate the autotoxic and allelopathic effects of the volatile and water soluble chemicals produced by an allelopathic shrub in a semiarid plant community. As allelopathic species we selected *Artemisia herba-alba* Asso. (desert wormwood; Escudero et al., 2000; Friedman et al., 1977), a dwarf shrub about 30-40 cm tall, and widespread in semiarid areas of the Mediterranean Basin, especially in the Iberian Peninsula, North Africa and the Middle East. Although, the effects of *A. herba-alba* aqueous extract have been assessed on different target species, the water soluble chemicals of the aqueous extract seldom have been characterized. On the other hand, the composition of the essential oils of *A. herba-alba* has been investigated from a biochemical perspective (Salido et al., 2004; Tilaoui et al., 2015; Younsi et al., 2016) although its phytotoxicity through volatilization has not been assessed before (but see Friedman et al., 1977). We hypothesized that i) both volatile and water soluble chemicals of *A. herba-alba* are involved in autotoxicity and allelopathy. We further hypothesized that ii) aqueous extract of *A. herba-alba* is rich in phenolic compounds and that iii) some of those phenolic compounds (*i.e.*, identified ones) have autotoxic and allelopathic activity.

To address the first hypothesis, germination and growth bioassays were conducted to assess allelopathic effects of volatile and water soluble chemicals on a set of target species. Target species included the shrub *Salsola vermiculata* L., the perennial grass *Lygeum spartum* L. (two dominant species that co-exist with *A. herba-alba* in natural semiarid plant communities) and *Pinus halepensis* Mill. (a species commonly used to reforest areas that have natural populations of *A. herba-alba*). *Artemisia herba-alba* was also included as target species to test for autotoxicity (Friedman and Orshan, 1975). To address the second hypothesis, we quantified the Total Phenolic Content (TPC) of the aqueous extract, and of the leaves and stems of *A. herba-alba* to estimate the fraction which is actually water soluble. Later, the phenolic compounds in the aqueous extract were identified based on High Performance Liquid Chromatography (HPLC). To address the third hypothesis, the potential phytotoxic effects of the phenolic compounds identified were assessed on seed germination in the target species.

Material and methods

Plant material collection and aqueous extraction

Mature seeds of *A. herba-alba* and *S. vermiculata*, and mature caryopses of *L. spartum* were collected in the field, in 2014, from a natural plant community in the Middle Ebro Valley, NE Spain. This area is characterized by a semiarid climate, and has an average annual precipitation that ranges between 300 and 400 mm year^{-1}. Mean annual temperature is about 15 ºC, with a pronounced continentality (data obtained from the Digital Climatic Atlas of Aragón; http://anciles.aragon.es/AtlasClimatico/). Seeds of *P. halepensis*, which were collected in 2011, were obtained from a local garden center. In addition, several shoots (aerial parts) were collected from a number of random *A. herba-alba* individuals in the same plant community. Specifically, aerial parts of *A. herba-alba* for the chemical analyses and the aqueous extract were collected in March 2015, while aerial parts used in the volatile bioassays were collected in December 2015. Aerial parts were dried at room temperature for 10 d. In the drying process, fresh material lost > 40% of its weight. Plant material was stored in a cold chamber at 4ºC until its utilization. Prior to the start of the volatile bioassays, the dried aerial parts used were ground, almost to powder, by a mechanical mill.

To obtain the aqueous extract of *A. herba-alba*, the dried aerial parts were soaked in demineralized water (100 g l^{-1}) for 24 h at room temperature (15ºC-20ºC) in total darkness. The solution was filtered, stored in small portions (30 ml) and frozen (-18 ºC) until its utilization.

Determination of the allelopathic potential of volatile and water soluble chemicals

Seed germination bioassays

The allelopathic potential of the volatile chemicals released from the aerial parts of *A. herba-alba* was determined by assessing seed germination in the four target species (*S. vermiculata*, *L. spartum*, *P. halepensis* and *A. herba-alba*). Seeds were placed on a layer of Joseph filter paper within 10-cm-diameter Petri dishes and were moistened with 5 ml of demineralized water. Aluminum foil containers (3-cm-diameter) with 0.05, 0.1 or 0.5 g (dry weight) of ground aerial parts were then placed into Petri dishes. Ten replicates (*i.e.*, Petri dishes) of *S. vermiculata, P. halepensis* and *A. herba-alba* (10 seed each), and *L. spartum* (5 caryopses each) were utilized at each treatment. The controls were performed placing empty aluminum containers within Petri dishes. Petri dishes were hermetically sealed with parafilm. Seeds were set to germinate in a greenhouse under controlled conditions (12 h of light at 23 ºC and 12 h of darkness at 18 ºC; Table S1).

The allelopathic potential of the water soluble chemicals was determined by assessing seed germination in the four target species following the same design mentioned above (Table S1). In this case, seeds were moistened with 5 ml of aqueous extract diluted to 0.5, 2 or 5 g l^{-1}, and controls were performed leaching seeds with demineralized water. In addition, seeds were set to germinate in a room that had a temperature that ranged between 24 ºC in the day and 20 ºC at night, and 12 h of light provided by cool light fluorescent tubes (204 µmol s^{-1} m^{-2}). Germination of *A. herba-alba* seeds failed in those conditions and required lower temperatures to succeed. Therefore, *A. herba-alba* seeds were set to germinate in a thermostatic growth chamber that had 12 h of light photoperiod (210 µmol s^{-1} m^{-2}), light period temperature 15 °C and dark period temperature 5°C (Table S1). The positions of the Petri dishes were changed randomly every few days and the number of germinated seeds (rupture of seed coats and protrusion of the radicle) was recorded daily for 21 d.

The allelopathic effects of a mixture of pure phenolic compounds were determined by assessing seed germination in the four target species. Specifically, catechol, protocatechuic and vanillic acids were used for the mixture because they were identified and quantified in aqueous extract (37.2, 9.4, and 2.2 mg l^{-1}, respectively). Seeds were moistened with 5 ml of three different concentrations of the phenolic mixture as treatments and with 5 ml of demineralized water in the control. The quantities of the pure phenolic compounds used in each concentration were the same as those found in the three treatments of aqueous extract of *A. herba-alba*. The number of replicates, number of seeds per treatment and germination conditions was the same as those in the germination bioassay of aqueous extract (Table S1).

Early seedling growth bioassays

Seeds of *S. vermiculata*, *L. spartum*, *P. halepensis* and *A. herba-alba* were sowed in 8 x 8 x 7 cm plastic pots (60 pots per species and bioassay) that were filled with approximately 100 g of substrate. To maximize the probability of having at least one seedling per pot, multiple seeds were sowed in each pot. Extra seedlings were removed once more than one seedling had emerged in a pot. Exceptionally, two seedlings of *P. halepensis* were left to grow in some pots because germination was low. *Pinus halepensis* seedlings have pivoting roots, with limited lateral development and, consequently, neighboring seedlings have negligible influence. Pot positions were randomized in space when seeds were set to germinate. In the germination period, pots were watered regularly with fresh water. After most of the seedlings had become well established (approximately two weeks), treatments were applied.

The allelopathic potential of the volatile chemicals released from aerial parts of A. herba-alba was determined by assessing early seedling growth in the four target species. Seedlings were set to grow in three portable plastic greenhouses (0.29 m3) under a 12 h of natural daylight photoperiod and a temperature that ranged between 20ºC and 25ºC on clear days (temperature did not differ significantly among greenhouses, F3,24 = 0.061, p>0.05, data not shown). Fifteen pots of each seedling species were randomly placed into each greenhouse (60 pots per greenhouse) and either, 3, 10 or 30 g of ground aerial parts (equivalent to 0.05, 0.17 or 0.5 g per pot respectively) were placed in a plastic support that hung about 20 cm above the pots. Artemisia herba-alba aerial parts were replaced twice in the bioassay. Controls pots (n = 60) were randomly placed into a fourth greenhouse without the aerial parts of A. herba-alba (Table S1). The positions of the portable greenhouses and pots were randomly changed every few days. Pots were watered regularly with fresh water. Greenhouses were open for about 10 min when pots were watered.

The allelopathic potential of the water soluble chemicals of *A. herba-alba* was determined by assessing early seedling growth in the four target species. Specifically, 15 pots per species were watered weekly with 40 ml of aqueous extract diluted to either, 0.5, 2 or 5 g l^{-1}. Occasionally, the treatment was reduced to 30 ml because the substrate was nearly saturated. In those instances, to ensure that the dosages of water soluble chemicals were maintained, the concentrations of the solutions were increased to 0.7, 2.7 and 6.7 g l^{-1} respectively. Irrigation with fresh water remained as control for the rest of pots. All pots were set to grow in a room that had a temperature of 24ºC in the day and 20ºC at night, and 12 hours of light provided by cool light fluorescent tubes (204 µmol s^{-1} m^{-2}; Table S1). The positions of the pots were changed randomly every few days. After eight weeks growing, seedlings were dried at 60 ºC for 3-4 days, and the biomass of the aerial parts and roots of the seedlings was measured.

Biochemical analyses

Total Phenolic Content (TPC) of the aqueous extract, leaves and stems of *A. herba-alba* was measured by spectrophotometry following the Folin-Ciocalteu method (Singleton and Rossi, 1965). Three 0.1-g samples of powdered leaves and stems from various individuals were extracted with 50 ml of 70% ethanol and heated at ebullition temperature for 30 min.

Ethanol extracts were filtered (filtration funnel with filter nº 2, 40-100 µm), and 0.1 ml of each extract was diluted in 3.4 ml of distilled water before 0.5 ml of Folin-Ciocalteu reagent was added. After 1 min incubation, 1 ml of Na_2CO_3 solution (20%) was added. The resultant solution was mixed thoroughly by mechanical shaking before being heated in a bain-marie at 40 ºC for 20 min. On the other hand, three 0.02-ml samples of aqueous extract of *A. herba-alba* were diluted in 3.48 ml distilled water, and the same above-mentioned protocol was followed. The absorbance of the samples was read by spectrophotometry at 760 nm (Shimadzu UV-160) against the blank test (distilled water). TPC of the samples was estimated based on a calibration curve that was prepared with standard gallic acid (1 to 10 µg ml^{-1}) and was expressed as mg of gallic acid equivalents (GAE) per g of plant material.

Phenolic compounds of the aqueous extract of *A. herba-alba* were identified by HPLC. Before the chemical analysis, an acid hydrolysis was performed to obtain free phenolic compounds. Specifically, 0.3 ml of concentrated hydrochloric acid was added to 1.5 ml aqueous extract of *A. herba-alba* in a glass tube, which was heated at 100 °C for 1 h. This solution was filtered through a 1-µm glass-microfiber disc before being injected into the HPLC system. A pre-column Waters µBondapak C18 Sentry Guard Cartridge, (10 µm, 3.9 mm x 20 mm) was placed immediately before a Waters column µBondapak 18 C (250 mm x 4.6 mm ID cartridge, 10 µm). The phenolic compounds were eluated with a flow rate of 1.5 ml min^{-1} and a linear gradient from 0 to 20% of solvent B (acetonitrile) in solvent A (distilled water with 0.5% glacial acetic acid), followed by 15 min re-equilibration with solvent A. The UV-visible spectrum was recorded at 280 nm (maximum absorbance of phenolic compounds) with a Waters 996 photodiode array detector. The compounds were identified and quantified by comparing the retention time, the absorption spectrum and the peak area to those of a standard.

Statistical analyses

Differences in germination rates among treatments were evaluated, for each target species, at three times: 7, 14 and 21 days (d7, d14 and d21 respectively) after the start of the germination bioassays (except *S. vermiculata* because germination had ended by d14). According to Chiapusio *et al*. (1997), simple comparisons between treatments and a control in the germination rates at each exposure time are more appropriate than is using multiple germination indices for assessing allelopathic effects. Generalized linear models (GLMs) with a binomial distribution were specified as data did not meet normality assumptions. Tukey´s *post hoc* honest significant difference (HSD) tests were used to detect significant differences between pairs of treatments.

Differences in the aerial and root biomass of seedlings among treatments in the growth bioassays were evaluated, for each target species, using one-way ANOVA. Tukey′s *post hoc* honest significant difference (HSD) tests were used to detect significant differences between pairs of treatments.

Statistical analyses were performed in R (R Core Team, 2015).

Results

Allelopathic effects of volatile and water soluble chemicals on seed germination

Significant effects of the volatile chemicals released from *A. herba-alba* aerial parts were not observed, at any moment, on germination of *S. vermiculata* and *L. spartum* (Table 1). However, the volatile chemicals released from the highest amount of *A. herba-alba* aerial parts inhibited germination of *P. halepensis* seeds at d14 and d21 (Table 1). Furthermore, at all three amounts of aerial parts, the volatile chemicals of *A. herba-alba* inhibited the germination of its own seeds at d7, d14 and d21 (Table 1).

The water soluble chemicals of *A. herba-alba* did not have a significant effect on the germination of *S. vermiculata* and *L. spartum* seeds at any moment (Table 2). However, at d14 and d21, the aqueous extract had a significant effect on the germination of *P. halepensis* seeds. Specifically, the intermediate concentration of aqueous extract promoted seed germination at d14, whereas, at d21, such promotion was significant only compared to the highest concentration of aqueous extract (Table 2). In addition, the water soluble chemicals of *A. herba-alba* inhibited the germination of *A. herba-alba* seeds. That inhibition was significant for the highest concentration of aqueous extract at d7, d14 and d21, whereas the lowest and intermediate concentrations only delayed its germination at d14 (Table 2).

Table 1. Seed germination (%; mean ± SE) and results of GLM analyses of the effects of volatile chemicals released from the aerial parts of *A. herba-alba* on seed germination in four target species at 7, 14 and 21 d after exposure. Significant effects ($p < 0.05$) are highlighted in bold. Different letters in the same row indicate significant differences among treatments (Tukey´s HSD test).

Species	Time	Treatments				Deviance (df=3)	p-value
		Control	0.05 g	0.1 g	0.5 g		
S. vermiculata							
	d7	83^{a} ± 3.35	79^{a} ± 3.79	82^{a} ± 3.59	91^{a} ± 3.14	6.43	0.093
	d14	83^{a} ± 3.35	80^{a} ± 3.33	82^{a} ± 3.59	91^{a} ± 3.14	5.83	0.120
L. spartum							
	d7	38^{a} ± 7.57	38^{a} ± 8.67	34^{a} ± 5.21	22^{a} ± 6.96	4.06	0.255
	d14	80^{a} ± 5.96	84^{a} ± 4.99	90^{a} ± 4.47	80^{a} ± 7.30	2.59	0.459
	d21	84^{a} ± 5.81	86^{a} ± 5.21	90^{a} ± 4.47	92^{a} ± 3.27	1.92	0.589
P. halepensis							
	d7	4^{a} ± 2.21	10^{a} ± 3.56	4^{a} ± 1.76	3^{a} ± 1.53	5.40	0.145
	d14	74^{a} ± 4.52	68^{a} ± 5.01	62^{a} ± 3.51	43^{b} ± 4.39	**23.91**	**< 0.001**
	d21	80^{a} ± 3.65	79^{a} ± 4.30	79^{a} ± 3.79	59^{b} ± 5.10	**14.69**	**< 0.01**
A. herba-alba							
	d7	23^{a} ± 6.49	2^{b} ± 1.47	4^{b} ± 1.63	2^{b} ± 1.33	**34.68**	**< 0.001**
	d14	40^{a} ± 6.73	3^{b} ± 1.67	7^{b} ± 2.60	5^{b} ± 3.07	**65.13**	**< 0.001**
	d21	45^{a} ± 7.96	4^{b} ± 1.76	9^{b} ± 3.14	6^{b} ± 3.07	**71.37**	**< 0.001**

Table 2. Seed germination (%; mean ± SE) and results of GLM analyses of the effects of aqueous extract of aerial parts of *A. herba-alba* on seed germination in four target species at 7, 14 and 21 d after exposure. Significant effects ($p < 0.05$) are highlighted in bold. Different letters in the same row indicate significant differences among treatments (Tukey´s HSD test).

Species	Time	Treatments				Deviance (df=3)	p-value
		Control	0.5 g/l	2 g/l	5 g/l		
S. vermiculata							
	d7	71^{a} ± 3.48	79^{a} ± 2.33	74^{a} ± 7.18	74^{a} ± 3.4	2.18	0.535
	d14	79^{a} ± 3.48	79^{a} ± 2.33	75^{a} ± 6.87	77^{a} ± 3.67	2.22	0.529
L. spartum							
	d7	68^{a} ± 5.33	44^{a} ± 5.81	60^{a} ± 10.33	62^{a} ± 6.96	6.46	0.091
	d14	82^{a} ± 5.54	84^{a} ± 4.99	76^{a} ± 9.80	82^{a} ± 5.54	1.14	0.769
	d21	82^{a} ± 5.54	88^{a} ± 4.42	80^{a} ± 7.89	90^{a} ± 3.33	2.70	0.440
P. halepensis							
	d7	7^{a} ± 3	14^{a} ± 3.71	16^{a} ± 3.05	15^{a} ± 3.42	4.87	0.181
	d14	48^{a} ± 5.12	52^{ab} ± 6.63	66^{b} ± 3.4	40^{a} ± 4.22	**15.02**	**< 0.01**
	d21	65^{ab} ± 5.82	64^{ab} ± 4	72^{a} ± 3.59	53^{b} ± 5.18	**8.63**	**< 0.05**
A. herba-alba							
	d7	56^{a} ± 4.56	52^{a} ± 5.12	47^{a} ± 3	25^{b} ± 5	**24.43**	**< 0.001**
	d14	97^{a} ± 1.53	83^{b} ± 3.69	85^{b} ± 1.67	56^{c} ± 4.27	**56.99**	**< 0.001**
	d21	99^{a} ± 1	90^{a} ± 3.65	91^{a} ± 2.77	71^{b} ± 5.47	**39.92**	**< 0.001**

Allelopathic effects of volatile and water soluble chemicals on early seedling growth

After 8 weeks, the volatile chemicals released from the intermediate and the highest amount of *A. herba-alba* aerial parts significantly reduced the root biomass of *S. vermiculata* seedlings ($F_{3,45} = 11.96$, $p < 0.001$), but non-significant effects were observed on the aerial biomass ($F_{3,45} = 1.64$, $p = 0.19$; Fig. 1). Conversely, the volatile chemicals did not have a statistically significant effect on the root biomass *L. spartum* seedlings ($F_{3,56} = 2.5$, $p = 0.07$), but aerial biomass differed significantly among treatments ($F_{3,56} = 3.84$, $p = 0.01$). Specifically, aerial biomass was lower in *L. spartum* seedlings exposed to the volatile chemicals released from the intermediate amount of ground aerial parts than it was in those exposed to the lowest amount. However, they did not differ significantly from seedlings in the control (Fig. 1). In *P. halepensis*, aerial and root biomass were significantly higher in seedlings exposed to the highest amount of ground aerial parts of *A. herba-alba* than they were in seedlings in the control ($F_{3,49} = 3.03$, $p = 0.04$ and $F_{3,49} = 5.12$, $p = 0.004$; Fig. 1). At all three treatments, the volatile chemicals of *A. herba-alba* aerial parts did not have a significant effect on the aerial ($F_{3,54} = 1$, $p = 0.4$) or the root biomass ($F_{3,51} = 1.74$, $p = 0.17$) of *A. herba-alba* seedlings (Fig. 1).

The effects of the aqueous extract of *A. herba-alba* on early seedling growth in *S. vermiculata* and *A. herba-alba* could not be quantified because mortality was very high and too few seedlings survived to the end of the bioassay (data not shown). The aerial biomass of *L. spartum* seedlings watered with the highest concentration of aqueous extract was significantly higher than the aerial biomass of seedlings in the control ($F_{3,55} = 2.72$, $p = 0.05$), but seedling root biomass did not differ significantly among the treatments ($F_{3,55} = 2.11$, $p = 0.11$; Fig. 2). In addition, both the aerial and root biomass were significantly higher in the seedlings of *P. halepensis* irrigated with either the intermediate or the highest concentration of aqueous extract than they were in the control seedlings or in those irrigated with the lowest concentration of aqueous extract ($F_{3,53} = 4.16$, $p = 0.01$ and $F_{3,52} = 4.29$, $p = 0.009$; Fig. 2).

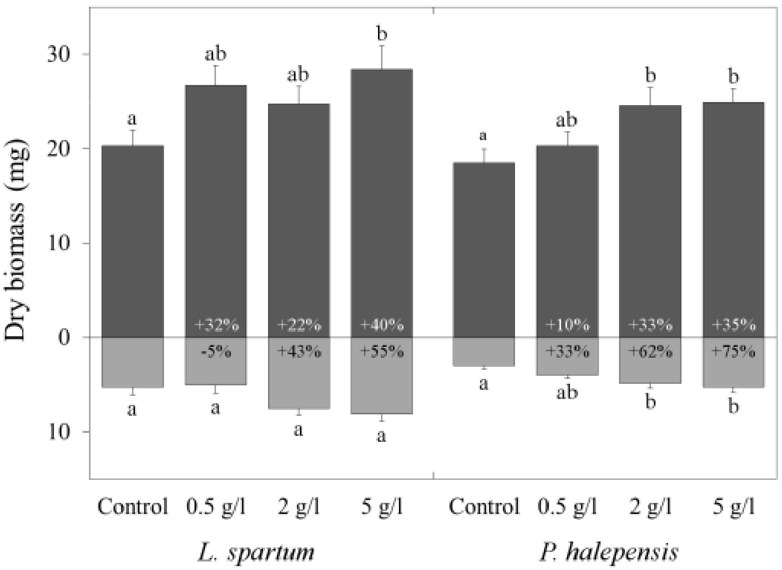


Fig. 1. Dry biomass (mean ± SE) of aerial parts (dark grey) and roots (light grey) of seedlings of *S. vermiculata*, *L. spartum*, *P. halepensis* and *A. herba-alba* (from left to right) after two months of exposure to either 0.05, 0.17 or 0.5 g per seedling of ground *A. herba-alba* aerial parts. Numbers in the bars indicate the percentage of variation from control. Different letters among treatments indicate significant differences.

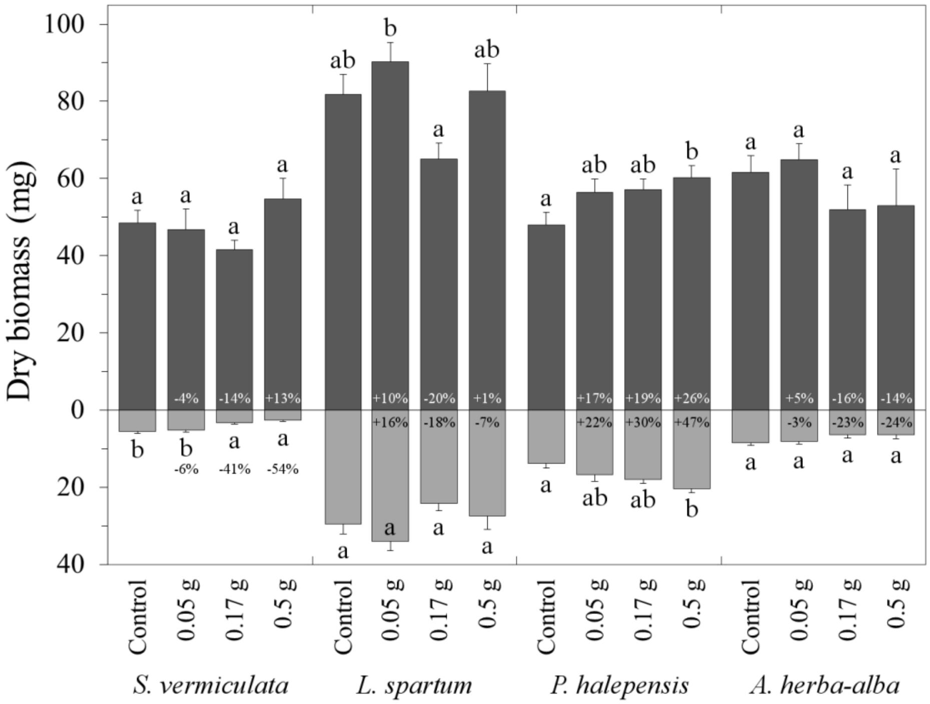


Fig. 2. Dry biomass (mean ± SE) of aerial parts (dark grey) and roots (light grey) of seedlings of *L. spartum* (left side) and *P. halepensis* (right side) after two months of watering with either 0.5, 2 or 5 g l^{-1} of aqueous extract of *A. herba-alba*. Numbers in the bars indicate the percentage of variation from control. Different letters among treatments indicate significant differences.

Phenolic compounds and the allelopathic effects of identified phenols

Chemical analyses determined that the TPC of *A. herba-alba* leaves (54.25 ± 1.15 mg GAE g^{-1}) and stems (36.15 ± 0.48 mg GAE g^{-1}) were approximately four and three times higher, respectively, than the TPC in the aqueous extract of *A. herba-alba* (12.39 ± 0.36 mg GAE per g of plant material used in aqueous extraction).

The HPLC chromatogram at 280 nm indicated that the aqueous extract of *A. herba-alba* contained at least 15 different phenolic compounds, one of which was very abundant. However, only three of the compounds were clearly identified and quantified in the aqueous extract: catechol, protocatechuic and vanillic acids (37.2, 9.4, and 2.2 mg l^{-1} respectively; Fig. 3).

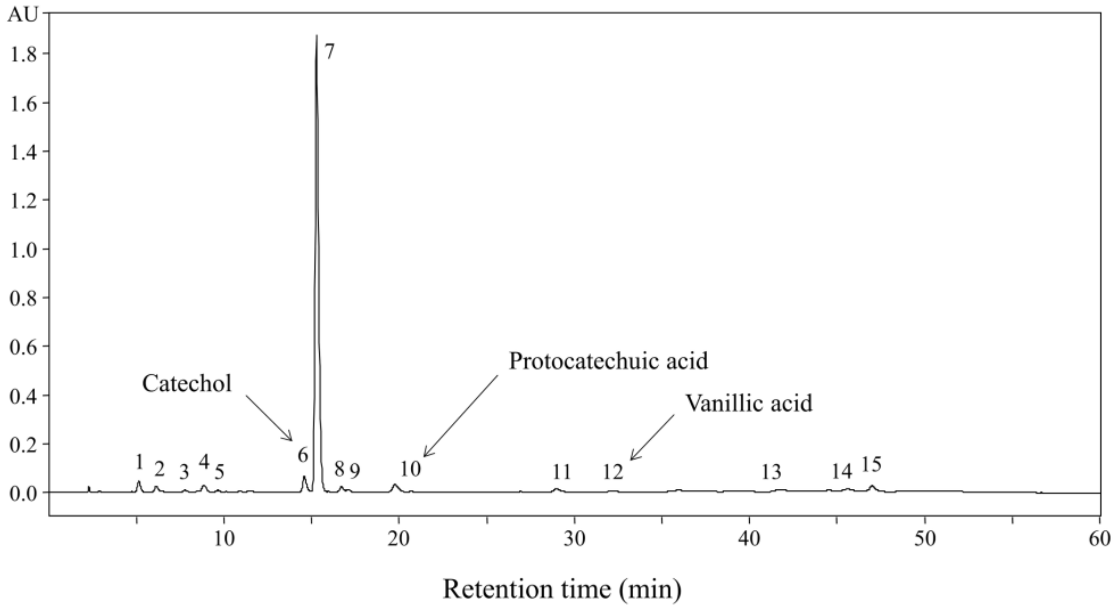


Fig. 3. HPLC chromatogram at 280 nm of *A. herba-alba* aqueous extract.

The mixture of pure phenolic compounds (*i.e.* catechol, protocatechuic and vanillic acids) did not have a significant effect on the germination of *S. vermiculata*, *L. spartum* and *P. halepensis* seeds, at all three concentration or time considered (Table 3). However, the highest concentration of the mixture significantly delayed germination of *A. herba-alba* seeds at d7 (Table 3).

Table 3. Seed germination (%; mean ± SE) and results of GLM analyses of the effects of a phenolic mixture of catechol, protocatechuic and vanillic acids on seed germination in four target species at 7, 14 and 21 d after exposure. The quantities of the pure phenolic compounds used at each treatment were the same than those found at each concentration of aqueous extract. Significant effects ($p < 0.05$) are highlighted in bold. Different letters in the same row indicate significant differences among treatments (Tukey´s HSD test).

		Treatments					
Species	Time	Control	0.5 g/l	2 g/l	5 g/l	Deviance (df=3)	p-value
S. vermiculata							
	d7	78^{a} ± 1.93	77^{a} ± 3.72	74^{a} ± 5.74	70^{a} ± 5.17	2.57	0.464
	d14	79^{a} ± 2.28	77^{a} ± 3.72	75^{a} ± 6.13	72^{a} ± 4.93	1.86	0.601
L. spartum							
	d7	54^{a} ± 11.57	66^{a} ± 9.45	70^{a} ± 6.15	66^{a} ± 6.70	3.08	0.380
	d14	78^{a} ± 6.96	76^{a} ± 8.33	86^{a} ± 6.70	90^{a} ± 4.47	4.69	0.196
	d21	84^{a} ± 5.81	86^{a} ± 6	88^{a} ± 6.80	92^{a} ± 4.42	1.68	0.642
P. halepensis							
	d7	1^{a} ± 1	3^{a} ± 1.53	6^{a} ± 3.06	3^{a} ± 1.53	4.23	0.237
	d14	10^{a} ± 2.58	12^{a} ± 3.89	22^{a} ± 4.90	20^{a} ± 4.22	7.85	< 0.05
	d21	14^{a} ± 2.67	24^{a} ± 3.05	26^{a} ± 4.76	24^{a} ± 4	5.62	0.132
A. herba-alba							
	d7	62^{a} ± 4.11	65^{a} ± 5	65^{a} ± 5.43	29^{b} ± 4.82	**38.57**	**< 0.001**
	d14	91^{a} ± 2.33	92^{a} ± 2.49	91^{a} ± 3.48	81^{a} ± 4.25	7.13	0.068
	d21	93^{a} ± 2.13	95^{a} ± 2.24	94^{a} ± 2.67	94^{a} ± 2.67	0.38	0.945

Discussion

In this study we investigated the autotoxic and allelopathic effects of the volatile and the water soluble chemicals produced by the shrub *A. herba-alba* on seed germination and early seedling growth in species that coexist in a natural semiarid plant community. As expected, both the volatile and water soluble chemicals strongly inhibited the germination of *A. herba-alba* seeds, highlighting the autotoxic nature of *A. herba-alba* (Friedman and Orshan, 1975). Previous studies also found autotoxic effects in *A. herba-alba*. Atoum et al. (2006) reported that exposure to aqueous extract obtained from *A. herba-alba* fruits might reduce initial elongation of *A. herba-alba* seedlings. In addition, it was found that water soluble chemicals can increase the mortality of *A. herba-alba* seedlings that emerge from the seed bank near *A. herba-alba* individuals (Arroyo et al., 2016). Autotoxicity has been observed in many crop systems (Singh et al., 1999), but also in natural environments such as Mediterranean forest of *P. halepensis* (Fernandez et al., 2008) and shrublands of *Cistus* *ladanifer* L. (Alías et al., 2006) and *Cistus clusii* Dunal (Foronda *et al. in rev*.). In a field study, Pueyo *et al.* (2016) found a pattern of segregation between adult *A. herba-alba* and other *A. herba-alba* individuals, independent of its ontogenetic stage (*i.e.* adults, juveniles or seedlings). Our study suggests that the volatile and the water soluble chemicals, in particular identified phenols, drive autotoxicity of *A. herba-alba* during germination, which may lead to the observed pattern among individuals in nature. Apart from the secondary metabolites, it has been argued that other mechanisms might also regulate the autotoxic process, such as the exposure to fragmented extracellular self-DNA (Mazzoleni et al., 2015). Given that individuals of the same species occupy a similar niche, a strong competition for resources might be expected to occur among them (Armas and Pugnaire, 2011). Therefore, in semiarid environments where resources are scarce (*e.g.*, water), autotoxicity may arise as an adaptive advantage to regulate population density, diminishing self-recruitment near parent species by inhibiting or delaying germination until rainfall and, thereby, preventing intraspecific competition (Armas and Pugnaire, 2011; Friedman and Waller, 1985; Singh et al., 1999). Indeed, autotoxicity (or biochemical recognition *sensu* Renne et al., 2014) might be a common strategy to avoid competitive environments (Renne et al., 2014). The dual role of some secondary metabolites in allelopathy and autotoxicity emphasize their complex roles on the structure of plant populations and communities (Ruan et al., 2011).

Although it was not possible a direct comparison between experiments, the autotoxic effect caused by the volatile chemicals was, apparently, higher than the autotoxic effect of the water soluble compounds. Often, the highest concentrations of allelochemicals cause the greatest inhibitory effects (Einhellig, 2004; Li et al., 2011; Reigosa and Pazos-Malvido, 2007). However, the results of our study suggest that, although the water soluble chemicals could act in a dose-dependent manner, the autotoxic effects of volatile chemicals on seed germination might be triggered by a threshold. Accordingly, we found that the higher concentration of water soluble chemicals inhibited the germination of *A. herba-alba* seeds while the low and intermediate concentrations only delayed it. Conversely, the germination inhibition caused by the volatile chemicals persisted over time and treatments. This might be caused because the volatile chemicals are released continuously from the ground aerial parts of *A. herba-alba*, whereas the amount of water soluble chemicals cannot be replaced when lost.

With respect to allelopathy, our results showed limited deleterious allelopathic effects of the volatile and water soluble compounds released from *A. herba-alba* on neighboring plant species. Those species might have some kind of tolerance to the allelochemicals produced by *A. herba-alba*. A long associational history between allelopathic plants (and their allelochemicals) and other coexisting species might lead some species to become adapted to its chemical neighbor (Ehlers and Thompson, 2004; Mallik and Pellissier, 2000; Renne et al., 2014). In such cases, a positive allelopathic effect of volatile and water soluble chemicals on plant growth might be expected. The latter situation might be exemplified by the perennial grass *L. spartum*. It has been previously suggested that *L. spartum* and other perennial grasses can take advantage of the benign microclimatic conditions that are created under the canopy of *A. herba-alba* individuals regardless of its allelopathic activity (Arroyo et al., 2015; Pueyo et al., 2016). The ratio of susceptible and tolerant species will dictate the impacts of an allelopathic species at community level. Consequently, allelopathic effects should be assessed from a multispecific perspective, including a number of target species covering several life forms and abundances in order to get reliable data on the impacts of an allelopathic species on plant community organization.

Interestingly, the volatile chemicals produced by *A. herba-alba* reduced germination of *P. halepensis* seeds, while the water soluble chemicals promoted it. Positive effects of allelochemicals have been reported too (Einhellig, 2004; Fernandez et al., 2013; Tsubo et al., 2012). Actually, low concentrations of a phytotoxic compound can result in stimulatory effects, which is known as hormetic effect (Duke et al., 2006). However, negative effects on the germination of neighboring species of higher concentrations of the water soluble chemicals of *A. herba-alba* were not found. Further studies investigating the joint effect of compounds from different chemical classes are necessary, as allelopathic effects in nature are expected to be the result of a number of compounds in a mixture acting jointly (Inderjit and Duke, 2003). In this sense, antagonistic effects of volatile and water soluble chemicals on seed germination could be very frequent when both classes of allelochemicals are simultaneously available for seeds (Dias and Moreira, 2002).

It has been reported the inhibitory effect of water soluble phenols on plant growth (Muscolo et al., 2013). However, in our study, water soluble chemicals from *A. herba-alba* had positive allelopathic effects on early seedling growth. Low concentrations of phenolic acids such as those identified in the aqueous extract of *A. herba-alba* can induce stimulatory responses (Einhellig, 2004). Similarly, it has been reported the negative effects that the volatile chemicals can have on seedling growth (Muller et al., 1964; Scognamiglio et al., 2013; Vokou et al., 2003). However, we found that the volatile chemicals of *A. herba-alba* had a positive effect on the early seedling growth of *P. halepensis*. In line with our results, Tsubo *et al.* (2012) found that exposure to low concentrations of the volatile chemicals released by *Artemisia adamsii* Besser stimulated photosynthetic rates in the grass *Stipa krilovii* Roshev., and, hence, its growth. Given the complex, and sometimes contradictory, effects of allelochemicals on germination and early seedling growth, these two processes should be separated in studies of allelopathy. Although those conditions would not occur in nature, that approach would allow the identification of the allelopathic effects of the volatile and water soluble chemicals purely on seedling growth.

We found an important total amount of phenolic compounds in the leaves and stems of *A. herba-alba*. Some have reported TPC values > 100 mg GAE g^{-1} in *A. herba-alba* (Khlifi et al., 2013) but others have reported values that were lower than those in our study (Bourgou et al., 2016; Younsi et al., 2016). The variation in TPC among studies might be caused by differences in the plant organs used in the analyses, in the season of plant material collection and in climatic conditions in different regions (Khlifi et al., 2013). Similarly, phenolic compounds including catechol, protocatechuic and vanillic acids were abundant in the aqueous extract of *A. herba-alba*. Protocatechuic and vanillic acids were already reported in *A. herba-alba* (Bourgou et al., 2016; Seddik et al., 2010). Our results showed that the pure phenolic mixture inhibited germination of *A. herba-alba* seeds, thus, suggesting that identified phenols might have contributed to the net autotoxic effect of the aqueous extract of *A. herba-alba*. However, some have argued that common phenolic acids are weakly phytotoxic (Dayan and Duke, 2009). Actually, in our study, autotoxic effects of the aqueous extract were apparently stronger than the effects of the mixture of pure phenols. On the one hand, there were other compounds in the aqueous extract of *A. herba-alba* that were unidentified, and might have contributed more to its autotoxicity than did the identified phenols. In this sense, it should be noted that the HPLC chromatogram at 280 nm indicated the presence of an extraordinarily abundant unidentified compound. *Artemisia herba-alba* is known to contain other phenolic compounds such as chlorogenic, ferulic and caffeic acids, apigenin, luteolin and quercetin (Saleh et al., 1985; Seddik et al., 2010; Younsi et al., 2016). In addition, it could be possible that there were very small amounts of certain compounds belonging to other chemical classes (*e.g.*, terpenes) in the aqueous extract, which might have inhibitory effects (Weidenhamer et al., 1993). On the other hand, pure compounds in the mixture probably degrade faster (Li et al., 2015) than they do in the aqueous extract, because those in the aqueous extract can bond to other molecules, which provide some protection against degradation.

We did not identify the chemical compounds released through volatilization in our study. However, the composition of the essential oils of *A. herba-alba* has been thoroughly investigated (Abad et al., 2012; Mohamed et al., 2010). The relative abundance of those compounds, even their presence is known to vary among geographic regions (Belhattab et al., 2014; Mohamed et al., 2010; Salido et al., 2004). Oxygenated monoterpenes are often found to be the major constituents. In particular, the presence of alfa/beta-thujones, camphor, chrysanthenone and 1,8-cineole is commonly reported in essential oils of *A. herba-alba* (Amri et al., 2013; Bourgou et al., 2016; Mohamed et al., 2010; Salido et al., 2004; Younsi et al., 2016). It should be noted that in our study some volatile compounds could be lost, at least partially, during the drying process (Verma et al., 2010). However, our results highlight the potential of *A. herba-alba* litter as a significant source of volatile phytotoxic compounds involved in autotoxicity and affecting the germination and early growth of some neighboring plant species once released into the environment (Araniti et al., 2016). Further research might use living plant material to investigate the autotoxic and allelopathic effects of the volatile chemicals.

In conclusion, our study highlighted that both the volatile and the water soluble allelochemicals are involved in intra- and inter-specific interactions in semiarid environments. We found only limited allelopathic effects of the volatile chemicals released from *A. herba-alba* on the germination of some neighboring plant species (*P. halepensis*) and generalized positive effects of the volatile and water soluble chemicals on early seedling growth. These findings suggested that target species selected in our study might have a certain tolerance to allelochemicals of *A. herba-alba* because of co-evolution. Regarding autotoxicity, both types of allelochemicals had strong autotoxic effects on the germination of *A. herba-alba* seeds. On the other hand, we found that phenolic compounds were abundant in the *A. herba-alba* aqueous extract. Our results pointed out that the identified phenolic compounds can be partially responsible for the autotoxicity of the aqueous extract. However, the identified phenols did not have allelopathic activity. Further studies should go in depth for the identification of the unknown phenolic compounds in the aqueous extract of *A. herba-alba* and evaluate, through new bioassays, the effects of mixtures of compounds that belong to different chemical classes acting jointly. In addition, field research is required to test our conclusions in natural conditions, and to fully understand the significance of chemical interference by allelopathic plants in the dynamics of vegetation and community organization.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

AIA, YP and FP conceived the study and designed the experiments. AIA, AM and FP performed chemical analyses. AIA, FP and JR performed the bioassays. AIA and YP analyzed the data and wrote the manuscript with contributions of all co-authors.

Funding

This research was funded by the Spanish Ministry of Education, Culture and Sport (AP-2012-4126 grant to A.I.A., FPU Program) and by the Spanish Ministry of Economy and Competitiveness (CGL-2012-37508 and CGL-2016-80783-R projects).

Acknowledgements

We especially thank to C. Gallet for her invaluable help with biochemical analyses, M.L. Giner and P. Bravo for their field assistance, help with the experimental set up and data collection and J. Cosculluela ("Viveros Ejea") for kindly providing *P. halepensis* seeds used in bioassays. We are grateful to two anonymous reviewers for their valuable comments, which helped to improve the manuscript.

Supplementary material

Supplementary Table S1 may be found in the online version of this article.

References

Abad, M. J., Bedoya, L. M., Apaza, L., and Bermejo, P. (2012). The Artemisia L. genus: a review of bioactive essential oils. *Molecules* 17, 2542–2566. doi:10.3390/molecules17032542.

Akula, R., and Ravishankar, G. A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signal. Behav.* 6, 1720–1731. doi:10.4161/psb.6.11.17613.

Alías, J. C., Sosa, T., Escudero, J. C., and Chaves, N. (2006). Autotoxicity against germination and seedling emergence in Cistus ladanifer L. *Plant Soil* 282, 327–332. doi:10.1007/s11104-005-6066-y.

Amri, I., De Martino, L., Marandino, A., Lamia, H., Mohsen, H., Scandolera, E., et al. (2013). Chemical composition and biological activities of the essential oil from Artemisia herba-alba growing wild in Tunisia. *Nat. Prod. Commun.* 8, 407–410.

Araniti, F., Gullì, T., Marrelli, M., Statti, G., Gelsomino, A., and Abenavoli, M. R. (2016). Artemisia arborescens L. leaf litter: phytotoxic activity and phytochemical characterization. *Acta Physiol. Plant.* 38. doi:10.1007/s11738-016-2141-7.

Araniti, F., Sorgonà, A., Lupini, A., and Abenavoli, M. R. (2012). Screening of Mediterranean wild plant species for allelopathic activity and their use as bio-herbicides. *Allelopathy J.* 29, 107–124.

Armas, C., and Pugnaire, F. I. (2011). Belowground zone of influence in a tussock grass species. *Acta Oecologica* 37, 284–289. doi:10.1016/j.actao.2011.02.013.

Arroyo, A. I., Pueyo, Y., Reiné, R., Giner, M. L., and Alados, C. L. (2016). Effects of the allelopathic plant Artemisia herba-alba Asso on the soil seed bank of a semi-arid plant community. *J. Plant Ecol.*, rtw120. doi:10.1093/jpe/rtw120.

Arroyo, A. I., Pueyo, Y., Saiz, H., and Alados, C. L. (2015). Plant-plant interactions as a mechanism structuring plant diversity in a Mediterranean semi-arid ecosystem. *Ecol. Evol.* 5, 5305–5317. doi:10.1002/ece3.1770.

Atoum, M., Al-Charchafchi, F., and Modallal, N. (2006). Biological activity and antimutagencity of water soluble phytotoxins from Artemisia herba alba Asso. *Pak. J. Biol. Sci.* 9, 1774–1778. doi:10.3923/pjbs.2006.1774.1778.

Bautista, I., Boscaiu, M., Lidón, A., Llinares, J. V., Lull, C., Donat, M. P., et al. (2016). Environmentally induced changes in antioxidant phenolic compounds levels in wild plants. *Acta Physiol. Plant.* 38. doi:10.1007/s11738-015-2025-2.

Belhattab, R., Amor, L., Barroso, J. G., Pedro, L. G., and Cristina Figueiredo, A. (2014). Essential oil from Artemisia herba-alba Asso grown wild in Algeria: variability assessment and comparison with an updated literature survey. *Arab. J. Chem.* 7, 243–251. doi:10.1016/j.arabjc.2012.04.042.

Bourgou, S., Tammar, S., Salem, N., Mkadmini, K., and Msaada, K. (2016). Phenolic composition, essential oil, and antioxidant activity in the aerial part of *Artemisia herba-alba* from several provenances: a comparative study. *Int. J. Food Prop.* 19, 549–563. doi:10.1080/10942912.2015.1040495.

Callaway, R. M. (2007). "Direct Mechanisms for Facilitation," in *Positive Interactions and Interdependence in Plant Communities* (Dordrecht: Springer Netherlands), 15–116.

Castaldi, S., Carfora, A., Fiorentino, A., Natale, A., Messere, A., Miglietta, F., et al. (2009). Inhibition of net nitrification activity in a Mediterranean woodland: possible role of chemicals produced by Arbutus unedo. *Plant Soil* 315, 273–283. doi:10.1007/s11104-008-9750-x.

Cerdà, A. (1997). The effect of patchy distribution of Stipa tenacissima L. on runoff and erosion. *J. Arid Environ.* 36, 37–51. doi:10.1006/jare.1995.0198.

Chen, S., Xiao, S., and Callaway, R. M. (2012). Light intensity alters the allelopathic effects of an exotic invader. *Plant Ecol. Divers.* 5, 521–526. doi:10.1080/17550874.2012.736548.

Chiapusio, G., Sánchez, A. M., Reigosa, M. J., González, L., and Pellissier, F. (1997). Do germination indices adequately reflect allelochemical effects on the germination process? *J. Chem. Ecol.* 23, 2445–2453. doi:10.1023/B:JOEC.0000006658.27633.15.

Chou, C.-H. (1999). Roles of allelopathy in plant biodiversity and sustainable agriculture. *Crit. Rev. Plant Sci.* 18, 609–636. doi:10.1080/07352689991309414.

Dayan, F. E., and Duke, S. O. (2009). "Biological Activity of Allelochemicals," in *Plant-derived Natural Products*, eds. A. E. Osbourn and V. Lanzotti (New York, NY: Springer US), 361–384.

Dias, L. S., and Moreira, I. (2002). Interaction between water soluble and volatile compounds of Cistus ladanifer L. *CHEMOECOLOGY* 12, 77–82. doi:10.1007/s00049-002-8329-x.

Duke, S. O., Cedergreen, N., Velini, E. D., and Belz, R. G. (2006). Hormesis: Is it an important factor in Herbicide use and Allelopathy? *Outlooks Pest Manag.* 17, 29–33. doi:10.1564/16feb10.

Ehlers, B. K., and Thompson, J. (2004). Do co-occurring plant species adapt to one another? The response of Bromus erectus to the presence of different Thymus vulgaris chemotypes. *Oecologia* 141, 511–518. doi:10.1007/s00442-004-1663-7.

Einhellig, F. A. (2004). “Mode of allelochemical action of phenolic compounds,” in *Allelopathy: chemistry and mode of action of allelochemicals*, eds. F. A. Macias, J. C. G. Galindo, J. M. G. Molinillo, and H. G. Cutler (Boca Raton, FL: CRC Press), 217–238.

Escudero, A., Albert, M. J., Pita, J. M., and Pérez-García, F. (2000). Inhibitory effects of Artemisia herba-alba on the germination of the gypsophyte Helianthemum squamatum. *Plant Ecol.* 148, 71–80. doi:10.1023/A:1009848215019.

Fernandez, C., Santonja, M., Gros, R., Monnier, Y., Chomel, M., Baldy, V., et al. (2013). Allelochemicals of Pinus halepensis as drivers of biodiversity in Mediterranean open mosaic habitats during the colonization stage of secondary succession. *J. Chem. Ecol.* 39, 298–311. doi:10.1007/s10886-013-0239-6.

Fernandez, C., Voiriot, S., Mévy, J.-P., Vila, B., Ormeño, E., Dupouyet, S., et al. (2008). Regeneration failure of Pinus halepensis Mill.: The role of autotoxicity and some abiotic environmental parameters. *For. Ecol. Manag.* 255, 2928–2936. doi:10.1016/j.foreco.2008.01.072.

Friedjung, A., Choudhary, S. P., Dudai, N., and Rachmilevitch, S. (2013). Physiological conjunction of allelochemicals and desert plants. *PLoS ONE* 8, e81580. doi:10.1371/journal.pone.0081580.

Friedman, J., and Orshan, G. (1975). The distribution, emergence and survival of seedlings of Artemisia herba-alba Asso in the Negev desert of Israel in relation to distance from the adult plants. *J. Ecol.* 63, 627. doi:10.2307/2258740.

Friedman, J., Orshan, G., and Ziger-Cfir, Y. (1977). Suppression of annuals by Artemisia herba-alba in the Negev desert of Israel. *J. Ecol.* 65, 413–426. doi:10.2307/2259491.

Friedman, J., and Waller, G. R. (1985). Allelopathy and autotoxicity. *Trends Biochem. Sci.* 10, 47–50. doi:10.1016/0968-0004(85)90224-5.

Hättenschwiler, S., and Vitousek, P. M. (2000). The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends Ecol. Evol.* 15, 238–243. doi:10.1016/S0169-5347(00)01861-9.

Herranz, J. M., Ferrandis, P., Copete, M. A., Duro, E. M., and Zalacaín, A. (2006). Effect of allelopathic compounds produced by Cistus ladanifer on germination of 20 Mediterranean taxa. *Plant Ecol.* 184, 259–272. doi:10.1007/s11258-005-9071-6.

Inderjit, and Duke, S. O. (2003). Ecophysiological aspects of allelopathy. *Planta* 217, 529–539. doi:10.1007/s00425-003-1054-z.

Inderjit, and Mallik, A. U. (1997). Effect of phenolic compounds on selected soil properties. *For. Ecol. Manag.* 92, 11–18. doi:10.1016/S0378-1127(96)03957-6.

Khlifi, D., Sghaier, R. M., Amouri, S., Laouini, D., Hamdi, M., and Bouajila, J. (2013). Composition and anti-oxidant, anti-cancer and anti-inflammatory activities of Artemisia herba-alba, Ruta chalpensis L. and Peganum harmala L. *Food Chem. Toxicol.* 55, 202–208. doi:10.1016/j.fct.2013.01.004.

Li, J., Zhang, Q., Hu, W., Yang, X., and He, H. (2015). Stability of phenolic acids and the effect on weed control activity. *J. Korean Soc. Appl. Biol. Chem.* 58, 919–926. doi:10.1007/s13765-015-0124-9.

Li, X. F., Wang, J., Huang, D., Wang, L. X., and Wang, K. (2011). Allelopathic potential of Artemisia frigida and successional changes of plant communities in the northern China steppe. *Plant Soil* 341, 383–398. doi:10.1007/s11104-010-0652-3.

Li, Z. H., Wang, Q., Ruan, X., Pan, C.-D., and Jiang, D.-A. (2010). Phenolics and plant allelopathy. *Molecules* 15, 8933–8952. doi:10.3390/molecules15128933.

Mallik, A. U., and Pellissier, F. (2000). Effects of Vaccinium myrtillus on spruce regeneration: testing the notion of coevolutionary significance of allelopathy. *J. Chem. Ecol.* 26, 2197–2209. doi:10.1023/A:1005528701927.

Mazzoleni, S., Bonanomi, G., Incerti, G., Chiusano, M. L., Termolino, P., Mingo, A., et al. (2015). Inhibitory and toxic effects of extracellular self-DNA in litter: a mechanism for negative plant-soil feedbacks? *New Phytol.* 205, 1195–1210. doi:10.1111/nph.13121.

Meiners, S. J. (2014). Functional correlates of allelopathic potential in a successional plant community. *Plant Ecol.* 215, 661–672. doi:10.1007/s11258-014-0331-1.

Mohamed, A. E.-H. H., El-Sayed, M. A., Hegazy, M. E., Helaly, S. E., Esmail, A. M., and Mohamed, N. S. (2010). Chemical constituents and biological activities of Artemisia herba-alba. *Rec. Nat. Prod.* 4, 1–25.

Moral, R. D., and Cates, R. G. (1971). Allelopathic potential of the dominant vegetation of western Washington. *Ecology* 52, 1030–1037. doi:10.2307/1933809.

Muller, C. H., Muller, W. H., and Haines, B. L. (1964). Volatile growth inhibitors produced by aromatic shrubs. *Science* 143, 471–473. doi:10.1126/science.143.3605.471.

Muscolo, A., and Sidari, M. (2010). "Source of soil phenolic compounds," in *Soil phenols*, eds. A. Muscolo and M. Sidari (New York, NY: Nova Science Publishers, Inc.), 1–30.

Muscolo, A., Sidari, M., and Texeira da Silva, J. A. (2013). Biological effects of water soluble soil phenol and soil humic extracts on plant systems. *Acta Physiol. Plant.* 35, 309–320. doi:10.1007/s11738-012-1065-0.

Noy-Meir, I. (1973). Desert ecosystems: environment and producers. *Annu. Rev. Ecol. Syst.* 4, 25–51. doi:10.1146/annurev.es.04.110173.000325.

Pedrol, N., González, L., and Reigosa, M. J. (2006). "Allelopathy and abiotic stress," in *Allelopathy*, eds. M. J. Reigosa, N. Pedrol, and L. González (Dordrecht: Springer Netherlands), 171–209. Available at: http://dx.doi.org/10.1007/1-4020-4280-9_9.

Pueyo, Y., Moret-Fernández, D., Arroyo, A. I., de Frutos, A., Kéfi, S., Saiz, H., et al. (2016). Plant nurse effects rely on combined hydrological and ecological components in a semiarid ecosystem. *Ecosphere* 7, e01514. doi:10.1002/ecs2.1514.

R Core Team (2015). *R: a language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing Available at: https://www.R-project.org/.

Reigosa, M. J., and Pazos-Malvido, E. (2007). Phytotoxic effects of 21 plant secondary metabolites on Arabidopsis thaliana germination and root growth. *J. Chem. Ecol.* 33, 1456–1466. doi:10.1007/s10886-007-9318-x.

Reigosa, M. J., Sánchez-Moreiras, A., and González, L. (1999). Ecophysiological approach in allelopathy. *Crit. Rev. Plant Sci.* 18, 577–608. doi:10.1080/07352689991309405.

Renne, I. J., Sinn, B. T., Shook, G. W., Sedlacko, D. M., Dull, J. R., Villarreal, D., et al. (2014). Eavesdropping in plants: delayed germination via biochemical recognition. *J. Ecol.* 102, 86–94. doi:10.1111/1365-2745.12189.

Rice, E. L. (1984). *Allelopathy (Second Edition)*. San Diego: Academic Press.

Ruan, X., Li, Z.-H., Wang, Q., Pan, C.-D., Jiang, D.-A., and Wang, G. G. (2011). Autotoxicity and allelopathy of 3,4-dihydroxyacetophenone isolated from Picea schrenkiana needles. *Molecules* 16, 8874–8893. doi:10.3390/molecules16108874.

Saleh, N. A. M., El-Negoumy, S. I., Abd-Alla, M. F., Abou-Zaid, M. M., Dellamonica, G., and Chopin, J. (1985). Flavonoid glycosides of Artemisia monosperma and A. herba-alba. *Phytochemistry* 24, 201–203. doi:10.1016/S0031-9422(00)80845-6.

Salido, S., Valenzuela, L. R., Altarejos, J., Nogueras, M., Sánchez, A., and Cano, E. (2004). Composition and infraspecific variability of Artemisia herba-alba from southern Spain. *Biochem. Syst. Ecol.* 32, 265–277. doi:10.1016/j.bse.2003.09.002.

Scognamiglio, M., D'Abrosca, B., Esposito, A., Pacifico, S., Monaco, P., and Fiorentino, A. (2013). Plant growth inhibitors: allelopathic role or phytotoxic effects? Focus on Mediterranean biomes. *Phytochem. Rev.* 12, 803–830. doi:10.1007/s11101-013-9281-9.

Seddik, K., Nadjet, I., Daoud, B. A. H., and Lekhmici, A. (2010). Antioxidant and antibacterial activities of extracts from Artemisia herba alba Asso. leaves and some phenolic compounds. *J. Med. Plants Res.* 4, 1273–1280. doi:10.5897/JMPR09.379.

Singh, H. P., Batish, D. R., and Kohli, R. K. (1999). Autotoxicity: concept, organisms, and ecological significance. *Crit. Rev. Plant Sci.* 18, 757–772. doi:10.1080/07352689991309478.

Singleton, V. L., and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16, 144–158.

Tilaoui, M., Ait Mouse, H., Jaafari, A., and Zyad, A. (2015). Comparative phytochemical analysis of essential oils from different biological parts of Artemisia herba alba and their cytotoxic effect on cancer cells. *PLoS ONE* 10, e0131799. doi:10.1371/journal.pone.0131799.

Tsubo, M., Nishihara, E., Nakamatsu, K., Cheng, Y., and Shinoda, M. (2012). Plant volatiles inhibit restoration of plant species communities in dry grassland. *Basic Appl. Ecol.* 13, 76–84. doi:10.1016/j.baae.2011.11.005.

van der Putten, W. H. (2009). A multitrophic perspective on functioning and evolution of facilitation in plant communities. *J. Ecol.* 97, 1131–1138. doi:10.1111/j.1365-2745.2009.01561.x.

Varela, M. C., Arslan, I., Reginato, M. A., Cenzano, A. M., and Luna, M. V. (2016). Phenolic compounds as indicators of drought resistance in shrubs from Patagonian shrublands (Argentina). *Plant Physiol. Biochem.* 104, 81–91. doi:10.1016/j.plaphy.2016.03.014.

Verma, R. S., Rahman, L., Chanotiya, C. S., Verma, R. K., Chauhan, A., Yadav, A., et al. (2010). Chemical composition of volatile fraction of fresh and Dry Artemisia capillaris Thunb. from Kumaon Himalaya. *J. Essent. Oil Bear. Plants* 13, 118–122. doi:10.1080/0972060X.2010.10643799.

Vilà, M., and Sardans, J. (1999). Plant competition in mediterranean-type vegetation. *J. Veg. Sci.* 10, 281–294. doi:10.2307/3237150.

Vokou, D., Douvli, P., Blionis, G. J., and Halley, J. M. (2003). Effects of monoterpenoids, acting alone or in pairs, on seed germination and subsequent seedling growth. *J. Chem. Ecol.* 29, 2281–2301.

Wang, J., Li, X., Zhang, J., Yao, T., Wei, D., Wang, Y., et al. (2012). Effect of root exudates on beneficial microorganisms—evidence from a continuous soybean monoculture. *Plant Ecol.* 213, 1883–1892. doi:10.1007/s11258-012-0088-3.

Weidenhamer, J. D., Macias, F. A., Fischer, N. H., and Williamson, G. B. (1993). Just how insoluble are monoterpenes? *J. Chem. Ecol.* 19, 1799–1807. doi:10.1007/BF00982309.

Younsi, F., Trimech, R., Boulila, A., Ezzine, O., Dhahri, S., Boussaid, M., et al. (2016). Essential oil and phenolic compounds of Artemisia herba-alba (Asso.): composition, antioxidant, antiacetylcholinesterase, and antibacterial activities. *Int. J. Food Prop.* 19, 1425–1438. doi:10.1080/10942912.2015.1079789.

Appendix 1

Table S1. Summary of the methods used in the germination and growth bioassays. See “Material and Methods” section for further details.

Bioassay	Plant material	Allelopathic agent	Treatments				Target species	Replicates *	Conditions **
			Control	Low	Mid	High			
Seed germination (21 days)	Ground *A. herba-alba* aerial parts	Volatile chemicals	0 g	0.05 g	0.1 g	0.5 g	*S. vermiculata*	10 seeds x 10 replicates x 4 treatments	Tª = 18-23ºC Light = 10h natural + 2h artificial
							L. spartum	5 seeds x 10 replicates x 4 treatments	
							P. halepensis	10 seeds x 10 replicates x 4 treatments	
							A. herba-alba	10 seeds x 10 replicates x 4 treatments	
	A. herba-alba aqueous extract	Water soluble chemicals	Demineralized water	0.5 g/l	2 g/l	5 g/l	*S. vermiculata*	10 seeds x 10 replicates x 4 treatments	Tª = 20-24ºC (5-15ºC) Light = 12h artificial
							L. spartum	5 seeds x 10 replicates x 4 treatments	
							P. halepensis	10 seeds x 10 replicates x 4 treatments	
							A. herba-alba	10 seeds x 10 replicates x 4 treatments	
	Mixture of pure phenolic compounds	Phenolic compounds	Demineralized water	0.5 g/l	2 g/l	5 g/l	*S. vermiculata*	10 seeds x 10 replicates x 4 treatments	Tª = 20-24ºC (5-15ºC) Light = 12h artificial
							L. spartum	5 seeds x 10 replicates x 4 treatments	
							P. halepensis	10 seeds x 10 replicates x 4 treatments	
							A. herba-alba	10 seeds x 10 replicates x 4 treatments	
Early seedling growth (8 weeks)	Ground *A. herba-alba* aerial parts	Volatile chemicals	0 g	3 g (0.05 g per pot)	10 g (0.17 g per pot)	30 g (0.5 g per pot)	*S. vermiculata*	1 seedling x 15 replicates x 4 treatments	Tª = 20-25ºC Light = 12h natural
							L. spartum	1 seedling x 15 replicates x 4 treatments	
							P. halepensis	1 seedling x 15 replicates x 4 treatments	
							A. herba-alba	1 seedling x 15 replicates x 4 treatments	
	A. herba-alba aqueous extract	Water soluble chemicals	Demineralized water	0.5 g/l	2 g/l	5 g/l	*S. vermiculata*	1 seedling x 15 replicates x 4 treatments	Tª = 20-24ºC Light = 12h artificial
							L. spartum	1 seedling x 15 replicates x 4 treatments	
							P. halepensis	1 seedling x 15 replicates x 4 treatments	
							A. herba-alba	1 seedling x 15 replicates x 4 treatments	

* In the growth bioassays, not all pots had a seedling. Therefore, for some target species the analyses were based on < 15 replicates.

** Particular conditions for *A. herba-alba* in brackets.

Evidence for allelopathic interference of neighboring plant species by *Artemisia herba-alba* Asso: a field study

Antonio I. Arroyo, Yolanda Pueyo, Mª Luz Giner, Ana Foronda,
Pedro Sanchez-Navarrete, Hugo Saiz, Concepción L. Alados

In revision

Evidence for allelopathic interference of neighboring plant species by Artemisia herba-alba Asso: a field study

Antonio I. Arroyo[1], Yolanda Pueyo[1], Mª Luz Giner[1], Ana Foronda[1], Pedro Sanchez-Navarrete[1], Hugo Saiz[2], Concepción L. Alados[1]

[1] Instituto Pirenaico de Ecología, Consejo Superior de Investigaciones Científicas, Av. Montañana 1005, 50059 Zaragoza, Spain.

[2] Departamento de Biología y Geología, Física y Química Inorgánica, Universidad Rey Juan Carlos, C/ Tulipán s/n, 28933 Móstoles, Spain.

Abstract

The role of facilitative and competitive interactions in driving the composition and structure of plant communities has been intensively studied over recent decades. However, allelopathy, the phenomenon whereby one plant interferes with neighboring plants through the release of inhibitory chemicals compounds into the local environment, has received much less attention. Although many studies have reported testing of the phytotoxicity of allelopathic compounds under controlled conditions, field studies are required to provide realistic evidences for the significance of chemical interference in natural communities. In this study, we conducted field experiments in a semiarid plant community in northeastern Spain. The experiments were designed to assess chemical interference of the growth, vitality, seed germination and seedling survival of plant species in the vicinity of the allelopathic species *Artemisia herba-alba* Asso., and to evaluate whether the allelopathic activity of *A. herba-alba* affects the outcome of its interactions with neighboring plants under various stress conditions. We planted juvenile individuals and sowed seeds of *Salsola vermiculata* L., *Lygeum spartum* L. and *A. herba-alba* beneath the canopy of adult individuals of *A. herba-alba* with and without the addition of activated carbon (AC) to the soil. In addition, juveniles and seeds of the same three test species were planted or sown in open bare soil and beneath the canopy of adults of *S. vemiculata* (a shrub similar to *A. herba-alba*, but non-allelopathic). We computed the Relative Interaction Index (RII) to estimate the net interaction outcome for plants located in *A. herba-alba* microsite and compared it with the interaction outcome for plants in *S. vermiculata* microsite. We found that seed germination and juvenile vitality were enhanced for plants beneath *A. herba-alba* individuals when AC was present. Furthermore, we found that the interaction outcome in *A. herba-alba* microsite was neutral whereas a positive outcome was found for *S. vermiculata* microsite. This suggests that allelopathy limits the potential facilitative effects of the enhanced microclimatic conditions in *A. herba-alba* microsite. Yet, *L. spartum* juveniles were facilitated in *A. herba-alba* microsite. In addition, the interaction outcome in *A. herba-alba* microsite was positive under conditions of very high abiotic stress, indicating that facilitative interactions predominated over the interference of allelopathic plants under those conditions. This is one of the few studies of allelopathy undertaken in a natural plant community. Our findings highlight that laboratory studies can overestimate the significance of allelopathy in nature, and that as a consequence, results obtained under controlled conditions should be interpreted carefully.

Keywords

Activated carbon (AC), allelopathy, auto-toxicity, facilitation, germination, *Lygeum spartum*, plant growth, Relative Interaction Index (RII), *Salsola vermiculata*, semiarid plant community.

Introduction

The composition and structure of plant communities in arid and semiarid environments are markedly influenced by positive or negative interactions that occur among plants (Tilman 1988; Schöb, Armas & Pugnaire 2013). Positive interactions occur when the presence of one plant (*e.g.* a nurse shrub) facilitates the establishment, growth or reproduction of neighboring plants, usually by modifying and improving the microclimatic conditions and resource availability for those plants (Callaway 2007; Macek *et al.* 2016). Negative interactions occur when one plant hinders the presence of neighboring plants. Negative interactions include competition for limiting resources, for instance water and nutrients (Tilman 1988), but also include allelopathy, which is a chemically mediated interaction that has received much less attention.

Allelopathy can be defined as the interference that a plant exerts over other plants through the production and release of "toxic" compounds into the local environment as a result of volatilization, root exudates, leaf leachates and plant litter decomposition (Rice 1984). These compounds can directly reduce germination, growth and survival of neighboring plants because of its biological effects on cell division, membrane permeability, respiration or photosynthesis (Gniazdowska & Bogatek 2005), but also indirectly by modifying the activity of associated soil microorganisms and their influence on ecosystem processes (Castaldi *et al.* 2009; Chomel *et al.* 2016). Allelopathic plants can cause a number of changes in vegetation patterning including bare rings, inhibition zones, monocultures and root segregation (Inderjit, Weston & Duke 2005; Armas & Pugnaire 2011). In addition, it has been reported that in species-rich Mediterranean shrublands the presence of an allelopathic shrub can lead to an increase in plant diversity through its negative effects on highly competitive plant species (indirect facilitation; Ehlers, Charpentier & Grøndahl 2014). Therefore, allelopathic interference should be considered as an important driver of community structure in arid and semiarid plant communities.

The net outcome of positive and negative interactions varies according to the degree of environmental harshness. The frequency and intensity of positive interactions increases with the level of abiotic stress (Bertness & Callaway 1994; López *et al.* 2016; but see Maestre *et al.* 2009). However, the synthesis, release, accumulation and phytotoxicity of allelopathic compounds are also enhanced under harsh abiotic conditions, including water scarcity, high temperatures or intense solar radiation (Friedman 1987; Einhellig 1994; Pedrol, González & Reigosa 2006). Thus, the potential benefits of the more benign microclimatic conditions that occur beneath allelopathic plants may be reduced or negated under more severe abiotic conditions. On the other hand, co-evolution may have led to local adaptation of some species to their chemical neighbor (Vivanco *et al.* 2004; Grøndahl & Ehlers 2008). Therefore, a net positive outcome from such interactions might be possible for tolerant species. Despite these processes operating simultaneously, the role of chemical interference modulating the net interaction outcome for allelopathic shrubs in arid and semiarid environments has been largely unexplored.

Several laboratory and greenhouse studies have confirmed the phytotoxicity of chemicals released from allelopathic plants, and this has usually been taken as direct evidence of allelopathy. However, experimental conditions (*i.e.* types, concentrations and exposure to allelopathic compounds, and the absence of soil and/or its microorganisms) are far from those found in nature, and consequently, field experiments are needed to provide a realistic understanding of the significance of allelopathy under natural conditions (Inderjit & Weston 2000). However, the methods required to assess allelopathic interference under natural conditions are challenging (Wardle *et al.* 1998; Inderjit & Callaway 2003). Activated carbon (AC) is commonly used as an effective way to reduce the chemical interference of allelopathic plants, because of its large capacity to adsorb biochemical compounds (Callaway & Aschehoug 2000; Inderjit & Callaway 2003; Hille & den Ouden 2005). Yet, AC may alter nutrient concentrations in the soil or modify other soil characteristics such as pH and water retention (Inderjit & Callaway 2003; Lau *et al.* 2008; Weißhuhn & Prati 2009). These effects can confuse interpretation of the beneficial effects of the release of chemical interference, and this possibility needs to be carefully assessed (Lau *et al.* 2008). Furthermore, most of the few field studies on allelopathy have been performed in managed ecosystems (*e.g.* Kulmatiski 2011; Li, Nie & Zhang 2016; Zhang *et al.* 2016) or communities invaded by exotic plant species (*e.g.* Cipollini, McClain & Cipollini 2008; Gómez-Aparicio & Canham 2008; Fabbro, Güsewell & Prati 2013), while almost no research has focused on undisturbed plant communities.

In this study we assessed the significance of chemical interference by an allelopathic plant in a natural semiarid plant community. Specifically, we conducted a 2-year field experiment to (1) determine the impacts of the allelopathic plant *Artemisia herba-alba* Asso. (desert wormwood; Friedman, Orshan & Ziger-Cfir 1977; Arroyo *et al.* 2016) on the growth, vitality, seed germination and seedling survival of neighboring plant species with and without the presence of chemical interference by the incorporation of AC, and to (2) assess the net interaction outcome for *A. herba-alba* in comparison with that for the similar but non-allelopathic shrub *Salsola vermiculata* L. (Mediterranean saltwort). *Artemisia herba-alba* and *S. vermiculata* have similar physiognomy and functional traits. Both are long-live shrubs having a moderately dense canopy that provides a similar degree of amelioration of abiotic conditions (Pueyo *et al.* 2016). We hypothesized that (i) allelopathic interference by *A. herba-alba* is an important process under field conditions. Specifically, we expected that plant growth, plant vitality, seed germination and seedling survival of neighboring species beneath the canopy of *A. herba-alba* would be enhanced through the addition of AC. Furthermore, we hypothesized that (ii) the allelopathic activity of *A. herba-alba* limits potential facilitative effects. In particular, we expected that the net interaction outcome for *S. vermiculata* would be positive, while that for *A. herba-alba* would be negative, even under very harsh abiotic stress conditions.

Material and methods

Study area

The study was conducted in the middle Ebro Valley (NE Spain), in the "El Planerón de Belchite" ornithological reserve (41º22´09´´N, 00º37´50´´W). This area is characterized by a semiarid Mediterranean climate, and has an average annual rainfall of 310 mm and average annual temperature of 15.4 ºC. Rainfall occurs mostly in spring and autumn, whereas winter and summer are usually dry (Fig. S1). In the study area the summers are particularly warm, with the mean maximum summer temperatures exceeding 30 ºC, and the absolute temperature peaks at over 40 ºC (data obtained from the digital climatic atlas of Aragón; http://anciles.aragon.es/AtlasClimatico/). Hence, the vegetation is periodically subjected to severe hydric stress. Moreover, the soils have a very high clay content, which results in poor water infiltration and exacerbates the water deficit. The main human activity in the area is traditional agro-pastoral land use (Pueyo 2005). The landscape consists of a mosaic of dry cereal crops and non-cultivated lands. The steppe plant community on non-cultivated lands is composed of small shrubs (*e.g. A. herba-alba*, *S. vermiculata* and *Suaeda vera* J.F.G.mel.), perennial grasses (*e.g. Dactylis glomerata* L. subsp. *hispanica* (Roth) Nyman, *Lygeum spartum* L. and *Stipa parviflora* Desf.) and several ephemeral herbs. The vegetation is clumped in patches within a matrix of bare soil.

Allelopathic effects of *A. herba-alba*

Juvenile plants (< 1 year old) of *S. vermiculata*, *L. spartum* and *A. herba-alba*, which are the three co-dominant species in the community, were transplanted beneath the canopy of *A. herba-alba* adults to assess the potential allelopathic impacts of *A. herba-alba* on plant growth and vitality. Specifically, 90 adults of *A. herba-alba* were randomly selected in 1.3 ha of the study area having homogeneous slope and soil type. In early February 2014, 15 juveniles of each test species (n = 45) were transplanted beneath the canopy of selected *A. herba-alba* adults with the addition of AC (Charcoal Activated powder; PanReac AppliChem; Cu < 50 ppm, Fe < 500 ppm, Ni < 50 ppm, Pb < 50 ppm) to the soil at a concentration of 2% (*i.e.* 2 g of AC per 100 g of soil). Another 15 juveniles of each test species were transplanted without the addition of AC (as a control). The AC was added to the soil removed to form the planting hole and was thoroughly hand-mixed with that soil in a bucket before the soil was returned to the hole. The juveniles were transplanted avoiding southern exposure and watered once following planting. Wild herbivores severely damaged some juveniles of *A. herba-alba* within few days of planting. These were replaced and fenced with a metallic mesh to prevent further grazing. The height and diameter of juveniles were measured at the beginning of the experiment and at the end of spring in three consecutive years; June 2014, June 2015 and June 2016. Then, the volume was calculated to estimate plant size. The volume of *S. vermiculata* and *A. herba-alba* juveniles was approximated to an inverted cone, while the volume of *L. spartum* juveniles was approximated to a cylinder. In addition, the vitality of juveniles (*i.e.* % of green aerial parts; 0 for a dead juvenile) was visually estimated in two contrasting drought conditions at the end of the experiment (at the end of spring, June 2016, and at the end of summer, October 2016), as an indicator of the general plant response to local stress conditions (Dobbertin

2005). While spring 2016 was relatively rainy, drought stress was extremely high in summer 2016 because there was almost no rainfall (Fig. S2). The estimation of plant vitality was always performed by the same person, who had been trained by repeatedly assessing the vitality of control plants (Dobbertin 2005). Juvenile plants of the three test species were supplied by a local garden center.

The potential allelopathic impacts of *A. herba-alba* on seed germination and seedling survival of the test species were also assessed. To this end, in December 2014 seeds of *S. vermiculata* (n = 90), *L. spartum* (n = 60) and *A. herba-alba* (n = 60) were sown in the same *A. herba-alba* individuals where juveniles of these test species had been transplanted (15 *A. herba-alba* individuals per target species and treatment). A higher number of *S. vermiculata* seeds was used because of the low establishment rate previously observed in a germination test. For the AC treatment the seeds were sown after thoroughly mixing 2 g of AC into 100 g of soil. In the control treatment the seeds were sown following mixing of the same soil volume by hand without the addition of AC. The seeds were sown at 1 cm depth, next to the transplanted juveniles, avoiding southern exposure. The position of each seed was marked by two nails to clearly identify the emerged seedling and distinguish it from other naturally emerging seedlings. Seed germination and the survival of emerged seedlings were recorded approximately every three months for 18 months. All seeds were collected in the field from natural populations in the study area.

Soil analyses

To control for potential side effects of AC, soil samples were collected in late October 2016 from 5 random juveniles per test species transplanted beneath *A. herba-alba* adults with the addition of AC (n=15) and from 5 random juveniles per test species transplanted without AC (n=15). Organic carbon (C), total nitrogen (N), the C:N ratio, available phosphorous (P) and pH were analyzed for all soil samples. The soil samples were dried and sieved through a 2-mm mesh sieve prior to analysis. The organic C content was measured using chromatic acid digestion (Heanes 1984). Total N and the C:N ratio were measured using a Vario MAX CN elemental analyzer. The available P was measured based on the absorbance at 430 nm (UNICAM 8625 UV/Vis Spectrometer) of samples extracted with Bray nº1 reagent

(Bray & Kurtz 1945). The soil pH in water following a dilution 1:2.5 was measured using a pH meter CRISON micropH 2001.

Net interaction outcome

Following the methodology described above, juvenile plants were transplanted and seeds were sown beneath adult individuals of the shrub *S. vermiculata* ($n = 45$) and in bare soil areas ($n = 45$; > 50 cm to the nearest vegetation patch) in order to compare the net interaction outcome for plants in *A. herba-alba* and *S. vermiculata* microsites. Specifically, 15 juveniles of each test species were transplanted in February 2014, and 45 seeds of *S. vermiculata* and 30 seeds of each of *L. spartum* and *A. herba-alba* were sown in December 2014. Because of herbivore damage to the transplanted juveniles of *A. herba-alba*, and to a lesser extent the juveniles of *S. vermiculata* in bare soil, the plants were protected from wild grazing using a metallic mesh surround. The size and vitality of transplanted juveniles, seed germination and the survival of emerged seedlings were measured in the *S. vermiculata* and the bare soil microsites at the same time they were measured in the *A. herba-alba* microsite. The net interaction outcome for *A. herba-alba* and *S. vermiculata* microsites was estimated by computing the Relative Interaction Index (RII; Armas, Ordiales & Pugnaire 2004) as:

$$\mathrm{RII}_{m,t} \quad \frac{V_{shrub} - V_{bare\ soil}}{V_{shrub} + V_{bare\ soil}},$$

where V_{shrub} is the value for the measured variables (plant size, plant vitality, seed germination and seedling survival) for each test species (t = *S. vermiculata*, *L. spartum* or *A. herba-alba*) in each shrub microsite (m = *A. herba-alba* or *S. vermiculata*) and $V_{bare\ soil}$ is the value for these variables for the test species in the bare soil areas. The final size of juveniles rather than growth was used to calculate RII, because a negative change in growth was observed for some juvenile plants and negative values cannot be used to calculate RII properly (Kikvidze & Armas 2010). Nevertheless, the initial size of the transplanted juveniles of the test species did not differ significantly among microsites ($p > 0.05$ for the three test species; Table S1). The RII for plant vitality was estimated under two drought stress conditions (*i.e.* spring and summer 2016). Measuring the size of juveniles after the summer of 2016 was not meaningful because they do not grow during this season. For seed germination and seedling survival, a global RII was calculated per test species and shrub microsite using the values at the end of the experiment.

The RII values range between 1 and -1. Positive RII values indicate that the measured variables are higher in the shrub microsite than in the bare soil, and thus, net facilitative effects can be inferred. Negative RII values indicate that the variables are lower in the shrub microsite than in the bare soil, indicating net interference. RII values of approximately zero indicate that the measured variables are similar in the shrub microsite and bare soil areas.

Statistical analysis

Differences in the annual growth of juveniles between treatments (*i.e.* with and without the addition of AC) were analyzed using linear mixed models (LMMs) for repeated measures. Treatment and the year of measurement were set as fixed factors, while the identity of each juvenile was included as a random factor. Only juveniles that survived to the end of the experiment were included in the assessment of plant growth. On the other hand, differences in juvenile vitality between treatments were analyzed using generalized linear mixed models (GLMMs) for repeated measures. Treatment and drought condition were set as fixed factors, while the identity of each juvenile was included as a random factor. As vitality was measured as a proportion, a binomial distribution of errors was specified in the models. Analyses were performed for all species pooled and for each test species separately. Species was included as a random factor in analyses when all species were pooled together. Best model selection was based on Akaike´s Information Criterion (AIC; Zuur *et al.* 2009).

Seed germination of test species and the survival of emerged seedlings were modeled, pooling all species together and for each test species separately, as time functions using the Kaplan-Meier method. The significance of differences in the curve shape between treatments (with and without AC) was tested using Cox regressions (Bewick, Cheek & Ball 2004).

Differences in soil organic C, total N, the C:N ratio, available P and the pH between treatments were analyzed using one-way ANOVA. The organic C and total N data was arcsine transformed to meet normality assumptions.

The presence of facilitative, neutral or negative interactions based on the RII values for the size and vitality of juveniles was analyzed by performing Student t-tests against a constant value (zero; Macek *et al.* 2016). Furthermore, significant differences in RII values between shrub microsites were analyzed by pooling all species together and for each target species separately, using two-way and one-way ANOVA respectively. Because only one RII value was calculated for seed germination and the survival of emerged seedlings for each combination of test species and shrub microsite, there was a lack of variability to statistically test differences between shrub microsites. To overcome this problem, the germination and survival were simulated 100 times for each target species and microsite using bootstrapping (Gardener 2014). Specifically, in each simulation, the germination was calculated by resampling the 15 individuals per shrub and bare soil microsites with replacement to obtain realistic values of germination in the microsites. The same resampling was performed to simulate survival, but only those shrub individuals and bare soil areas that had at least one germinated seed were considered (if germination was zero, it was not possible to calculate the survival). The RII was subsequently computed for each simulation. The RII values between shrub microsites were compared by calculating the z-score for the differences between microsites and simulations (Gardener 2014).

Statistical analyses were performed in R (R Core Team 2015). LMMs were performed using the *lme* function in the *nlme* package (Pinheiro *et al.* 2015). GLMMs were performed using the *lmer* function in the *lme4* package (Bates *et al.* 2015). Germination and survival curves were performed using the *survfit* function in the *survival* package (Therneau 2015).

Results

Allelopathic effects of *A. herba-alba*

The overall growth of juveniles of all test species transplanted beneath *A. herba-alba* individuals did not differ significantly between treatments (such differences were marginally significant; Fig. 1; Table 1). Similarly, growth did not differ significantly between treatments for any of the three test species individually (Fig. 1; Table 1). On the other hand, the growth of juveniles planted beneath *A. herba-alba* did not differ significantly among years of measurement, except in the case of *L. spartum*, where growth was significantly less in 2015 than in 2014 and 2016 (Fig. S2; Table 1).

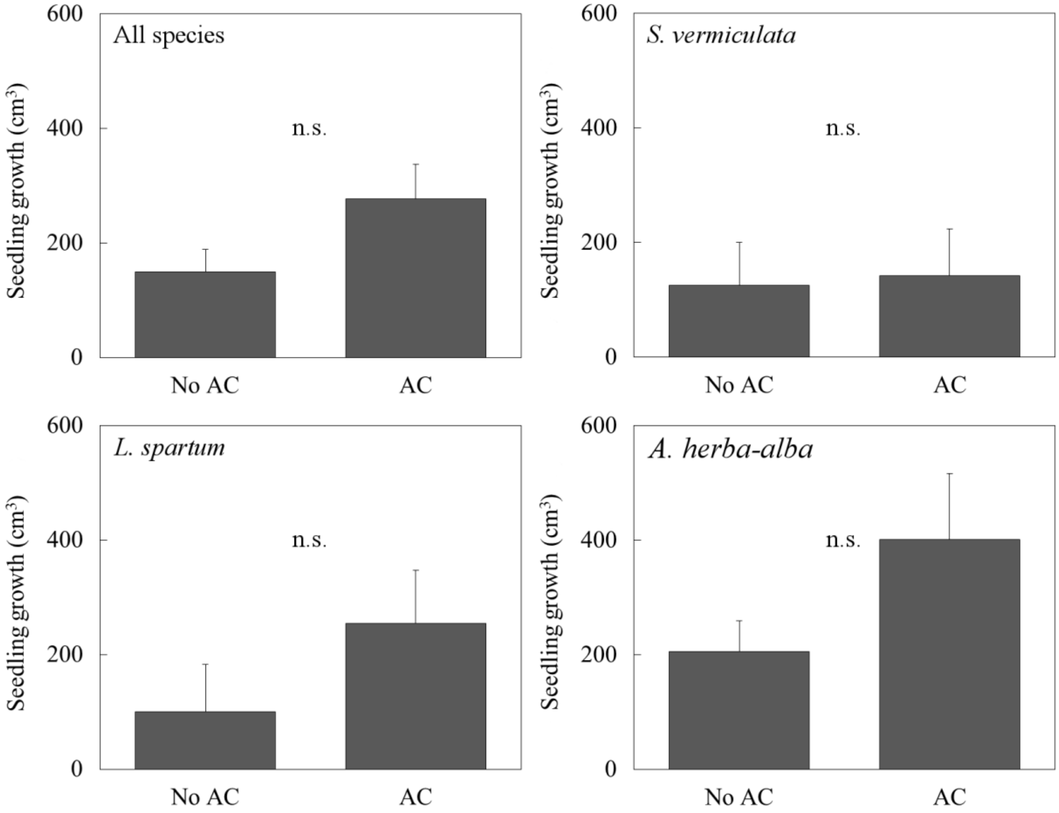


Fig. 1. Annual growth (mean ± SE) of juveniles of the test species transplanted beneath *A. herba-alba* with and without the addition of AC. n.s.: not significant.

The vitality (% green plant parts) of juveniles transplanted beneath *A. herba-alba* individuals amended with AC was significantly higher than in the absence of AC, when the three test species were considered together (Fig. 2; Table 2). Similarly, the vitality of *A. herba-alba* juveniles transplanted beneath the canopy of *A. herba-alba* individuals was significantly higher in the presence of AC than in its absence (Fig. 2; Table 2). The vitality of *S. vermiculata* and *L. spartum* juveniles transplanted beneath *A. herba-alba* individuals in the presence of AC did not differ significantly from the vitality of those transplanted without the addition of AC (Fig. 2; Table 2). On the other hand, the vitality of juveniles transplanted beneath *A. herba-alba* was significantly lower under more severe drought stress conditions (*i.e.* after summer), except for *S. vermiculata* juveniles (Fig. S3; Table 2).

Table 1. Result of LMM analyses for the effect of AC treatment and year of measurement on the growth of test species. Significant effects ($p < 0.05$) are highlighted in bold.

	Df	Sum Sq	F value	p (>F)
All species				
Treatment	1	9.141×10^5	3.113	0.08
Year	2	5.104×10^5	0.906	0.41
Residuals	218	6.210×10^7		
S. vermiculata				
Treatment	1	5.245×10^3	0.016	0.90
Year	2	2.680×10^4	0.072	0.93
Residuals	71	1.657×10^7		
L. spartum				
Treatment	1	3.553×10^5	1.460	0.24
Year	2	2.143×10^6	6.050	**<0.001**
Residuals	56	1.111×10^7		
A. herba-alba				
Treatment	1	8.275×10^5	2.234	0.15
Year	2	4.270×10^5	0.576	0.57
Residuals	83	3.074×10^7		

For the three test species combined, seed germination beneath adult individuals of *A. herba-alba* amended with AC was significantly higher than under individuals without the addition AC (Fig. 3). However, significant differences in seed germination between treatments were not found for any of the three target species individually (Fig. 3), although germination of *A. herba-alba* seeds was marginally higher with the addition of AC. On the other hand, seedling survival of the test species did not differ significantly between the two treatments, either when the test species were considered individually or in combination (Fig. 4).

Soil analyses

The values for soil organic C, the C:N ratio and the available P content were significantly higher in *A. herba-alba* microsite having added AC than in *A. herba-alba* microsite without added AC (Table 3). The total N content in soil did not differ significantly between treatments, but the soil pH was significantly lower in *A. herba-alba* microsite amended with AC (Table 3).

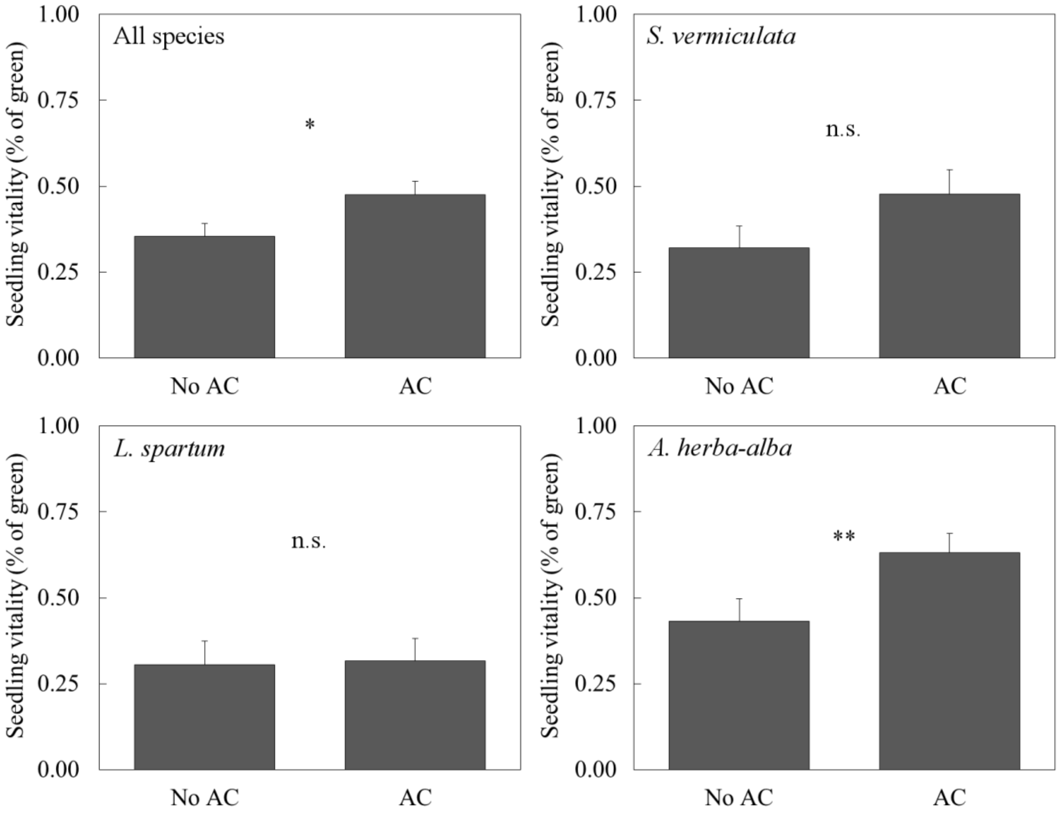


Fig. 2. Vitality (mean % ± SE) of juveniles of the test species transplanted beneath *A. herba-alba* with and without the addition of AC. n.s.: not significant; *: p<0.05; **: p<0.01.

Net interaction outcome

The RII value for the size of juveniles indicated a neutral interaction outcome for *A. herba-alba* and *S. vermiculata* microsites (*t*-test, $p > 0.05$; Fig. S4A). Furthermore, non-significant differences were observed in the RII values between the two microsites ($p > 0.05$; Fig. S4A). On the other hand, the RII values for the vitality of juveniles indicated an overall neutral interaction outcome for the effect of *A. herba-alba* on test species, but a net positive outcome for the effect of *S. vermiculata* (Fig. 5). The difference in the RII values between the two microsites was marginally significant (Table 4). Furthermore, the interaction outcome for the *A. herba-alba* microsite changed from neutral in spring to positive in the summer, when it was similar to the summer interaction outcome for the *S. vermiculata* microsite (Fig. 5; Table 4). This general pattern varied slightly depending on the test species. Specifically, *A. herba-alba* and *S. vermiculata* had neutral net effects on *S. vermiculata* juveniles in both drought stress conditions (Fig. 5), although in spring the RII value was significantly higher for the *S. vermiculata* microsite than for the *A. herba-alba* microsite (Table 4). Similarly, we found neutral effects of each shrub species on *A. herba-alba* juveniles in spring. However, the net interaction outcome for these juveniles became positive in the summer in the *S. vermiculata* microsite, while a neutral net effect was found in summer for *A. herba-alba* juveniles in the *A. herba-alba* microsite. Differences in RII values between the two microsites were non-significant (Table 4). On the other hand, the *A. herba-alba* and *S. vermiculata* microsites had net facilitative interactions on *L. spartum* juveniles. This positive interaction outcome remained constant under the more severe drought stress conditions (Fig. 5; Table 4).

Table 2. Result of GLMM analyses for the effect of AC treatment and drought condition on vitality (% of green parts) of test species. Significance of the change in deviance was tested by comparing models with and without the effect of each term, assuming a χ^2 distribution. Significant effects ($p < 0.05$) are highlighted in bold.

	Null Deviance	Res. Deviance	df	χ^2	p
All species					
Treatment	221.89	217.99	1	3.897	**0.05**
Drought	240.51	217.99	1	22.519	**<0.001**
S. vermiculata					
Treatment	75.698	74.226	1	1.472	0.23
Drought	75.243	74.226	1	1.016	0.31
L. spartum					
Treatment	65.041	64.953	1	0.089	0.77
Drought	74.843	64.953	1	9.89	**<0.01**
A. herba-alba					
Treatment	65.216	57.315	1	7.901	**<0.01**
Drought	75.849	57.315	1	18.534	**<0.001**

The RII values calculated for seed germination and seedling survival indicated a neutral interaction outcome in the *A. herba-alba* and *S. vermiculata* microsites, regardless of whether the test species were considered in combination or individually. In addition, in no case did RII values calculated for these two variables differ significantly between the *A. herba-alba* and *S. vermiculata* microsites (p > 0.05; Fig. S4B and S4C).

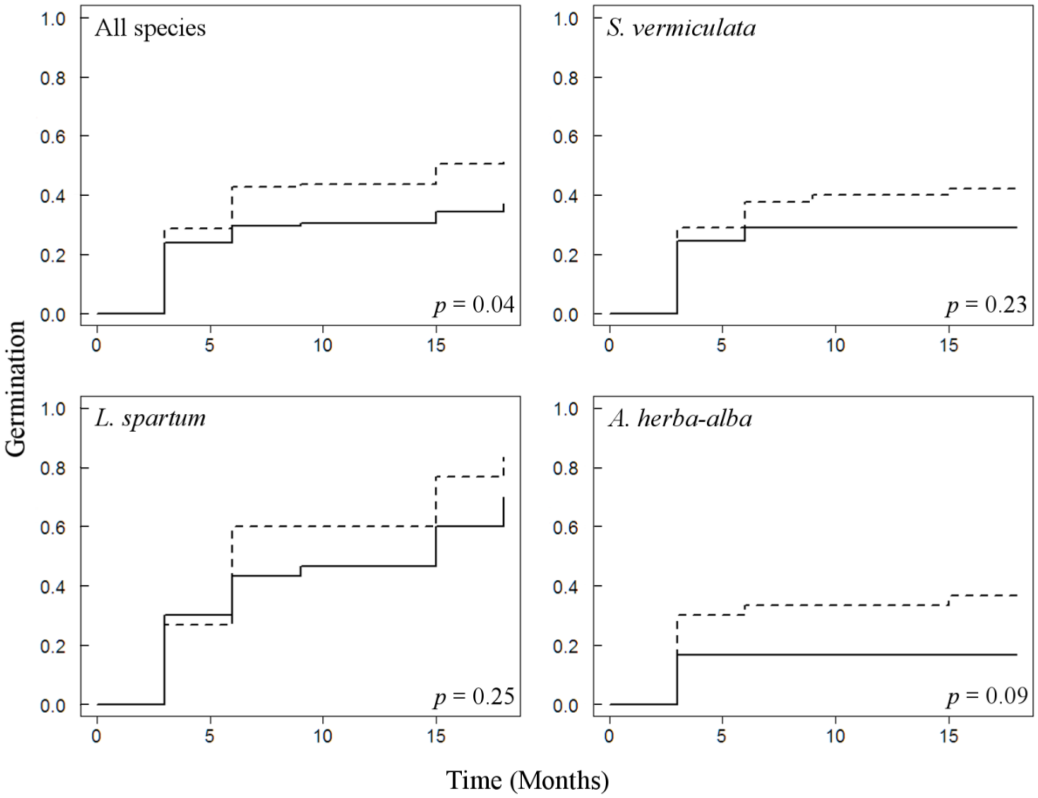


Fig. 3. Seed germination (Kaplan-Meier curves) of the test species over time following sowing in soil beneath *A. herba-alba* with (dashed line) and without (solid line) the addition of AC.

Discussion

We conducted a 2-year field experiment to determine the significance of chemical interference by the allelopathic plant *A. herba-alba* in a semiarid plant community, by assessing its impacts on the growth, vitality, seed germination and seedling survival of neighboring plant species. We also investigated how allelopathy modulates the net interaction outcome for *A. herba-alba*. Previous research has shown that *A. herba-alba* exerts a negative influence on surrounding vegetation. In an observational study, Friedman *et al.* (1977) found that germination of annuals plants was suppressed in the vicinity of *A. herba-alba*. In the same line, Pueyo *et al.* (2016) found a generalized spatial pattern of segregation between adult individuals of *A. herba-alba* and juvenile individuals of perennial plant species, and also found that *A. herba-alba* had a relatively low density of other plants beneath its canopy. In addition, Arroyo *et al.* (2015) found that *A. herba-alba* caused a diversity repulsion in its vicinity. In these studies the authors argued that the negative effects of *A. herba-alba* on vegetation might be a consequence, at least partially, of its allelopathic activity. This was based on a number of laboratory studies that demonstrated the phytotoxicity of the compounds released by this species (Friedman *et al.* 1977; Escudero *et al.* 2000; Tilaki *et al.* 2013). However, these studies provided no direct evidence of allelopathic interference, as they lacked an adequate control to remove the inhibitory compounds (*e.g.* AC). In our field study, we found that the chemical interference by *A. herba-alba* lessened seed germination and caused a reduction in the vitality of some neighboring plant species beneath its canopy, supporting previous conclusions.

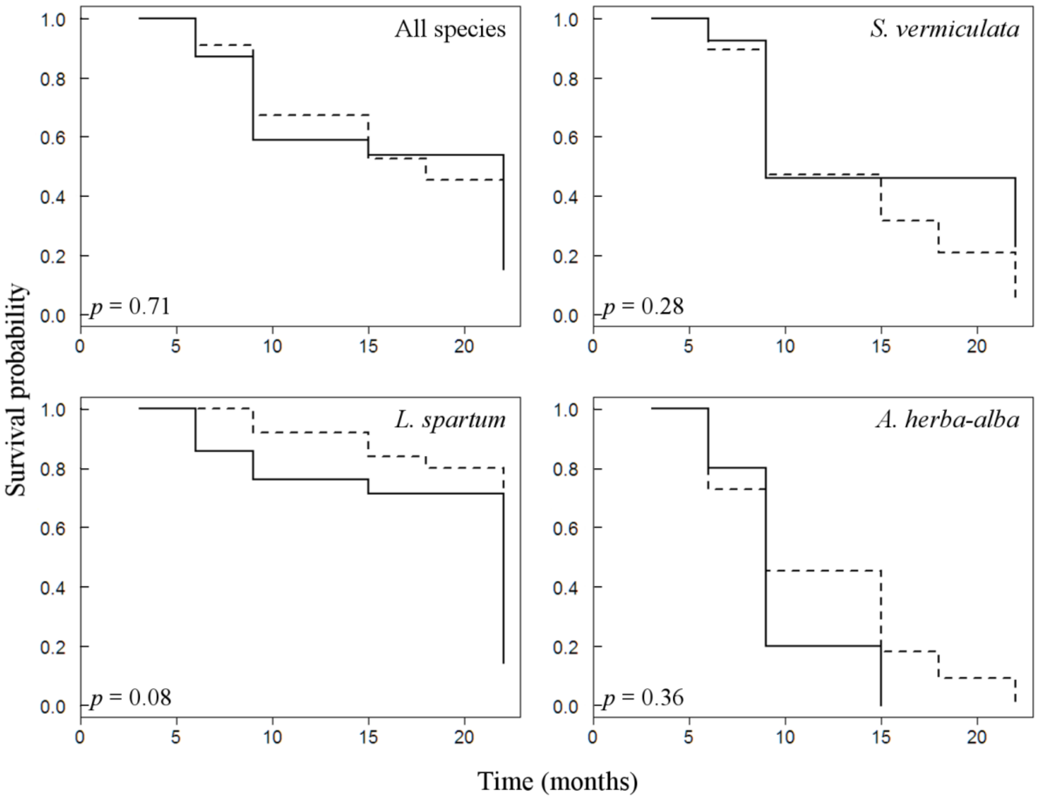


Fig. 4. Survival (Kaplan-Meier curves) of emerged seedlings of the test species in soil beneath *A. herba-alba* with (dashed line) and without (solid line) the addition of AC.

The germination of plant species in the *A. herba-alba* microsite was enhanced by amending the soil with AC, although this effect was weak and only detected when all test species were considered together in the analyses. This finding is in line with a previous study conducted under controlled conditions, which showed that volatile and water soluble chemicals released from *A. herba-alba* inhibited the germination of *A. herba-alba* seeds, although these compounds did not inhibit germination of *S. vermiculata* and *L. spartum* seeds (Arroyo *et al.* in rev.). In the field, seedling survival in the *A. herba-alba* microsite was not hampered in control treatment (no added AC) in comparison with the AC treatment. However, a previous laboratory study reported that the aqueous extract of *A. herba-alba* increased mortality of emerging *A. herba-alba* seedlings from the seedbank (Arroyo *et al.* 2016). Similarly, we did not observe any effect of the *A. herba-alba* microsite on growth of juvenile plants, despite a previous laboratory study showing that both volatile and water soluble chemicals released by *A. herba-alba* enhanced early seedling growth (Arroyo *et al.* in rev.). Differences in the allelopathic effects of *A. herba-alba* between previous laboratory studies and our field experiment might be because of differences in the experimental conditions, including the soil microbial community, level of exposure to the allelopathic compounds, the specific mixture or the relative concentrations of compounds acting jointly under natural conditions. For instance, it is known that in the field, associated soil microorganisms can metabolize chemical compounds, and thus diminish their allelopathic effects (Kaur *et al.* 2009; Li *et al.* 2017). This illustrates the need for further field studies to better understand the impacts of allelopathic species in natural plant communities. Furthermore, our findings highlight that laboratory studies can overestimate the significance of allelopathy in nature, as noted in previous studies (Stowe 1979; Pisula & Meiners 2010; da Silva *et al.* 2015). Consequently, results obtained under controlled conditions should be interpreted carefully.

Table 3. Organic C, total N, the C:N ratio, available P content and the soil pH (mean ± SE) in *A. herba-alba* microsite with and without the addition of AC (15 samples per treatment). Significant differences ($p < 0.05$) are highlighted in bold.

	Treatment			
	No AC	AC	F value	p (>F)
Organic C (%)	1.25 ± 0.13	2.02 ± 0.10	21.678	**<0.001**
Total N (%)	0.119 ± 0.011	0.124 ± 0.013	0.056	0.82
C:N	10.86 ± 0.69	18.24 ± 1.51	19.716	**<0.001**
Available P (mg/kg)	41.67 ± 4.69	64.40 ± 6.28	8.420	**<0.01**
pH	7.88 ± 0.03	7.68 ± 0.02	33.047	**<0.001**

Addition of AC to soil in studies of allelopathy has been used to reduce or prevent allelopathic interference, because it adsorbs the phytotoxic compounds released by allelopathic plants (Inderjit & Callaway 2003; Yuan *et al.* 2014). However, it is imperative to control for other potential effects of the use of AC which could confuse interpretation of the positive effects resulting from release from allelopathic interference (Kabouw, Nab & van Dam 2010). We found that the addition of AC (2%) modified the soil pH and increased the organic C content and amount of P available for plants. It is likely that if AC affects nutrient availability, it may also affect subsequent plant growth (Lau *et al.* 2008). However, after more than two years we did not find any effect, either positive or negative, of the addition of AC on the growth of test species. Actually, the most limiting nutrient for plants in semiarid environments is N (Whitford 2002), and the addition of AC to the soil did not increase the N content. Furthermore, the C:N ratio is negatively correlated with the rate of N mineralization (Plymale, Boerner & Logan 1987; Finzi, Breemen & Canham 1998). Consequently, it is likely that the addition of AC did not have direct positive effects on plant performance. Moreover, in these environments it has been observed that germination does not always vary among microsites (*e.g.* shrub vs. open bare soil), even though the amount of nutrients (*e.g.* amount of P) varies (Sotomayor, Lortie & Lamarque 2014; Woods & Miriti 2016; Pueyo *et al.* 2016). This suggests that the enhanced germination in *A. herba-alba* microsite amended with AC was because of release from chemical interference. On the other hand, the addition of AC to the soil can result in texture changes that increase water retention capacity, because of the finer particle size (Inderjit & Callaway 2003). However, germination of the test species is triggered very rapidly following rainfall (from hours to 2 or 3 days; personal observation; Parsons 2012). Furthermore, increased retention of water might have favored seedling survival in *A. herba-alba* microsite treated with AC, but we did make any observations suggesting this effect. Therefore, although AC altered some soil characteristics, they were probably insufficient to confound the benefits of release from chemical interference.

Despite the similarities between *A. herba-alba* and *S. vermiculata* in ameliorating resources availability (*e.g.* nutrients) and abiotic conditions (*e.g.* temperature; Pueyo *et al.* 2016), we expected to find differences in plant-plant biotic interactions involving these two species, because of the allelopathic activity of *A. herba-alba* (Friedman *et al.* 1977;

Escudero *et al.* 2000; Arroyo *et al.* 2016). However, we found no differences in the RII values calculated for plant growth, seed germination and seedling survival between *A. herba-alba* and *S. vermiculata*. Only the RII values for plant vitality indicated different effects of these two species in plant biotic interactions. Specifically, our results indicated a neutral net interaction outcome for *A. herba-alba* in this semiarid plant community. This finding is consistent with that of Arroyo *et al.* (2015), who found that *A. herba-alba* had a neutral net effect on plant diversity in the same semiarid community. On the other hand, the similar, but non-allelopathic, shrub *S. vermiculata* had a net positive interaction outcome on test species, in agreement with previous studies showing that *S. vermiculata* is an effective nurse plant (Arroyo *et al.* 2015; Pueyo *et al.* 2016). Hence, it appears that allelopathic interference can limit somewhat the potential facilitative effects of enhanced microclimatic conditions in *A.herba-alba* microsite.

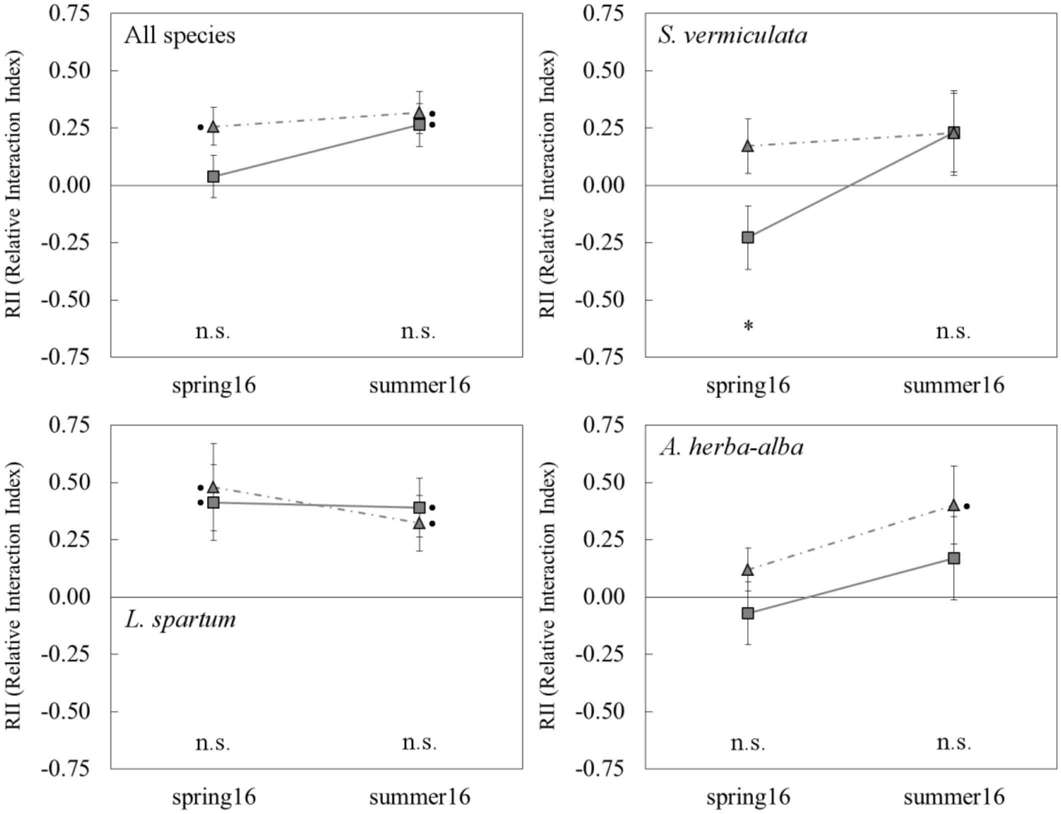


Fig. 5. Relative interaction index (RII; mean ± SE) calculated for the vitality of juveniles in *A. herba-alba* (square symbols) and *S. vermiculata* (triangle symbols) microsites in spring and summer 2016. Black dots indicate RII values significantly different from zero (t-test, $p<0.05$). n.s.: not significant; *: $p<0.05$.

During summer 2016 the drought stress conditions became extreme, as almost no rainfall occurred in the study area between June and September 2016. It has been reported that a shift from neutral (or even positive) to negative effects occurs in the interaction outcome for allelopathic species under very harsh drought stress conditions (Jankju 2013), in accordance with predictions of the refined stress gradient hypothesis (SGH; Maestre *et al.* 2009). However, we found that the outcome of interactions between *A. herba-alba* and neighboring species shifted from neutral to positive. Soil water scarcity may have limited the diffusion of water-borne allelopathic compounds to neighboring plants (Kobayashi 2004). Consequently, allelopathy did not prevent a shift to a positive outcome under the more severe drought conditions. Thus, facilitative interactions predominated over interference (*i.e.* competition + allelopathy) by the allelopathic plant under very harsh abiotic stress, regardless of whether production or the phytotoxicity of allelopathic compounds increased under those conditions (Friedman 1987; Einhellig 1994).

Table 4. Result of ANOVA analyses for the net interaction outcome (quantified using the RII index) between shrub microsites under two drought stress conditions (spring and summer). Significant differences ($p < 0.05$) are highlighted in bold.

	Spring16				Summer16			
	Df	Sum Sq	F value	p (>F)	Df	Sum Sq	F value	p (>F)
All species								
Microsite	1	1.080	3.107	0.08	1	0.065	0.169	0.68
Species	2	4.044	6.552	**<0.01**	2	0.247	0.318	0.73
Residuals	88	30.588			88	33.667		
S. vermiculata								
Microsite	1	1.194	4.793	**0.04**	1	1×10^{-4}	1×10^{-4}	0.99
Residuals	28	6.977			28	13.408		
L. spartum								
Microsite	1	0.034	0.072	0.79	1	0.035	0.150	0.70
Residuals	28	13.290			28	6.588		
A. herba-alba								
Microsite	1	0.273	1.307	0.26	1	0.404	0.867	0.36
Residuals	28	5.846			28	13.050		

Our results showed that the balance between facilitative interactions and interference by *A. herba-alba* was species-specific. We found a neutral net effect on *S. vermiculata* and *A. herba-alba* juveniles, but, interestingly, net facilitative interactions were found for *L. spartum* juveniles in *A. herba-alba* microsite, independently of drought severity. Indeed, other perennial grasses in addition to *L. spartum* may tolerate the allelopathic compounds of *A. herba-alba* (Arroyo *et al.* 2015, 2016; Pueyo *et al.* 2016). Perhaps species of this group have become adapted to the chemicals of *A. herba-alba* as a consequence of a long associational history (Vivanco *et al.* 2004; Thorpe *et al.* 2011). Thus, in this semiarid plant community *A. herba-alba* may act as a nurse plant facilitating the establishment of perennial grasses, which once established may replace their own nurse plants (Armas & Pugnaire 2005). This highlights the effects that an allelopathic species can have on the dynamics of a semiarid plant community, beyond chemical interference of some neighboring species.

In conclusion, this study of allelopathy constitutes one of the few examples performed in natural plant communities (but see Nilsson 1994; Nilsson *et al.* 2000; da Silva *et al.* 2015). Our findings provide novel evidence for the significance of the chemical interference of neighboring plant species by an allelopathic shrub in a semiarid plant community. Our results indicate that under natural conditions allelopathic interference can result in inhibition of seed germination and reduction of plant vitality. They also suggest that allelopathy can limit potential facilitative effects of enhanced microclimatic conditions beneath allelopathic shrubs in semiarid environments, although, tolerant species (*e.g. L. spartum*) may still being facilitated. This highlights the importance of a multispecific perspective in studies of allelopathy. Our results also show that net facilitative interactions should be expected for allelopathic shrubs under very harsh drought stress conditions. Our study links the observed effects in the field of *A. herba-alba* on vegetation patterns in its vicinity, with laboratory studies that have demonstrated the phytotoxic properties of this plant. Although the addition of AC slightly modified some soil characteristics, this was probably insufficient to confound the interpretation that AC resulted in better plant performance by release from chemical interference. Although methods to test chemical interference may result challenging, more field studies are required, because of the results obtained under controlled conditions may overestimate the significance of allelopathy in natural communities.

Acknowledgements

This research was funded by the Spanish Ministry of Education, Culture and Sport (grant AP-2012-4126 to A.I.A.; FPU Program) and the Spanish Ministry of Economy, Industry and Competitiveness (projects CGL-2012-37508 and CGL-2016-80783-R). H.S. is supported by the European Research Council (ERC Grant agreement 647038 [BIODESERT]). We especially thank P. Bravo, C. Capistrós and G. Enguita for their field assistance and J. Cosculluela ("Viveros Ejea") for kindly providing the juvenile plants used in the experiments.

Supplementary material

Supplementary Table S1 and Figures S1, S2, S3 and S4 may be found in the online version of this article.

References

Armas, C., Ordiales, R. & Pugnaire, F.I. (2004) Measuring plant interactions: a new comparative index. *Ecology*, **85**, 2682–2686.

Armas, C. & Pugnaire, F.I. (2005) Plant interactions govern population dynamics in a semi-arid plant community. *Journal of Ecology*, **93**, 978–989.

Armas, C. & Pugnaire, F.I. (2011) Belowground zone of influence in a tussock grass species. *Acta Oecologica*, **37**, 284–289.

Arroyo, A.I., Pueyo, Y., Reiné, R., Giner, M.L. & Alados, C.L. (2016) Effects of the allelopathic plant Artemisia herba-alba Asso on the soil seed bank of a semi-arid plant community. *Journal of Plant Ecology*, rtw120.

Arroyo, A.I., Pueyo, Y., Saiz, H. & Alados, C.L. (2015) Plant-plant interactions as a mechanism structuring plant diversity in a Mediterranean semi-arid ecosystem. *Ecology and Evolution*, **5**, 5305–5317.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, **67**, 1–48.

Bertness, M.D. & Callaway, R. (1994) Positive interactions in communities. *Trends Ecol Evol*, **9**, 191–3.

Bewick, V., Cheek, L. & Ball, J. (2004) Statistics review 12: Survival analysis. *Critical Care*, **8**, 389–394.

Bray, R.H. & Kurtz, L.T. (1945) Determination of total, organic, and available forms of phosphorus in soils. *Soil Science*, **59**, 39–46.

Callaway, R.M. (2007) *Positive Interactions and Interdependence in Plant Communities*. Springer, Dordrecht, Netherlands.

Callaway, R.M. & Aschehoug, E.T. (2000) Invasive Plants Versus Their New and Old Neighbors: A Mechanism for Exotic Invasion. *Science*, **290**, 521–523.

Castaldi, S., Carfora, A., Fiorentino, A., Natale, A., Messere, A., Miglietta, F. & Cotrufo, M.F. (2009) Inhibition of net nitrification activity in a Mediterranean woodland: possible role of chemicals produced by Arbutus unedo. *Plant and Soil*, **315**, 273–283.

Chomel, M., Guittonny-Larchevêque, M., Fernandez, C., Gallet, C., DesRochers, A., Paré, D., Jackson, B.G. & Baldy, V. (2016) Plant secondary metabolites: a key driver of litter decomposition and soil nutrient cycling (ed R Aerts). *Journal of Ecology*, **104**, 1527–1541.

Cipollini, K.A., McClain, G.Y. & Cipollini, D. (2008) Separating Above- and Belowground Effects of Alliaria Petiolata and Lonicera Maackii on The Performance of Impatiens Capensis. *The American Midland Naturalist*, **160**, 117–128.

Dobbertin, M. (2005) Tree growth as indicator of tree vitality and of tree reaction to environmental stress: a review. *European Journal of Forest Research*, **124**, 319–333.

Ehlers, B.K., Charpentier, A. & Grøndahl, E. (2014) An allelopathic plant facilitates species richness in the Mediterranean garrigue. *Journal of Ecology*, **102**, 176–185.

Einhellig, F.A. (1994) Allelopathy: Current Status and Future Goals. *Allelopathy* (eds Inderjit, K.M.M. Dakshini & F.A. Einhellig), pp. 1–24. American Chemical Society, Washington, DC.

Escudero, A., Albert, M.J., Pita, J.M. & Pérez-García, F. (2000) Inhibitory effects of Artemisia herba-alba on the germination of the gypsophyte Helianthemum squamatum. *Plant Ecology*, **148**, 71–80.

Fabbro, C.D., Güsewell, S. & Prati, D. (2013) Allelopathic effects of three plant invaders on germination of native species: a field study. *Biological Invasions*, **16**, 1035–1042.

Finzi, A.C., Breemen, N.V. & Canham, C.D. (1998) Canopy Tree-Soil Interactions within Temperate Forests: Species Effects on Soil Carbon and Nitrogen. *Ecological Applications*, **8**, 440.

Friedman, J. (1987) Allelopathy in Desert Ecosystems. *Allelochemicals: Role in Agriculture and Forestry* (ed G.R. Waller), pp. 53–68. American Chemical Society, Washington, DC.

Friedman, J., Orshan, G. & Ziger-Cfir, Y. (1977) Suppression of annuals by Artemisia herba-alba in the Negev desert of Israel. *Journal of Ecology*, **65**, 413–426.

Gardener, M. (2014) *Community Ecology: Analytical Methods Using R and Excel*. Pelagic Publishing Ltd, Exeter.

Gniazdowska, A. & Bogatek, R. (2005) Allelopathic interactions between plants. Multi site action of allelochemicals. *Acta Physiologiae Plantarum*, **27**, 395–407.

Gómez-Aparicio, L. & Canham, C.D. (2008) Neighbourhood analyses of the allelopathic effects of the invasive tree Ailanthus altissima in temperate forests. *Journal of Ecology*, **96**, 447–458.

Grøndahl, E. & Ehlers, B.K. (2008) Local adaptation to biotic factors: reciprocal transplants of four species associated with aromatic Thymus pulegioides and T. serpyllum. *Journal of Ecology*, **96**, 981–992.

Heanes, D.L. (1984) Determination of total organic-C in soils by an improved chromic acid digestion and spectrophotometric procedure. *Communications in Soil Science and Plant Analysis*, **15**, 1191–1213.

Hille, M. & den Ouden, J. (2005) Charcoal and activated carbon as adsorbate of phytotoxic compounds - a comparative study. *Oikos*, **108**, 202–207.

Inderjit & Callaway, R.M. (2003) Experimental designs for the study of allelopathy. *Plant and Soil*, **256**, 1–11.

Inderjit & Weston, L.A. (2000) Are Laboratory Bioassays for Allelopathy Suitable for Prediction of Field Responses? *Journal of Chemical Ecology*, **26**, 2111–2118.

Inderjit, Weston, L.A. & Duke, S.O. (2005) Challenges, achievements and opportunities in allelopathy research. *Journal of Plant Interactions*, **1**, 69–81.

Jankju, M. (2013) Role of nurse shrubs in restoration of an arid rangeland: Effects of microclimate on grass establishment. *Journal of Arid Environments*, **89**, 103–109.

Kabouw, P., Nab, M. & van Dam, N.M. (2010) Activated carbon addition affects substrate pH and germination of six plant species. *Soil Biology and Biochemistry*, **42**, 1165–1167.

Kikvidze, Z. & Armas, C. (2010) Plant Interaction Indices Based on Experimental Plant Performance Data. *Positive Plant Interactions and Community Dynamics* (ed F. Pugnaire), pp. 17–38. CRC Press.

Kobayashi, K. (2004) Factors affecting phytotoxic activity of allelochemicals in soil. *Weed Biology and Management*, **4**, 1–7.

Kulmatiski, A. (2011) Changing Soils to Manage Plant Communities: Activated Carbon as a Restoration Tool in Ex-arable Fields. *Restoration Ecology*, **19**, 102–110.

Lau, J.A., Puliafico, K.P., Kopshever, J.A., Steltzer, H., Jarvis, E.P., Schwarzlander, M., Strauss, S.Y. & Hufbauer, R.A. (2008) Inference of allelopathy is complicated by effects of activated carbon on plant growth. *New Phytol*, **178**, 412–23.

Li, S., Nie, Z. & Zhang, D. (2016) Competition between cocksfoot (Dactylis glomerata L.) and companion species: Evidence for allelopathy. *Field Crops Research*, **196**, 452–462.

López, R.P., Squeo, F.A., Armas, C., Kelt, D.A. & Gutiérrez, J.R. (2016) Enhanced facilitation at the extreme end of the aridity gradient in the Atacama Desert: a community-level approach. *Ecology*, **97**, 1593–1604.

Macek, P., Prieto, I., Macková, J., Pistón, N. & Pugnaire, F.I. (2016) Functional Plant Types Drive Plant Interactions in a Mediterranean Mountain Range. *Frontiers in Plant Science*, **7**.

Maestre, F.T., Callaway, R.M., Valladares, F. & Lortie, C.J. (2009) Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology*, **97**, 199–205.

Nilsson, M.-C. (1994) Separation of allelopathy and resource competition by the boreal dwarf shrub Empetrum hermaphroditum Hagerup. *Oecologia*, **98**, 1–7.

Nilsson, M.-C., Zackrisson, O., Sterner, O. & Wallstedt, A. (2000) Characterisation of the differential interference effects of two boreal dwarf shrub species. *Oecologia*, **123**, 122–128.

Parsons, R.F. (2012) Incidence and ecology of very fast germination. *Seed Science Research*, **22**, 161–167.

Pedrol, N., González, L. & Reigosa, M.J. (2006) Allelopathy and abiotic stress. *Allelopathy* (eds M.J. Reigosa, N. Pedrol & L. González), pp. 171–209. Springer Netherlands, Dordrecht.

Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. (2015) *Nlme: Linear and Nonlinear Mixed Effects Models*.

Plymale, A.E., Boerner, R.E.J. & Logan, T.J. (1987) Relative nitrogen mineralization and nitrification in soils of two contrasting hardwood forests: Effects of site microclimate and initial soil chemistry. *Forest Ecology and Management*, **21**, 21–36.

Pueyo, Y. (2005) *Evaluación de los factores ambientales y del uso antrópico como condicionantes de la conservación de la vegetación del sector central de la depresión del Ebro*. Zaragoza, Departamento de Agricultura y Economía Agraria.

Pueyo, Y., Moret-Fernández, D., Arroyo, A.I., de Frutos, A., Kéfi, S., Saiz, H., Charte, R., Giner, M. de la L. & Alados, C.L. (2016) Plant nurse effects rely on combined hydrological and ecological components in a semiarid ecosystem. *Ecosphere*, **7**, e01514.

R Core Team. (2015) *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna, Austria.

Rice, E.L. (1984) *Allelopathy (Second Edition).* Academic Press, San Diego.

Schöb, C., Armas, C. & Pugnaire, F.I. (2013) Direct and indirect interactions co-determine species composition in nurse plant systems. *Oikos*, **122**, 1371–1379.

da Silva, E.R., Ferreira, P.M.A., Overbeck, G.E. & Soares, G.L.G. (2015) Does the phytotoxic shrub Heterothalamus psiadioides affect a plant community through allelopathy? *Plant Ecology*, **216**, 87–97.

Sotomayor, D.A., Lortie, C.J. & Lamarque, L.J. (2014) Nurse-plant effects on the seed biology and germination of desert annuals. *Austral Ecology*, **39**, 786–794.

Therneau, T. (2015) *A Package for Survival Analysis in S.*

Thorpe, A.S., Aschehoug, E.T., Atwater, D.Z. & Callaway, R.M. (2011) Interactions among plants and evolution. *Journal of Ecology*, **99**, 729–740.

Tilaki, G.A.D., Gholami, F., Behtari, B. & Bezdi, K.G. (2013) Chemical composition and allelopathic effect of the essential oil of Artemisia herba-alba Asso. on seed germination and early seedling growth of legumes and grasses species. *Legume Research*, **36**, 33–40.

Tilman, D. (1988) *Plant Strategies and the Dynamics and Structure of Plant Communities*. Princeton University Press, Princeton, New Jersey.

Vivanco, J.M., Bais, H.P., Stermitz, F.R., Thelen, G.C. & Callaway, R.M. (2004) Biogeographical variation in community response to root allelochemistry: novel weapons and exotic invasion. *Ecology Letters*, **7**, 285–292.

Wardle, D.A., Nilsson, M.-C., Gallet, C. & Zackrisson, O. (1998) An ecosystem-level perspective of allelopathy. *Biological Reviews*, **73**, 305–319.

Weißhuhn, K. & Prati, D. (2009) Activated carbon may have undesired side effects for testing allelopathy in invasive plants. *Basic and Applied Ecology*, **10**, 500–507.

Whitford, W.G. (2002) Decomposition and Nutrient Cycling. *Ecology of desert systems*, pp. 235–274. Academic Press, London, UK.

Woods, N.N. & Miriti, M.N. (2016) Ubiquitous germination among common perennial species in response to facilitated and unfacilitated microhabitats. *Journal of Arid Environments*, **124**, 72–79.

Yuan, Y., Tang, J., Leng, D., Hu, S., Yong, J.W.H. & Chen, X. (2014) An Invasive Plant Promotes Its Arbuscular Mycorrhizal Symbioses and Competitiveness through Its Secondary Metabolites: Indirect Evidence from Activated Carbon (ed M Moora). *PLoS ONE*, **9**, e97163.

Zhang, C., Li, X., Chen, Y., Zhao, J., Wan, S., Lin, Y. & Fu, S. (2016) Effects of Eucalyptus litter and roots on the establishment of native tree species in Eucalyptus plantations in South China. *Forest Ecology and Management*, **375**, 76–83.

Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A. & Smith, G.M. (2009) *Mixed Effects Models and Extensions in Ecology with R.* Springer New York, New York, NY.

Appendix 1

Table S1. Initial size (cm^3) of juveniles of the three test species (*S. vermiculata*, *L. spartum* and *A. herba-alba*) transplanted beneath adults of the allelopathic shrub *A. herba-alba*, the non-allelopathic shrub *S. vermiculata* and in bare soil (15 juveniles per microsite). Significant differences among microsites ($p < 0.05$) are highlighted in bold.

	Microsite				
Target species	*A. herba-alba*	*S. vermiculata*	Bare Soil	F value	p (>F)
S. vermiculata	12.68 ± 7.14	26.71 ± 19.43	28.37 ± 20.11	0.267	0.77
L. spartum	246.94 ± 44.57	191.09 ± 79.23	322.75 ± 40.78	1.320	0.28
A. herba-alba	9.11 ± 4.20	37.97 ± 26.54	27.28 ± 15.52	0.664	0.52

Appendix 2

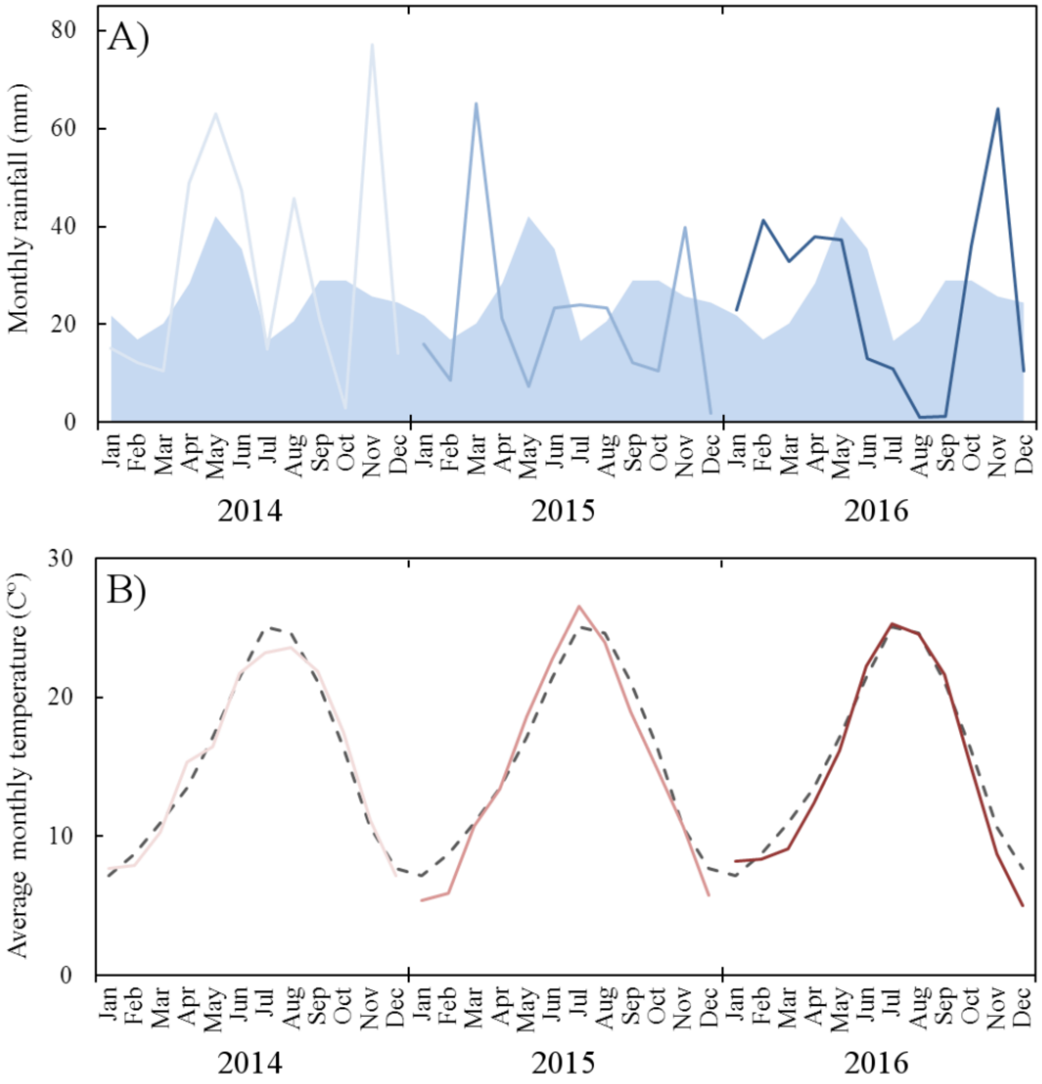


Fig. S1. A) Monthly rainfall (solid lines) in the study area during the period 2014-2016. The shaded area represents the average rainfall over 30 years (1970-2000). B) Average monthly temperature (solid lines) in the study area during the period 2014-2016. The dashed line represents the average temperature over 30 years (1970-2000). Data for the period 2014-2016 were obtained from the nearest meteorological station (Belchite station; http://eportal.magrama.gob.es/websiar/SeleccionParametrosMap.aspx?dst=1). Data for the period 1970-2000 were obtained from the digital climatic atlas of Aragón (http://anciles.aragon.es/AtlasClimatico/).

Appendix 3

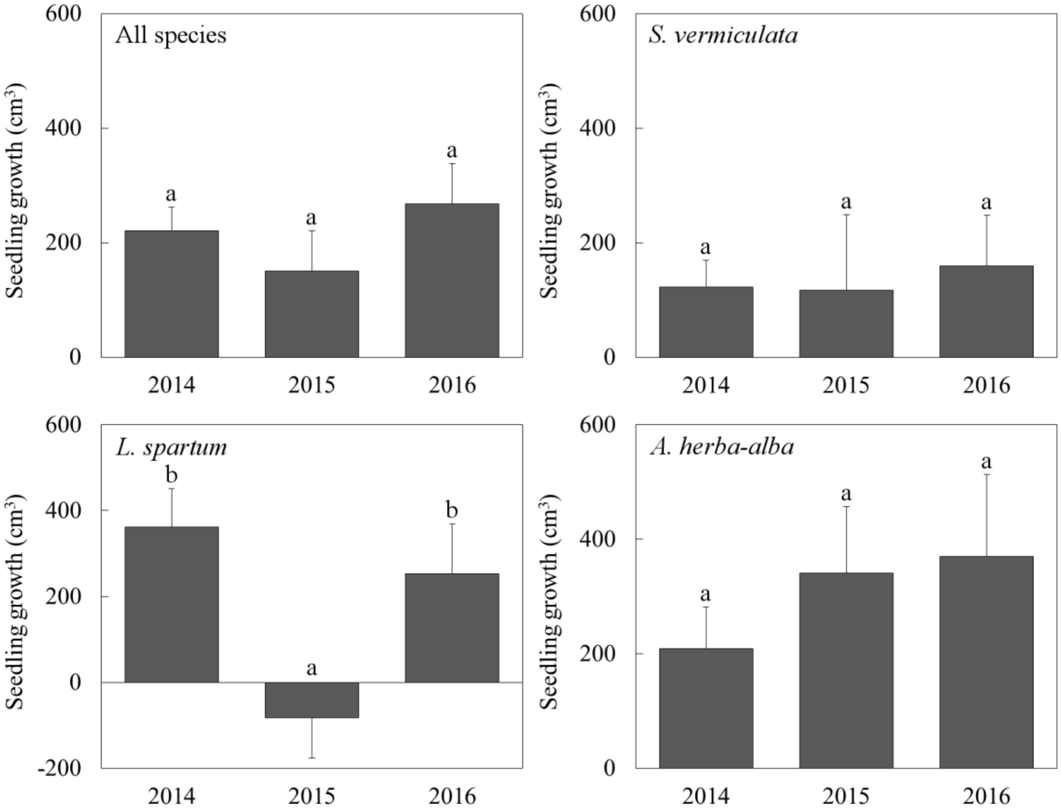


Fig. S2. Annual growth (mean ± SE) of juveniles of the test species transplanted beneath *A. herba-alba*. Different letters indicate significant differences among years (Tukey´s HSD test; $p < 0.05$).

Appendix 4

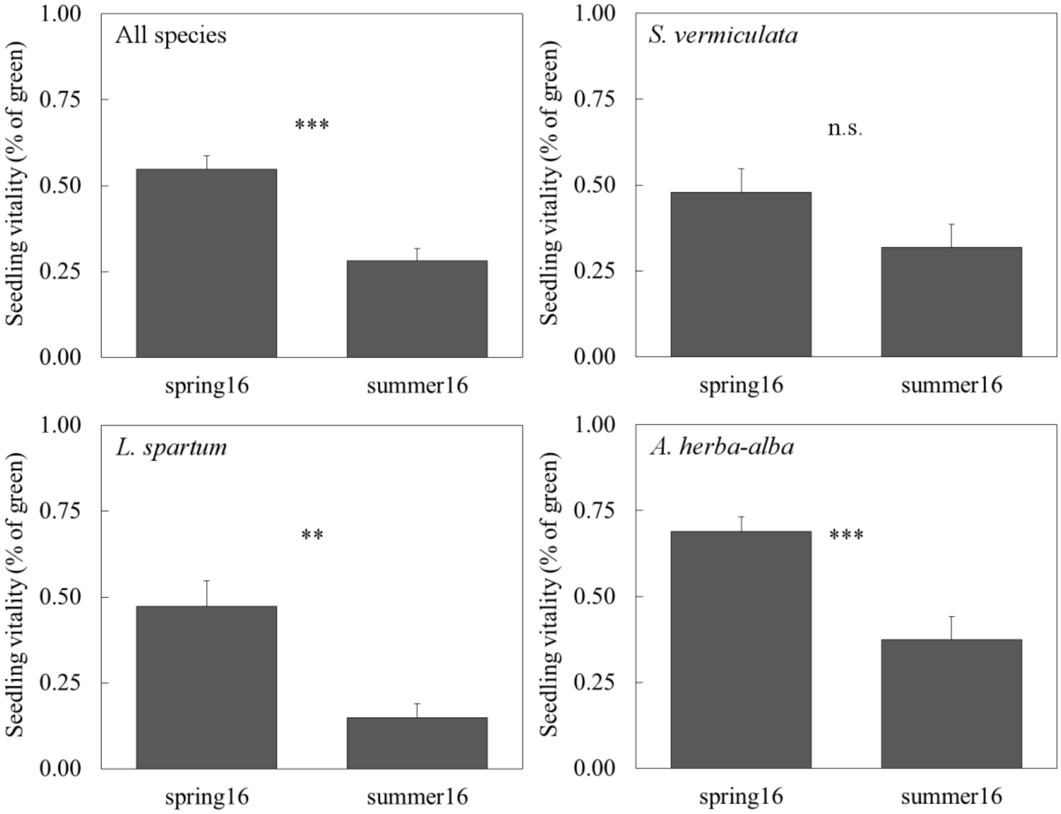


Fig. S3. Vitality (mean % ± SE) of juveniles of the test species transplanted beneath *A. herba-alba* under the two drought stress conditions. n.s.: not significant; **: $p<0.01$; ***: $p<0.001$.

Appendix 5

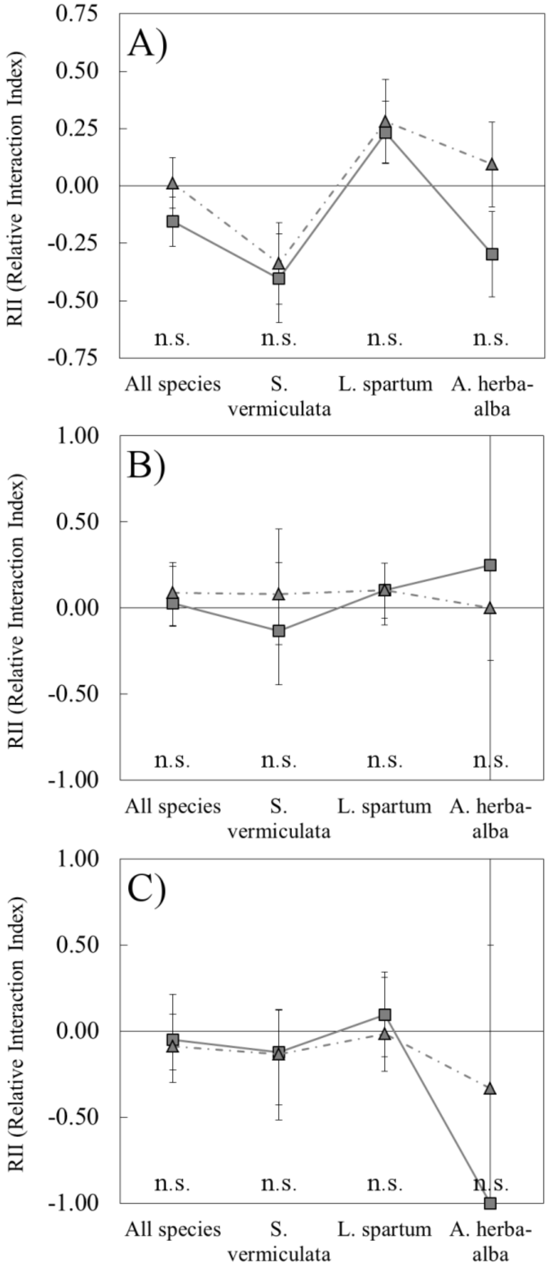


Fig. S4. Relative interaction index (RII) calculated for A) the size (mean ± SE), B) germination and C) survival of the test species in *A. herba-alba* (square symbols) and *S. vermiculata* (triangle symbols) microsites in spring 2016. Bars in B) and C) indicate the 95% confidence interval obtained using the bootstraping (see material and methods section for further details). n.s.: not significant.

Discusión general

En la presente tesis doctoral se ha profundizado en la importancia de la interferencia alelopática de *A. herba-alba* para la organización y la dinámica de las comunidades vegetales semiáridas de la depresión media del Ebro con respecto a otras interacciones bióticas entre plantas (p. ej. facilitación). Para ello se ha combinado el análisis del patrón de la vegetación en las comunidades de estudio con experimentos realizados tanto en laboratorio como en la propia comunidad vegetal en condiciones naturales. Concretamente, se ha pretendido dar respuesta a las siguientes preguntas: ¿Cuál es la relación espacial que existe entre las plantas alelopáticas y el resto de especies que componen la comunidad vegetal semiárida? ¿Favorecen las plantas alelopáticas un patrón menos agregado de la vegetación en su entorno? (Capítulos 1 y 2). ¿Cuál es el efecto de la interferencia química en los distintos estadios del ciclo vital de las plantas que cohabitan con las plantas alelopáticas? ¿Afectan los compuestos químicos liberados por las plantas alelopáticas a los individuos de su propia especie? (Capítulos 1, 3, 4, 5). ¿Cuál es la importancia relativa de la alelopatía en condiciones naturales frente a otras interacciones bióticas? En su caso, ¿varía esta importancia relativa con el nivel de estrés ambiental? (Capítulos 1, 2 y 5).

Willis (1985) estableció hasta seis criterios metodológicos que deben ser cumplidos para poder sugerir que la alelopatía es un fenómeno relevante: (1) debe existir un patrón de inhibición de unas plantas sobre otras, (2) la planta causante de la inhibición, o a través de su hojarasca, debe producir una sustancia fitotóxica, (3) la planta debe liberar esta sustancia al medio, (4) debe existir un modo de transporte y/o acumulación de la toxina en el medio, (5) las plantas afectadas deben tomar de algún modo la sustancia y (6) el patrón de inhibición observado no puede ser explicado solamente por otros factores como la competencia o la herbivoría (ver también Blum, Shafer & Lehman 1999). Sin embargo, lo habitual es que los estudios de alelopatía apenas aborden dos o tres de estos puntos, y a menudo se refieran al potencial alelopático de una especie vegetal basándose únicamente en la evaluación de la fitotoxicidad de un extracto, que ha sido concentrado o diluido de forma arbitraria, en condiciones de laboratorio y sobre especies que no siempre coexisten con la supuesta especie alelopática (Willis 1985). La aproximación integral en esta tesis ha permitido abordar de forma conjunta gran parte de los criterios metodológicos propuestos. Se ha observado que *A. herba-alba* genera en su entorno un patrón espacial de la vegetación más disperso y menos rico en especies de lo que se esperaría de un arbusto dominante que mejora las condiciones microclimáticas en su entorno. Además, se ha encontrado que *A. herba-alba* es rica en metabolitos secundarios, de los cuales se han identificado algunos compuestos fenólicos, que pueden ser liberados tanto por volatilización como disueltos en agua. De los experimentos llevados a cabo se deduce que estos compuestos actúan sobre todo inhibiendo la germinación de muchas especies del banco de semillas, incluyendo las propias semillas de *A. herba-alba*. Otras especies (principalmente gramíneas perennes) parecen haber desarrollado tolerancia a los compuestos alelopáticos de *A. herba-alba*. Un mayor entendimiento de las interferencia alelopática pasa por fortalecer la relación entre la información obtenida en los experimentos desarrollados en laboratorio y el verdadero significado de los compuestos alelopáticos en condiciones naturales (Inderjit & Callaway 2003; Aschehoug *et al.* 2016). En este sentido, una de las mayores aportaciones de la aproximación empleada para el estudio de la alelopatía ha sido la realización de experimentos en el campo. Estos experimentos indican que los compuestos alelopáticos producidos y emitidos por *A. herba-alba* se distribuyen y acumulan en concentraciones suficientes como para que sean tomados por las plantas afectadas, pese a que estas variables no se hayan cuantificado directamente. Además, han permitido comprender que la actividad alelopática de *A. herba-alba* es, al margen del resto de interacciones bióticas, una interacción relevante en las comunidades naturales semiáridas estudiadas, aunque sus efectos en la vegetación pueden ser fácilmente sobreestimados basándose exclusivamente en experimentos de laboratorio. Por tanto, dado que *A. herba-alba* mejora las condiciones microclimáticas bajo sus dosel del mismo modo que otros arbustos facilitadores de la comunidad, la alelopatía parece ser la razón más plausible para explicar el patrón de la vegetación y la diversidad observados a su alrededor.

Evaluación de la relación espacial entre *A. herba-alba* y la vegetación de comunidades semiáridas de la depresión media del Ebro

En ecosistemas sometidos a un elevado nivel de estrés ambiental, como los ecosistemas alpinos o de zonas áridas y semiáridas, la presencia de determinadas especies vegetales facilita el establecimiento de otras plantas en sus proximidades debido a que modifican el microambiente local, de tal manera que resulta menos estresante que las zonas más abiertas (Aguiar & Sala 1997; Callaway & Walker 1997; Cavieres *et al.* 2007; Macek *et al.* 2016). Se considera que generalmente los arbustos crean zonas favorables para el establecimiento de otras especies en las zonas áridas y semiáridas (Gómez-Aparicio 2008, 2009). En el caso de las comunidades vegetales semiáridas de la depresión media del Ebro se ha observado que tanto la disponibilidad de agua en el suelo (por una mayor infiltración en los eventos de lluvia), como la cantidad de nutrientes (p. ej. porcentaje de materia orgánica o nitrógeno (N)), son sustancialmente mayores en el suelo bajo los individuos adultos de *Artemisia herba-alba* Asso. y *Salsola vermiculata* L. (las dos especies arbustivas más abundantes de la comunidad) que en el suelo desnudo (capítulo 1). De forma análoga, las temperaturas máximas diurnas en verano debajo de estas especies de plantas son entre 2.5 y 3.2 ºC inferiores a las que hay en el suelo desnudo debido a una menor radiación solar (capítulo 1). Numerosos trabajos han relacionado el efecto positivo que tienen los arbustos en la vegetación de las comunidades de zonas áridas y semiáridas con la mejora de las condiciones microclimáticas y el aumento de la disponibilidad de recursos bajo su dosel con respecto al suelo desnudo (Pugnaire, Armas & Valladares 2004; Callaway 2007). Por ejemplo, Pugnaire et al. (2004) encontraron que en general la concentración de nutrientes y la humedad del suelo bajo las seis especies arbustivas más abundantes de una comunidad vegetal semiárida en el sureste de la península ibérica fueron mayores que en el suelo desnudo y que se relacionan positivamente con la biomasa de plantas anuales que albergan debajo. Por su parte, Valiente-Banuet y Ezcurra (1991) encontraron que varias especies suculentas que habitan en el Desierto de Vizcaíno y Gran Desierto de Altar, ambos en México, se establecen preferentemente en la cara norte de las especies arbustivas nodriza que es más sombreada. En concordancia con la mejora de las condiciones microclimáticas, se ha encontrado que *S. vermiculata* actúa como una planta nodriza muy eficiente facilitando el establecimiento y la supervivencia de otras plantas, creando así parches de vegetación con una alta diversidad y manteniendo un patrón espacial agrupado de la vegetación. *Salsola vermiculata* facilita sobretodo anuales y también gramíneas perennes (capítulos 1 y 2). La interacción positiva que se establece entre arbustos y anuales ha sido observada en las zonas áridas y semiáridas a lo largo del mundo (Holzapfel *et al.* 2006; López, Larrea-Alcázar & Teresa 2009).

Dada la mejora microclimática bajo las plantas de *A. herba-alba* con respecto al suelo desnudo, cabría esperar que esta especie tuviera un efecto en la vegetación similar al encontrado para *S. vermiculata*. Sin embargo, las plantas adultas de *A. herba-alba* no funcionan como plantas nodriza y presentan, en general, un patrón espacial segregado con respecto a otras plantas perennes (capítulo 1; H1.1). Los compuestos producidos y liberados por *A. herba-alba* inhiben la germinación de nuevas plántulas del banco de semillas, lo que puede conducir a la baja diversidad y densidad de especies encontradas bajo sus individuos (capítulo 3). Otras especies arbustivas alelopáticas de zonas áridas y semiáridas, como *Thymus hyemalis* Lange, también presentan un efecto predominantemente negativo en la vegetación (p. ej. una menor riqueza de especies y biomasa), a pesar de que mejoran las condiciones microclimáticas con respecto al suelo desnudo (Pugnaire *et al.* 2004; Hortal *et al.* 2015). La presencia de plantas con actividad alelopática puede debilitar de alguna manera el efecto de retroalimentación positiva que mantiene la organización de las comunidades vegetales de zonas áridas y semiáridas, desagregando el patrón espacial agrupado en los parches de vegetación y haciendo la comunidad menos productiva (Ludwig, Tongway & Marsden 1999; Alados *et al.* 2003; Kefi *et al.* 2007b). Por tanto, de cara a las labores de gestión, conservación y restauración de las comunidades vegetales semiáridas, la generalización "arbusto = planta facilitadora" no es válida cuando está implicada la alelopatía (Pueyo *et al.* 2009).

La facilitación indirecta se considera un mecanismo importante para la coexistencia de especies que conduce a un aumento de la diversidad (Brooker *et al.* 2008; McIntire & Fajardo 2014). Ehlers et al. (2014) encontraron que en comunidades vegetales Mediterráneas la especie alelopática *Thymus vulgaris* L. aumenta la diversidad de especies en su entorno, llegando a tener un efecto en la comunidad vegetal en conjunto, debido a que los compuestos químicos que produce suprimen a *Bromus erectus* Huds., una especie muy competitiva. En otras palabras, la alelopatía resulta en un aumento de la diversidad por la facilitación indirecta. En las comunidades vegetales semiáridas de la depresión media del Ebro, el arbusto alelopático *A. herba-alba* no tiene dicho efecto positivo en la diversidad observado en otras comunidades mediterráneas menos xéricas. Al contrario, la diversidad alrededor de las plantas de *A. herba-alba* es menor de lo que cabría esperar (capítulo 2; H1.2). La actividad alelopática de *A. herba-alba* actúa como un filtro biótico que reduce el "*pool*" de especies que pueden coexistir en los parches de vegetación dominados por *A. herba-alba*. En la línea de estos resultados, Hortal et al. (2015) encontraron que la riqueza de especies debajo de *T. hyemalis*, un arbusto alelopático de poco porte encontrado en comunidades vegetales semiáridas del suroeste de España, es bastante más baja que la que se puede encontrar bajo otros arbustos facilitadores como *Retama sphaerocarpa* (L.) Boiss o, incluso, que en el suelo desnudo. Por tanto, el predominio de plantas alelopáticas en comunidades semiáridas podría conducir a un empobrecimiento de la comunidad (Bazzaz 1975), y por consiguiente a una disminución en su funcionalidad (Zavaleta *et al.* 2010; Maestre *et al.* 2012b), haciendo estas comunidades más vulnerables a los procesos de desertificación (Safriel & Adeel 2005; Maestre *et al.* 2016).

Es importante señalar que la mera existencia de una zona de inhibición de la vegetación y la diversidad alrededor de las plantas alelopáticas no debe tomarse como prueba inequívoca de la presencia de interferencia química, ya que otros mecanismos, como una fuerte competencia por los recursos, pueden conducir a un patrón de la vegetación similar (Inderjit *et al.* 2005; Armas & Pugnaire 2011; Pescador *et al.* 2014). De hecho, en las comunidades vegetales semiáridas de la depresión media del Ebro, las plantas adultas de *L. spartum* también presentan a su alrededor una menor diversidad de especies de la que cabría esperar (capítulo 2). Sin embargo, hasta donde se sabe *L. spartum* no es una especie alelopática. En este caso, el patrón observado está causado, muy probablemente, por la fuerte competencia por el agua ejercida por el denso sistema radicular de esta gramínea (Jackson *et al.* 1996; Armas & Pugnaire 2011). Por otra parte, es importante señalar que además de su actividad alelopática, *A. herba-alba* tiene unas raíces menos profundas en comparación con las de *S. vermiculata* (Guerrero-Campo 1998), con lo que es menos probable que use agua de capas más profundas del suelo, o favorezca el "*hydraulic lift*"

(Dawson 1993) en el que el agua es redistribuida pasivamente desde capas más profundas de suelo hasta capas más superficiales, aumentando la ventana de máxima disponibilidad de agua bajo las plantas, lo que ayudaría a explicar también su asociación espacial negativa con otras especies vegetales. Por esta razón, el análisis del patrón de la vegetación alrededor de *A. herba-alba* se ha combinado con multitud de experimentos de laboratorio y, sobretodo, de campo.

Evaluación de la interferencia alelopática de *A. herba-alba*

En zonas áridas y semiáridas, el banco de semillas es un elemento crucial que determina la persistencia y coexistencia de muchas especies en la comunidad, y en última instancia, la composición de la vegetación (Olano *et al.* 2005; Caballero *et al.* 2008b; Martinez-Duro *et al.* 2012). En estas zonas, la estructura espacial del banco de semillas sigue el patrón parcheado de la vegetación (Aguiar & Sala 1997). Así, el número de especies y la densidad del banco de semillas es mayor en los parches de vegetación que en el suelo desnudo, ya que los parches actúan como fuente y como sumidero acumulando semillas (Guo, Rundel & Goodall 1998; Giladi, Segoli & Ungar 2013). En concordancia, se ha encontrado que la riqueza y la densidad del banco de semillas son mayores bajo *A. herba-alba* y *S. vermiculata* que en el suelo desnudo (capítulo 3). Además, se ha encontrado que la estructura del banco de semillas (en términos de densidad de semillas y riqueza de especies) de *A. herba-alba* no presenta grandes diferencias con respecto al banco de semillas de *S. vermiculata* (capítulo 3). Aunque los parches de vegetación formados por *S. vermiculata* son más diversos que los de *A. herba-alba* (capítulo 2), una dispersión de semillas muy efectiva puede prevenir diferencias en la riqueza del banco de semillas entre estas dos especies (Saatkamp, Poschlod & Venable 2014). El “cierzo” podría favorecer tal dispersión en las comunidades vegetales semiáridas de la depresión media del Ebro (Longares 1997). Por otra parte, el papel de los parches de vegetación atrapando y acumulando semillas depende principalmente de la altura de los mismos (Caballero *et al.* 2008a). La extrema similitud fisionómica entre *A. herba-alba* y *S. vermiculata* hace pensar que la tasa de captura de semillas debe ser similar, lo que explicaría que la densidad del banco de semillas no sea diferente entre estas dos especies. Únicamente se ha observado una mayor densidad de semillas de *A. herba-alba* bajo individuos de *A. herba-alba* que de *S. vermiculata* (capítulo 3). La explicación más plausible para este hallazgo podría estar relacionada con el hecho de que las muestras del banco de semillas se recogieron justo después del periodo de dispersión de semillas de *A. herba-alba*. Alternativamente, es posible que las semillas de *A. herba-alba* permanezcan junto a las plantas adultas debido a la ausencia de estructuras de dispersión (atelecoria). Sus características, redondas y relativamente pesadas (aprox. 11 mg por semilla), no favorecen la dispersión por el viento (Tackenberg, Poschlod & Bonn 2003; Liu, Zhu & Deng 2012).

Se ha comprobado que los compuestos alelopáticos del extracto acuoso de *A. herba-alba* reducen la emergencia de plántulas desde el banco de semillas (capítulo 3; H2.2). Posteriormente, este resultado ha sido confirmado por los experimentos realizados en condiciones naturales. Concretamente, se ha encontrado que la tasa de germinación de las semillas sembradas bajo plantas adultas de *A. herba-alba* con la adición de carbón activo (CA), un agente muy eficaz adsorbiendo compuestos alelopáticos (Inderjit & Callaway 2003; Hille & den Ouden 2005), es superior a la germinación de las semillas sembradas bajo plantas de *A. herba-alba* sin CA, esto es, con la presencia de los compuestos alelopáticos (capítulo 5). Por tanto, el patrón observado de la vegetación alrededor de las plantas alelopáticas en las comunidades vegetales semiáridas estudiadas puede ser atribuido a sus efectos inhibitorios en el banco de semillas antes que a otras heterogeneidades en la estructura del banco de semillas con respecto a arbustos no alelopáticos. Se ha sugerido que los mecanismos responsables de la inhibición de la germinación están más relacionados con una alteración del metabolismo celular que con el daño celular (Gniazdowska & Bogatek 2005). Por ejemplo, los compuestos alelopáticos pueden disminuir la actividad de encimas como α-amilasa (que regula la descomposición del almidón), isocitrato liasa (implicada en la movilización de los lípidos) o glucosa-6-fosfato deshidrogenasa (necesaria para proveer a la semilla con energía) y por tanto, inhibir la germinación (Gniazdowska & Bogatek 2005).

Cabe señalar que el uso del banco de semillas supone una buena alternativa para suplir algunos de los aspectos más criticados de la mayoría de los estudios de alelopatía que testan los efectos fitotóxicos de determinados compuestos químicos en experimentos realizados bajo condiciones controladas sobre la germinación de una o dos especies de interés (Gniazdowska & Bogatek 2005; Scognamiglio *et al.* 2013). Por un lado, se evita el uso de especies modelo (p. ej. la lechuga; *Lactuca sativa* L.; Escudero *et al.* 2000; Zhang *et al.* 2014) que poco o nada tienen que decir acerca del significado de la alelopatía en comunidades vegetales naturales, ya que no coexisten con la especie alelopática (Inderjit & Callaway 2003). Por otro, el uso de multitud de especies (todas las que forman banco de semillas) es adecuado para poder observar los efectos especie-específicos de la alelopatía (Linhart *et al.* 2015).

Algunos experimentos han mostrado que en ocasiones la inhibición de la germinación por parte de los compuestos alelopáticos no es total, sino que éstos causan sólo un retraso en la velocidad de germinación. Por ejemplo, Preston et al. (2002) encontraron que la exposición de semillas de la especie *Nicotiana attenuata* Steud. a la acción del metil jasmonato, un compuesto emitido por *Artemisia tridentata* Nutt., resulta en un retraso de su germinación de hasta seis días. También se ha observado un retraso en la velocidad de germinación de semillas expuestas a otros compuestos alelopáticos como el BOA (2-benzoxazolinona; Chiapusio *et al.* 1997). En esta línea, se ha encontrado que concentraciones bajas (0.5 g/l) e intermedias (2 g/l) de extracto acuoso de *A. herba-alba* inhiben la germinación de las semillas de *A. herba-alba* durante las dos primeras semanas de exposición, mientras que dicha inhibición ya no se observa posteriormente, lo que indica un retraso en su germinación (capítulo 4). En ecosistemas áridos y semiáridos, el momento de emergencia de las plántulas es un factor clave que determina el destino de las mismas. Cuanto más tarde aparezcan las plántulas, menor será su probabilidad de sobrevivir y establecerse tras la sequía estival (Escudero *et al.* 1999; Castro 2006) por la falta de un sistema radicular lo suficientemente profundo como para alcanzar las capas húmedas del suelo (Padilla & Pugnaire 2007). Por tanto, el patrón espacial segregado de la vegetación y la diversidad alrededor de las plantas alelopáticas de *A. herba-alba* podría ser consecuencia, no sólo de una inhibición de la germinación, sino también de un retraso de la germinación y la subsecuente reducción del éxito en el establecimiento de las plántulas. Sin embargo, en el experimento con el banco de semillas, el extracto acuoso de *A. herba-alba* no retrasó, sino que inhibió la germinación del banco de semillas (capítulo 3). Esta aparente contradicción entre los resultados obtenidos en uno y otro experimentos (capítulos 3 y 4) podría explicarse por el simple hecho de que los compuestos alelopáticos del extracto acuoso en el experimento de germinación en placas de Petri no se reponen a medida que se van degradando (Li *et al.* 2015), mientras que en el experimento con el banco de semillas el riego con extracto acuoso era continuo. Este hecho parece señalar que el efecto fitotóxico de los compuestos presentes en el extracto acuoso de *A. herba-alba* no resulta letal para la viabilidad de las semillas, sino que la presencia y acumulación de estos compuestos es lo que induce la inhibición de la germinación, y cuando éstos son eliminados (p. ej. se degradan o son arrastrados por una lluvia intensa) se produce la germinación tardía (Reigosa *et al.* 1999). De hecho, se ha sugerido que en muchos casos la inhibición de la germinación no se debe a la exposición a determinadas sustancias fitotóxicas, sino a la capacidad de las semillas de reconocer su entorno químico (y reconocer por tanto si son entornos muy competitivos) y posponer la germinación hasta que se den unas condiciones más favorables (reconocimiento bioquímico; Renne *et al.* 2014).

Los compuestos alelopáticos también pueden afectar a otros estadios de las plantas como el crecimiento y la mortalidad. Por ejemplo, Li et al. (2011) encontraron que los compuestos químicos volátiles y solubles en agua de *Artemisia frigida* Willd. inhiben fuertemente el crecimiento temprano de las raíces y de las partes aéreas de las plántulas de tres especies (*Leymus chinensis* (Trin.) Tzvelev, *Stipa krylovii* Roshev. y *Cleistogenes squarrosa* (Trin.) Keng) que coexisten en las mismas comunidades que *A. frigida*. En el caso de *A. herba-alba* se ha encontrado que, en condiciones de laboratorio, los compuestos volátiles que libera pueden reducir la biomasa radicular de las plántulas de ciertas especies, como *S. vermiculata*, que cohabitan en las mismas comunidades vegetales semiáridas. Sin embargo, los resultados fueron especie-específicos, y el efecto observado de los compuestos alelopáticos de *A. herba-alba* en el crecimiento de algunas especies de estudio en condiciones de laboratorio ha sido positivo (capítulo 4; ver discusión más adelante). Además, en otro experimento de laboratorio también se ha observado que el riego con extracto acuoso de *A. herba-alba* a una concentración de 5 g/l resulta en un incremento de la mortalidad de las propias plántulas de *A. herba-alba* que emergen desde el banco de semillas (capítulo 3). Probablemente, esto es el resultado del efecto simultáneo de los compuestos alelopáticos en varios procesos como la estructura y división celular, la permeabilidad de las membranas, la apertura estomatal, la fotosíntesis y la respiración (Reigosa *et al.* 1999; Gniazdowska & Bogatek 2005). Basándose en los resultados obtenidos en los experimentos realizados en condiciones controladas de laboratorio, podría concluirse que el efecto de los compuestos alelopáticos de *A. herba-alba* en la mortalidad y el crecimiento temprano de las plántulas son relevantes para entender el impacto de la alelopatía en las comunidades naturales.

Sin embargo, tales resultados no han podido ser confirmados por los experimentos llevados a cabo en condiciones naturales. Concretamente, no se ha encontrado que la supervivencia de las plántulas de las especies de estudio sea mayor bajo individuos adultos de *A. herba-alba* tratados con CA, esto es, sin interferencia química, que bajo individuos a los que no se ha añadido CA, y por tanto, con la presencia de compuestos alelopáticos. Por otra parte, no se han observado diferencias de crecimiento entre los juveniles de las especies plantadas bajo adultos de *A. herba-alba* con CA y los plantados bajo plantas adultas de *A. herba-alba* sin CA (capítulo 5). En su conjunto, estos resultados indican que los experimentos de laboratorio sobreestiman el verdadero impacto de las plantas alelopáticas en las comunidades vegetales. De hecho, otros trabajos han llegado previamente a la misma conclusión (Stowe 1979; Pisula & Meiners 2010; da Silva *et al.* 2015). Por ejemplo, Lorenzo et al. (2016) encontraron que en condiciones naturales el papel que juegan los compuestos alelopáticos liberados por la especie invasora *Acacia dealbata* Link. en el establecimiento de plántulas de especies nativas en las comunidades invadidas es prácticamente despreciable, mientras que estudios previos de laboratorio e invernadero observaron un efecto fitotóxico de los compuestos liberados por esta especie (Lorenzo *et al.* 2016). Las diferencias observadas en el efecto alelopático de *A. herba-alba* entre los experimentos de laboratorio y de campo podrían estar causadas por las diferencias en las condiciones experimentales. Así, por ejemplo, no es muy habitual que se incluya la actividad de los microorganismos del suelo en los experimentos de laboratorio (p. ej. usando suelo natural; Grove, Haubensak & Parker 2012), mientras que en el campo pueden metabolizar los compuestos alelopáticos, particularmente los ácidos fenólicos, disminuyendo sus efectos de inhibitorios (Blum *et al.* 2000; Kaur *et al.* 2009). Además, en el campo también intervienen los efectos positivos de la facilitación, mientras que en laboratorio la alelopatía se estudia en aislamiento de otras interacciones. Por consiguiente, los resultados obtenidos en estudios de alelopatía realizados bajo condiciones controladas de laboratorio deberían ser interpretados con cierta cautela, ya que podrían llevar a conclusiones erróneas. Por esta razón, todavía son necesarios muchos más estudios de campo para obtener evidencias realistas del significado de la alelopatía en condiciones naturales (Inderjit & Weston 2000).

Tanto en los experimentos de laboratorio como en los experimentos de campo se ha encontrado sistemáticamente un efecto durante la germinación de las semillas de los compuestos producidos y liberados por *A. herba-alba* (capítulos 3, 4 y 5). Por tanto, este parece ser el efecto más evidente de la actividad alelopática de *A. herba-alba* en la dinámica de las comunidades vegetales estudiadas. Aunque la mayoría de los estudios de alelopatía sólo consideran un punto temporal para testar el potencial alelopático de una planta, existe una variación estacional en la síntesis de compuestos alelopáticos, y por tanto en la fitotoxicidad de una planta (Ben-Hammouda *et al.* 1995; Filep *et al.* 2016). Por ejemplo, Ben-Hammouda et al. (1995) encontraron que existe una relación positiva entre el potencial alelopático del sorgo (*Sorghum bicolor* L. Moench) y la concentración en ácidos fenólicos del mismo (Filep *et al.* 2016). En las comunidades vegetales semiáridas de la depresión media del Ebro la germinación de las semillas ocurre sobretodo en primavera, y también en otoño, coincidiendo con la época de lluvias (observación personal). Por lo tanto, cabría esperar que la concentración de compuestos alelopáticos de *A. herba-alba* fuera más elevada durante la época de germinación de las semillas, ya que serían más efectivos inhibiendo la germinación. Además, dado que las semillas de *A. herba-alba* pueden germinar durante el invierno (observación personal), se evitaría también la autotoxicidad (ver discusión más adelante).

Naturaleza química de la interferencia alelopática de *A. herba-alba*

Dada la abundancia de especies aromáticas en las comunidades vegetales semiáridas mediterráneas (Thompson 2005; Scognamiglio *et al.* 2013), se tiende a generalizar que la alelopatía en zonas con escasez de agua está mediada principalmente por el efecto de compuestos volátiles, por ejemplo muchos terpenos, mientras que los compuestos alelopáticos que actúan disueltos en agua, como los fenoles, serían frecuentes de zonas más templadas (Moral & Cates 1971; Chou 1999). Sin embargo, las plantas de zonas áridas y semiáridas producen una gran cantidad de metabolitos secundarios con potencial alelopático más allá de los compuestos volátiles (Bautista *et al.* 2016; Varela *et al.* 2016), muchos de los cuales son solubles en agua (p. ej. compuestos fenólicos). Además, el aumento de la humedad del suelo que genera la presencia de vegetación (Callaway 2007) facilitaría su actividad, al menos dentro de los parches de vegetación. De hecho, el agua es el solvente más utilizado en los estudios de alelopatía para la extracción de los compuestos alelopáticos, y además es el principal solvente bajo condiciones naturales (Scognamiglio *et al.* 2013). En el caso de *A. herba-alba*, se ha observado que tanto los compuestos volátiles como los solubles en agua participan en su interferencia química, sobre todo durante la germinación de las especies vegetales (capítulos 3 y 4; H2.1). Los análisis químicos han revelado que las ramas y las hojas de *A. herba-alba* son ricas en compuestos fenólicos, del mismo modo que el extracto acuoso también lo es (capítulo 4). Estos compuestos son introducidos en el entorno a través de la descomposición de la hojarasca y de los lixiviados de las plantas (Hättenschwiler & Vitousek 2000), y sus efectos abarcan desde efectos biológicos en la permeabilidad de las células y durante la fotosíntesis hasta la interacción con el ciclo de nutrientes (Hättenschwiler & Vitousek 2000; Li *et al.* 2010). La presencia de los ácidos protocatechuico y vanílico en *A. herba-alba* ha sido descrita también por otros trabajos (Seddik *et al.* 2010; Bourgou *et al.* 2016). Por el contrario, los compuestos volátiles emitidos por *A. herba-alba* no han sido identificados, aunque la literatura provee suficiente información en este aspecto. En concreto, se ha encontrado que los monoterpenos oxigenados como alfa y beta tujonas, canfor o crisantenona son los constituyentes principales de la fracción volátil de *A. herba-alba* (Salido *et al.* 2004; Bourgou *et al.* 2016; Younsi *et al.* 2016). Es importante señalar en este punto que la composición y abundancia relativa de estos compuestos puede variar entre regiones geográficas (Salido *et al.* 2004; Belhattab *et al.* 2014). Aunque el diseño experimental no ha permitido una comparación directa de los efectos de ambos tipos de compuestos en las especies que cohabitan en las mismas comunidades que *A. herba-alba*, aparentemente el efecto de los compuestos químicos volátiles es mayor que el efecto de los compuestos solubles en agua, si bien no se puede despreciar el efecto que tienen estos últimos sobretodo en la autotoxicidad (capítulos 3 y 4).

Autotoxicidad de *A. herba-alba* y su significado en zonas áridas y semiáridas

Las plantas que producen compuestos alelopáticos presentan ciertos mecanismos para evitar sus efectos nocivos, como por ejemplo, la compartimentalización de los metabolitos secundarios en vacuolas u otras estructuras especializadas (Friedman & Waller 1985). Sin embargo, una vez que los compuestos químicos son liberados al medio, éstos pueden afectar también a la germinación, el crecimiento y la supervivencia de las propias plantas que los producen. Cuando la alelopatía ocurre dentro de la propia especie se denomina autotoxicidad (Singh *et al.* 1999; Ruan *et al.* 2011). En el campo de la agricultura es de sobra conocido que el cultivo repetido de determinadas especies, como por ejemplo la alfalfa (*Medicago sativa* L.), el arroz (*Oryza sativa* L.) o el espárrago (*Asparagus officinalis* L.), produce una disminución en la producción, a pesar de que las condiciones de humedad del suelo, nutrientes o luz sean óptimas (Chung *et al.* 2000; Amb & Ahluwalia 2016; Kato-Noguchi *et al.* 2017). Junto con la acción de los patógenos, la acumulación de compuestos autotóxicos en el suelo se ha descrito como una de las causas que conduce a este fenómeno ("*soil sickness*" en inglés; Singh *et al.* 1999). Además de en sistemas agrícolas, también se ha observado la existencia de autotoxicidad en comunidades de plantas naturales como en bosques Mediterráneos de P. *halepensis* (Fernandez *et al.* 2008) o matorrales de *Cistus ladanifer* L. (Alías *et al.* 2006) y *Cistus clusii* Dunal (Foronda, A. en revisión). En esta tesis se han encontrado indicios muy sólidos del fuerte carácter autotóxico de *A. herba-alba* (H2.3). En los experimentos que se han desarrollado bajo condiciones controladas de laboratorio se ha observado que tanto los compuestos químicos volátiles que emanan de las ramas y de las hojas de *A herba-alba* como los compuestos solubles en agua presentes en el extracto acuoso de *A. herba-alba*, incluso las concentraciones más bajas, inhiben de manera notoria la germinación de las semillas de *A. herba-alba*, incluyendo las que forman parte del banco de semillas (capítulos 3 y 4). En concreto, se ha identificado al catechol y a los ácidos vanílico y protocatechuico, tres compuestos presentes en el extracto acuoso de *A. herba-alba*, como posibles responsables directos de su autotoxicidad. Además, en los experimentos llevados a cabo en condiciones naturales durante más de dos años, se ha encontrado que la vitalidad de las plantas de *A. herba-alba* plantadas bajo individuos adultos de *A. herba-alba* con la adición de CA es mayor que la vitalidad de las plantas de *A. herba-alba* plantadas bajo adultos de su misma especie sin el uso de CA (capítulo 5). En consonancia, el análisis del patrón espacial de la vegetación en una comunidad semiárida de la depresión media del Ebro señala que las plantas adultas de *A. herba-alba* aparecen segregadas espacialmente de otras plantas de *A. herba-alba*, independientemente de su estado ontogenético (adultos, juveniles o plántulas), es decir, que las nuevas plántulas de *A. herba-alba* se establecen mejor lejos de la presencia de individuos adultos y los compuestos que estos producen (capítulo 1). Estos resultados concuerdan con estudios anteriores. Friedman y Orshan (1975) observaron que aunque las semillas de *A. herba-alba* son dispersadas cerca de los individuos parentales, pocas plántulas nuevas de *A. herba-alba* emergen debajo de su dosel y, además, las pocas que lo hacía sufrían una alta mortalidad durante el verano. De forma similar, Atoum et al. (2006) encontró que el extracto acuoso realizado a partir de frutos de *A. herba-alba* puede reducir la elongación inicial de las plántulas de *A. herba-alba*. Por lo tanto, queda patente que los compuestos producidos y liberados por *A. herba-alba* tienen efectos deletéreos significativos en sus propias plántulas y semillas.

A pesar de que la autotoxicidad es un fenómeno bien descrito en la bibliografía, su significado ecológico no está muy claro. Por ejemplo, se ha especulado con que la autotoxicidad es un coste derivado de las defensas químicas contra la herbivoría o los patógenos, o que fuerza una mayor dispersión de las semillas al no poder éstas germinar cerca de los individuos parentales por la presencia de los compuestos químicos, favoreciendo así la colonización de nuevas áreas y evitando la endogamia (Reigosa *et al.* 1999; Singh *et al.* 1999; Nilsen 2003). Alternativamente, en zonas áridas y semiáridas, la autotoxicidad puede constituir una ventaja adaptativa para autoregular la densidad de población, disminuyendo el reclutamiento de individuos conespecíficos cerca de los individuos adultos o, al menos, retrasando la germinación hasta que las condiciones sean más favorables (p. ej. tras una lluvia; Friedman & Waller 1985; Singh *et al.* 1999). De este modo, se prevendría la competencia intraespecífica por los recursos, que se espera que sea muy elevada debido a que los individuos de la misma ocupan el mismo nicho ecológico (Armas & Pugnaire 2011), y a que los recursos (p. ej. el agua) son escasos. Aparte de los metabolitos secundarios, se han propuesto otros mecanismos que podrían regular el proceso autotóxico. Por ejemplo, recientemente Mazzoleni et al. (2015) encontraron evidencias de que la exposición a fragmentos de ADN extracelular procedentes de la descomposición de la hojarasca inhibe la germinación y el crecimiento de la raíz de los individuos conespecíficos, mientras que no tiene efecto alguno en individuos heteroespecíficos. A menudo los estudios de alelopatía no exploran los efectos autotóxicos de los compuestos químicos (Nilsen 2003; Dorning & Cipollini 2005), y es evidente que todavía quedan avances por hacer para entender el significado ecológico y los mecanismos de la autotoxicidad. En cualquier caso, parece que la autotoxicidad de *A. herba-alba* juega un papel importante determinando su organización espacial y dinámica en las comunidades semiáridas de la depresión media del Ebro.

Tolerancia a los compuestos alelopáticos como mecanismo de coexistencia junto a las plantas alelopáticas de *A. herba-alba*

La magnitud del efecto que tienen las plantas de *A. herba-alba* en la estructura y organización de las comunidades vegetales semiáridas de la depresión media del Ebro estará determinada por la cantidad de especies vegetales dentro de la comunidad capaces de tolerar los compuestos químicos producidos y liberados por ella. En este sentido, uno de los resultados más consistentes que se ha obtenido en esta tesis, es la tolerancia de muchas especies de gramíneas perennes, como *Lygeum spartum* L., a los compuestos alelopáticos de *A. herba-alba* (H2.4). En ninguno de los experimentos de laboratorio o de campo llevados a cabo se ha observado un efecto negativo de los compuestos volátiles o solubles en agua liberados por *A. herba-alba* en la germinación de las cariopses de *L. spartum* (capítulos 4 y 5). Otros trabajos han informado sobre la adaptación de ciertas especies a su “vecino químico”. Por ejemplo, Grøndahl y Ehlers (2008) encontraron que especies que crecen habitualmente junto con plantas de tomillo que producen mayoritariamente el terpeno carvacrol, se desarrollan mejor en suelos tratados con este terpeno que en suelos tratados con β-caryophylleno, mientras que las plantas que viven junto con tomillos que producen mayoritariamente β-caryophylleno se desarrollan mejor en suelos tratados con este último que con carvacrol. De forma similar, Jensen y Ehlers (2010) encontraron que tres especies que crecen en localidades con presencia de *Thymus pulegioides* L. se desarrollan mejor en suelos tratados con carvacrol (un monoterpeno producido por *T.*

pulegioides) que esas mismas tres especies cuando proceden de localidades en las que *T. pulegioides* no está presente, lo que indica que *T. pulegioides* actúa como un agente selectivo en las especies asociadas, favoreciendo los genotipos con un mayor *fitness* en presencia de los compuestos alelopáticos que produce. Probablemente, la adaptación de estas especies a la presencia de las plantas alelopáticas y sus compuestos químicos sea el resultado de una larga historia evolutiva conjunta (Vivanco *et al.* 2004; Thorpe *et al.* 2011).

En los experimentos de laboratorio se ha observado un efecto estimulante de los compuestos químicos, tanto volátiles como solubles en agua, producidos por *A. herba-alba* en algunas especies de gramíneas. Más precisamente, se ha encontrado que estos compuestos estimulan el crecimiento de las plántulas de *L. spartum*, así como la supervivencia de *Dactylis glomerata* L. subsp. *hispánica* (Roth) Nyman (capítulos 3, 4). Efectos positivos de los compuestos alelopáticos han sido reportados también en la bibliografía (Tsubo *et al.* 2012; Fernandez *et al.* 2013). Un buen ejemplo es el trabajo de Tsubo et al. (2012) en el que observaron que la exposición a bajas concentraciones de los compuestos químicos emitidos por *Artemisia adamsii* Besser resultó en una estimulación de las tasas fotosintéticas de la gramínea *Stipa krilovii* Roshev., y por tanto de su crecimiento. Este fenómeno, es decir, el efecto estimulante que tiene una pequeña dosis de una sustancia tóxica, se conoce como hormesis (Duke *et al.* 2006). El efecto positivo de los compuestos alelopáticos de *A. herba-alba* en el crecimiento y la supervivencia de las gramíneas no ha podido ser confirmado en los experimentos realizados en condiciones naturales (capítulo 5). Sin embargo, en las comunidades vegetales semiáridas de la depresión media del Ebro, el patrón espacial de las gramíneas perennes es más agrupado y su diversidad es mayor entorno las plantas adultas de *A. herba-alba* de lo que cabría esperar (capítulos 1 y 2). Además, se ha encontrado una interacción neta de facilitación para las plántulas de *L. spartum* que fueron plantadas bajo el dosel de individuos adultos de *A. herba-alba* (capítulo 5). Esto significa que la tolerancia que exhiben las gramíneas perennes frente a los compuestos químicos de *A. herba-alba* les permite coexistir con ella, y beneficiarse de las condiciones microclimáticas más benignas bajo el dosel de *A. herba-alba* que en el suelo desnudo. Este hecho resalta el carácter especie específico de la alelopatía (Herranz *et al.* 2006; Linhart *et al.* 2015) y la importancia de una aproximación multiespecífica para obtener evidencias realistas acerca de los efectos de la alelopatía en la organización y dinámica de las comunidades vegetales, más allá de los efectos negativos.

Importancia relativa de la alelopatía frente al resto de interacciones bióticas

El balance neto de interacciones bióticas es el resultado entre la facilitación y la interferencia, donde la interferencia incluye tanto la competencia por los recursos como la alelopatía (Muller 1969). El uso de especies muy similares a las plantas alelopáticas y que coexisten con éstas en las mismas comunidades vegetales, pero que no muestran interferencia química, ha sido propuesto como un control adecuado para comprobar el efecto de la actividad alelopática, especialmente en estudios de campo (da Silva *et al.* 2015). En las comunidades vegetales semiáridas de la depresión media del Ebro, *A. herba-alba* y *S. vermiculata* son dos arbustos con una fisionomía muy parecida. Ambas son especies arbustivas, longevas, con un dosel moderadamente denso y que mejoran de forma muy similar las condiciones microclimáticas (mayor humedad del suelo y concentración de nutrientes) con respecto al suelo desnudo (capítulo 1), creando así un entorno favorable para el establecimiento de la vegetación de estas comunidades (capítulo 1; Drezner 2007; Pueyo *et al.* 2013). En el experimento de trasplantes realizado en condiciones naturales se ha observado que la interferencia química de *A. herba-alba* reduce la vitalidad de las plantas de su entorno. Como consecuencia, el resultado del balance neto de las interacciones de *A. herba-alba* no es positivo, a diferencia del arbusto *S. vermiculata* que coexiste en las mismas comunidades vegetales semiáridas, sino que es neutral, o incluso negativo (capítulo 5; H3.2). Esto significa que la vitalidad de las plantas debajo de *S. vermiculata* es mayor que en el suelo desnudo, mientras que la vitalidad de las plantas debajo de *A. herba-alba* no. Mientras que docenas de trabajos han demostrado la capacidad fitotóxica de los compuestos producidos y liberados por *A. herba-alba* (capítulos 3 y 4; Escudero *et al.* 2000; Mohamed *et al.* 2010), experimentos de laboratorio preliminares realizados con extracto acuoso de *S. vermiculata* no han indicado en efecto fitotóxico del mismo. Por tanto, parece que la actividad alelopática de *A. herba-alba* es suficiente como para contrarrestar o limitar sus potenciales efectos de facilitación.

Recientemente, se ha sugerido que mecanismos que pueden tener efectos negativos en otras plantas, como la alelopatía, pueden ser considerados como estrategias para no convertirse en plantas facilitadoras intra- (ver apartado anterior) e interespecíficamente (van der Putten 2009). La mayoría de los trabajos estudian únicamente los efectos positivos que las plantas nodrizas tienen sobre las plantas facilitadas (p. ej. Holzapfel *et al.* 2006; Callaway 2007). Sin embargo, una alta densidad de plantas facilitadas puede suponer un coste en el *fitness* de la planta facilitadora debido al incremento de la competencia por los recursos (Holzapfel & Mahall 1999; Schöb *et al.* 2014; pero ver Tirado *et al.* 2015). Además, se ha encontrado que las plantas facilitadas pueden terminar sustituyendo a las plantas nodriza (Armas & Pugnaire 2005). En las comunidades vegetales semiáridas de la depresión media del Ebro se ha observado que *A. herba-alba* puede facilitar a las gramíneas, incluyendo las plántulas de *L. spartum* (capítulos 1, 2 y 5), pero *L. spartum* no facilita el establecimiento de *A. herba-alba* (capítulo 1), lo que estaría sugiriendo que *L. sparutm* termina sustituyendo a *A. herba-alba*. En tal caso, cuando los individuos con un menor potencial alelopático sufren una mayor reducción en su *fitness*, la selección favorecerá aquellos individuos con un mayor potencial alelopático (van der Putten 2009). Por otra parte, si la inversión en la producción de compuestos alelopáticos también implica una reducción en el *fitness*, entonces deberá existir un equilibrio entre el coste de producir una gran cantidad de estos compuestos y el coste de tener una alta densidad de plantas alrededor (van der Putten 2009).

El balance neto de interacciones varía en función del grado de estrés ambiental. Así, la hipótesis del gradiente de estrés (SGH) propone que la frecuencia y la intensidad de las interacciones positivas con respecto a las interacciones negativas aumentan conforme lo hace el nivel de estrés (Bertness & Callaway 1994; He & Bertness 2014). Pero, por otra parte, tanto la producción de compuestos alelopáticos como el impacto de la alelopatía también se ven influenciados por el grado de estrés ambiental (Pedrol *et al.* 2006; Rivoal *et al.* 2011). Normalmente, la producción de compuestos alelopáticos, su acumulación y su fitotoxicidad son mayores bajo mayores condiciones de estrés ambiental como elevadas temperaturas, una intensa radiación solar o la escasez de agua (Einhellig 1994; Tang *et al.* 1995; Pedrol *et al.* 2006). Por ejemplo, en un experimento de laboratorio Zhang et al. (2014) encontraron que el efecto alelopático del extracto acuoso obtenido a partir de hojas frescas del arbusto invasor *Lantana camara* L. inhibiendo la germinación y el crecimiento de plántulas de lechuga fue mayor conforme se aumentó la temperatura. Además, un mayor nivel de estrés de origen biótico puede conducir al mismo efecto. En este sentido, Karban (2007) encontró que la germinación de diversas especies herbáceas y gramíneas expuestas a los compuestos emitidos por *Artemisia tridentata* Nutt cuyas hojas habían sido dañadas experimentalmente simulando la acción de los herbívoros, fue menor que aquellas expuestas a *A. tridentata* sin dañar. De forma similar, Rivoal et al. (2011) encontraron que un mayor nivel de competencia intraespecífica en *P. halepensis* se traduce en un incremento en la producción de compuestos alelopáticos como ácidos fenólicos y alifáticos, aunque la producción decrece para niveles muy altos de competencia por los recursos. Sin embargo, de entre la multitud de estudios que examinan el balance neto de las interacciones bióticas en comunidades vegetales de zonas áridas y semiáridas, prácticamente ninguno incluye especies alelopáticas (Jankju 2013; Hortal *et al.* 2015).

En un trabajo previo, Jankju (2013) observó que el balance neto de interacciones de la especie alelopática *Artemisia kopetdaghensis* Krasch cambió desde neutral (o incluso positivo) hacia negativo bajo unas mayores condiciones de aridez. Sin embargo, en contra de la hipótesis planteada (H3.3), en las comunidades vegetales semiáridas de la depresión media del Ebro se ha observado un cambio en el balance neto de interacciones de *A. herba-alba* desde la neutralidad (o negativo) hacia netamente positivo con mayor aridez, de acuerdo con la predicción de la SGH (capítulos 2 y 5). Dicho de otro modo, no se han encontrado pruebas que indiquen que la importancia relativa de la alelopatía aumenta con el grado de estrés ambiental, sino que las interacciones positivas predominan sobre la interferencia de las plantas alelopáticas bajo condiciones más severas, independientemente de si la producción o la fitotoxicidad de los compuestos alelopáticos también aumentan bajo estas condiciones (Friedman 1987; Einhellig 1994). En la línea de estos resultados, Hortal et al. (2015) reportaron que el balance neto de interacciones de la especie alelopática *T. hyemalis* es menos negativo conforme la humedad del suelo decrece y la temperatura aumenta. Una posible explicación sería que la escasez en el contenido de agua del suelo haya limitado la difusión de los compuestos alelopáticos solubles en agua hasta las plantas vecinas (Kobayashi 2004), y que al mismo tiempo no haya sido suficiente como para que las competencia por el agua haya contrarrestado las interacciones positivas (Maestre *et al.*

2009; Holmgren & Scheffer 2010). De acuerdo con esto último, el balance de interacciones de *S. vermiculata* también fue netamente positivo bajo las condiciones de mayor escasez de agua (capítulo 5). El hecho de que las interacciones positivas predominen sobre la interferencia de *A. herba-alba* bajo condiciones de mayor estrés ambiental sugiere que los parches dominados por esta especie podrían poseer cierta resiliencia frente al cambio a un nuevo estado de degradación (Kefi *et al.* 2007b), lo que resultaría de gran utilidad de cara a las labores de conservación y restauración de estos ecosistemas ante los distintos escenarios de cambio climático (IPCC 2014). No obstante, todavía es necesaria una mayor investigación sobre cómo varía el balance neto de interacciones de las plantas alelopáticas a lo largo de un gradiente más amplio de estrés ambiental (López *et al.* 2016) para poder obtener conclusiones definitivas.

Perspectivas y direcciones futuras

Los ecosistemas áridos y semiáridos se encuentran entre los más vulnerables al cambio global (Schroter 2005; Kefi *et al.* 2007a). El aumento de la aridez combinado con el sobrepastoreo suponen las principales amenazas que promueven la desertificación en estos ecosistemas (Safriel & Adeel 2005; Kefi *et al.* 2007a), entendida como una reducción o pérdida en su productividad y funcionalidad (Safriel & Adeel 2005; Maestre *et al.* 2016). El cambio de un estado relativamente saludable del ecosistema a un estado desertificado, lejos de ser gradual, a menudo se produce de manera abrupta, y no resulta fácil de recuperar (Schlesinger *et al.* 1990; Scheffer *et al.* 2001; Pueyo *et al.* 2009). Sin embargo, se ha encontrado que la facilitación disminuye el riesgo de que se produzca un cambio abrupto a un estado de degradación (Kefi *et al.* 2007b). En zonas áridas y semiáridas los arbustos a menudo actúan como plantas facilitadoras para el resto de especies de la comunidad vegetal, y por eso han sido recomendados como herramienta durante las labores de restauración (Gómez-Aparicio *et al.* 2004; Padilla & Pugnaire 2006; Gómez-Aparicio 2009). En el caso de las comunidades vegetales semiáridas estudiadas se ha encontrado que, más allá de la reducción de las condiciones de estrés ambiental y el aumento de fertilidad bajo su dosel, *A. herba-alba* no actúa como un arbusto facilitador. La presencia de compuestos alelopáticos emitidos por *A. herba-alba* actúa como un filtro biótico que reduce el nicho que el resto de las especies pueden ocupar, y por lo tanto la plantas adultas de *A. herba-alba* mantienen un patrón espacial segregado respecto a otras especies y una menor diversidad de especies a su alrededor de la que cabría esperar (capítulos 1 y 2). De este modo, las comunidades vegetales dominadas por *A. herba-alba* y, por extensión, por otras especies alelopáticas pueden ser especialmente frágiles y vulnerables ante el cambio global (Kefi *et al.* 2007b). Las labores de restauración en estas comunidades deberían centrarse más en el uso de otros arbustos facilitadores de la comunidad como *S. vermiculata* (Pueyo *et al.* 2009). No obstante, los parches formados por *A. herba-alba* pueden mostrar cierta resiliencia frente al cambio climático, ya que bajo unas mayores condiciones de aridez se ha observado una interacción netamente positiva por parte de *A. herba-alba*, sobre todo para las especies tolerantes a los compuestos que produce (capítulo 5). Además, se han encontrado indicios de que el pastoreo moderado, como el que se practica en la región de la depresión media del Ebro (Pueyo 2005), no parece potenciar el efecto alelopático de *A. herba-alba* en la vegetación (capítulo 2) y, por lo tanto, no se consideraría como un elemento que esté aumentando la fragilidad de estas comunidades.

El hecho de que a menudo la alelopatía se pase por alto respecto a otras interacciones bióticas, puede estar relacionado con la dificultad de separar la interferencia de una planta sobre otra debida a la competencia por los recursos, de la interferencia debida a alelopatía (Inderjit & del Moral 1997). Es posible que tal separación no sea realista, ya que en la naturaleza ocurren las dos a la vez (Inderjit & del Moral 1997). Sin embargo, cuantificar la importancia relativa de la alelopatía frente a la competencia por los recursos puede resultar clave para comprender su importancia en los ecosistemas naturales. Una de las formas que se emplea para discernir entre competencia y alelopatía es mediante el uso de CA para secuestrar los compuestos alelopáticos (Inderjit & Callaway 2003). Sin embargo, el uso del CA no está exento de críticas o limitaciones. Por ejemplo, el CA se ha asociado a un aporte extra de nutrientes que puede interferir con los resultados encontrados y conducir a interpretaciones erróneas sobre el papel de la alelopatía en la comunidad vegetal (Lau *et al.* 2008). Otra manera de discernir experimentalmente entre alelopatía y competencia por los recursos se basa en la naturaleza denso dependiente de los efectos fitotóxicos de los compuestos alelopáticos (Weidenhamer, Hartnett & Romeo 1989; Weidenhamer 2008). La naturaleza denso dependiente de los efectos fitotóxicos de los compuestos alelopáticos resulta de que las plantas “compiten” por las sustancias alelopáticas del mismo modo que lo hacen por los recursos. Así, ante la exposición a una determinada cantidad de sustancias alelopáticas, las plantas que crecen en bajas densidades disponen de una mayor cantidad de tales sustancias por planta, y por tanto sufrirán un mayor impacto negativo en su crecimiento, que aquellas que crecen en altas densidades (Weidenhamer 2008). La realización de futuros experimentos en este sentido ayudaría a determinar definitivamente cuál es el tipo de interferencia que predomina en *A. herba-alba*.

Además de los efectos biológicos directos que los compuestos alelopáticos puedan tener en las plantas susceptibles, existen cada vez más evidencias acerca de sus efectos indirectos modificando las comunidades de microrganismos del suelo y su actividad (Inderjit *et al.* 2011; Cipollini *et al.* 2012; Grove *et al.* 2012; Chomel *et al.* 2016). En esta tesis no se ha incluido el papel que pueden jugar los microrganismos del suelo en la alelopatía de *A. herba-alba*. Sin embargo, su estudio podría ser fundamental para comprender de forma completa la importancia de la alelopatía determinando la estructura de la vegetación de las comunidades semiáridas estudiadas. Los microorganismos de la rizosfera juegan un papel clave en la supervivencia de las plantas. Estos microrganismos protegen a las plantas de los patógenos, intervienen en la provisión a la planta de N y P, y en la mejora del balance hídrico (Augé 2001; Sikes, Cottenie & Klironomos 2009; Palacio *et al.* 2012). Se ha documentado como la presencia de compuestos alelopáticos puede causar una inhibición de estos microrganismos (p. ej. micorrizas y bacterias formadoras de nódulos; Cipollini *et al.* 2012). Por ejemplo Grove et al. (2012) encontraron que la abundancia de micorrizas en las plántulas de *Pseudotsuga menziesii* (Mirb.) Franco (un abeto originario de Norteamérica) es menor en aquellas que crecen en suelos colonizados por el arbusto alelopático *Cytisus scoparius* (L.) Link y en suelos a los que se añaden restos de la especie alelopática, que en las plántulas de *P. menziesii* que crecen en suelos no colonizados por *C. scoparius*. La eliminación de los microrganismos por parte de la planta alelopática se traduce en un efecto negativo en la planta huésped, y en una ventaja competitiva para la planta alelopática (Cipollini *et al.* 2012). Por otra parte, los compuestos producidos y liberados por las plantas alelopáticas pueden ser alterados por la actividad metabólica de los microrganismos del suelo, aumentando o disminuyendo así su potencial alelopático. Por un lado, la actividad metabólica de los microorganismos puede degradar determinados compuestos que no son tóxicos en otras formas más tóxicas (Gagliardo &

Chilton 1992). En otras ocasiones, compuestos con un elevado efecto fitotóxico para las plantas pueden ser degradados por los microrganismos en formas menos activas. Por ejemplo, Zhu et al. (2011) encontraron que los microrganismos del suelo pueden reducir los efectos alelopáticos de la especie invasora *Eupatorium adenophorum* Spreng en las comunidades naturales invadidas. En este sentido, sería muy interesante comprobar si la tolerancia que muestran muchas especies de gramíneas perennes a la actividad alelopática de *A. herba-alba* está relacionada con la presencia de microrganismos en su rizosfera capaces de degradar los compuestos alelopáticos de *A. herba-alba* en formas menos activas.

Por otra parte, cabe mencionar que los resultados y las conclusiones presentados en esta tesis doctoral sobre la importancia de la alelopatía para la estructura y la dinámica de las comunidades vegetales semiáridas, están basados en las plantas alelopáticas de una única especie y en unas comunidades concretas. Por lo tanto, futuros trabajos deberían comprobar la generalidad de estos hallazgos en otras especies alelopáticas a lo largo de la gran variedad de condiciones que componen las zonas áridas y semiáridas. Por ejemplo, el grupo de trabajo cuenta con abundantes datos de vegetación (en forma de transectos como los utilizados en el capítulo 2) de varias comunidades semiáridas del valle del Ebro y el sureste peninsular (Parque Nacional del Cabo de Gata-Níjar). Utilizando la metodología empleada en el capítulo 2 de esta tesis, se podría analizar el efecto en la diversidad de distintas especies arbustivas que habitan en estas comunidades y que podrían ser alelopáticas, como es el caso de *C. clusii* (Foronda *et al.* en revisión). Esto, combinado con el análisis de la frecuencia de la coexistencia entre pares de especies (capítulo 2) podría ayudar a identificar posibles especies en riesgo de ser excluidas de las comunidades dominadas por especies alelopáticas. Además, la alelopatía puede afectar a la sucesión vegetal, de modo que los compuestos alelopáticos liberados por las plantas alelopáticas pueden evitar la entrada de especies más tardías, por ejemplo inhibiendo su germinación, y por tanto permanecer más tiempo en la sucesión (Muller 1969; Reigosa *et al.* 1999). La abundancia de parcelas de cultivo que fueron abandonadas en distintas épocas en muchas áreas de la zona de estudio ofrece un marco ideal para que nuevos trabajos amplíen el estudio de la importancia de la alelopatía para la estructura y la dinámica de las comunidades vegetales en los distintos estadios de la sucesión vegetal. Por ejemplo, *A. herba-alba* es pionera en la colonización de las parcelas abandonadas (Cerdà 1997a) y su actividad alelopática podría causar una ralentización en la sucesión secundaria en comparación con parcelas abandonadas en localidades donde *A. herba-alba* no esté presente o no sea tan abundante. Aunque, alternativamente, la autotoxicidad podría reducir la dominancia inicial de *A. herba-alba* (Muller 1969). Por último, también sería interesante comprobar el papel que puede jugar la alelopatía reduciendo parcialmente el éxito de repoblaciones de *P. halepensis* llevadas a cabo en comunidades vegetales semiáridas de la depresión media del Ebro dominadas localmente por *A. herba-alba* (Jesús Csculluela, comunicación personal).

Conclusiones

1. Las condiciones microclimáticas bajo *A. herba-alba* son más benignas que en el suelo desnudo por el incremento en la humedad del suelo, la infiltración de agua tras una lluvia y la concentración de nutrientes y la reducción en la radiación solar. Sin embargo, las plantas de *A. herba-alba* no funcionan, en general, como plantas nodrizas facilitando el establecimiento de otras plántulas bajo su dosel, debido a su actividad alelopática. Este hecho pone de manifiesto que la identificación de una especie como nodriza no puede ser inferida simplemente a partir de las condiciones microclimáticas bajo su dosel.

2. La interferencia química de *A. herba-alba* favorece una disminución de la diversidad alrededor de sus individuos, al contrario de lo que se ha observado para las plantas alelopáticas de *Thymus vulgaris* L. en una comunidad vegetal mediterránea menos xérica. No obstante, hay determinadas especies (p. ej. muchas gramíneas perennes) que aparecen más agrupadas alrededor de *A. herba-alba* de lo que cabría esperar por azar, resaltando la capacidad de estas especies para tolerar los compuestos químicos liberados por *A. herba-alba*.

3. El patrón espacial de la vegetación observado alrededor de los individuos de *A. herba-alba* no se debe a diferencias en la densidad de semillas y riqueza de especies del banco de semillas con respecto a otros arbustos de la misma comunidad vegetal, sino a que los compuestos químicos liberados por *A. herba-alba* inhiben la emergencia de nuevas plántulas desde el banco de semillas. Esto señala que los efectos de las plantas alelopáticas en la organización de las comunidades vegetales semiáridas están controlados, al menos en parte, por sus efectos en el banco de semillas del suelo.

4. *Artemisia herba-alba* es una especie con un fuerte carácter autotóxico. Los compuestos químicos producidos y liberados por *A. herba-alba* dificultan el establecimiento de sus propias plántulas en las inmediaciones de plantas

adultas, inhibiendo la germinación de las semillas y aumentando la mortalidad de las plántulas de *A. herba-alba* que emergen desde el banco de semillas. Es posible que en zonas áridas y semiáridas la autotoxicidad represente una ventaja adaptativa para autoregular la densidad de poblacional y disminuir, en última instancia, la competencia intraespecífica por los recursos.

5. Tanto los compuestos químicos volátiles como los solubles en agua producidos y liberados por *A. herba-alba* artemisia son responsables de su autotoxicidad. El extracto acuoso de *A. herba-alba* es rico en sustancias fenólicas y algunos de estos fenoles (p. ej. ácidos fenólicos) participan en su autotoxicidad. Aunque aparentemente los efectos de los compuestos volátiles son más fuertes, el papel de los compuestos alelopáticos solubles en agua podría ser más importante en las zonas áridas y semiáridas de lo que se ha pensado anteriormente.

6. La actividad alelopática de *A. herba-alba* constituye un filtro biológico que determina qué especies son capaces o no de coexistir con las plantas alelopáticas y, en general, es más relevante durante la fase de germinación de las semillas que durante el crecimiento de las plántulas. No obstante, algunas especies que cohabitan en las mismas comunidades que *A. herba-alba* (p. ej. *Lygeum spartum*) muestran una elevada tolerancia a los compuestos alelopáticos producidos por esta especie, probablemente debido a una larga historia evolutiva conjunta.

7. En condiciones naturales la interferencia alelopática de *A. herba-alba* es una interacción relevante inhibiendo la germinación y reduciendo la vitalidad de las especies vegetales. Sin embargo, mientras que en condiciones de laboratorio se ha encontrado que los compuestos químicos producidos y liberados por *A. herba-alba* afectan a la mortalidad y al crecimiento de las plántulas, tales efectos no se han observado en condiciones naturales. Esto señala que los resultados obtenidos en experimentos de laboratorio deben ser interpretados con cautela, al mismo tiempo que más experimentos de campo

son necesarios para obtener evidencias realistas acerca del significado de la alelopatía en las comunidades vegetales.

8. El balance neto de interacciones del arbusto alelopático *A. herba-alba* es de signo negativo, debido al predominio de interacciones negativas con el resto de especies de la comunidad vegetal. Hasta cierto punto, la alelopatía contrarresta los potenciales efectos facilitadores por la mejora de las condiciones microclimáticas bajo las plantas alelopáticas en comunidades vegetales semiáridas. No obstante, el signo del balance neto de interacciones de *A. herba-alba* es positivo para las especies tolerantes a los compuestos químicos producidos por ella.

9. El balance neto de interacciones de *A. herba-alba* cambia hacia positivo bajo condiciones de mayor estrés ambiental (p. ej. una mayor aridez), independientemente de si la producción de compuestos alelopáticos también se incrementa o las especies son más vulnerables a sus efectos debido a que están sometidas a la acción de múltiples factores de estrés. Esto pone de manifiesto que la importancia relativa de la interferencia alelopática disminuye con el grado de estrés ambiental, en favor de las interacciones positivas.

Conclusions

1. Microclimatic conditions beneath *A. herba-alba* are more benign than in the bare soil due to an increase in soil moisture, water infiltration after a rainfall and nutrients concentration and a reduction in solar radiation. However, *A. herba-alba* does not act as a nurse plant facilitating the establishment of seedlings beneath its canopy because of its allelopathic activity. This fact highlights that the identification of a species as a nurse plant cannot be inferred solely from the enhanced microclimatic conditions under its canopy.

2. The chemical interference of *A. herba-alba* leads to a diversity repulsion around its individuals, contrary to what has been found for the allelopathic species *Thymus vulgaris* L. in a less arid Mediterranean plant community. Nevertheless, there are some species (*e.g.* many perennial grasses) that appear more frequently around *A. herba-alba* individuals than what it may be expected by chance, suggesting the ability of those species to tolerate the allelopathic compounds released by *A. herba-alba* plants.

3. The vegetation patterning found around *A. herba-alba* individuals is not caused by any differences in seed density and species richness of the seed bank with respect to similar shrubs of the plant community, but the chemicals compounds produced and released by *A. herba-alba* inhibit the seedling emergence from the seed bank. This underscores that the impacts of allelopathic plants in community organization are driven, at least partially, through their effect on the seed bank.

4. *Artemisia herba-alba* shows a strong autotoxic nature. The chemical compounds produced and released by *A. herba-alba* hamper the establishment of their own seedlings in the vicinity of parent plants, inhibiting seed germination and increasing the mortality of *A. herba-alba* seedlings emerging form the seed bank. It is possible that in arid and semiarid environments, autotoxicity may constitute an adaptive advantage to self-regulate the

population density and, ultimately, decrease intraspecific competition for resources.

5. Both the volatile and the water soluble chemicals produced and released by *A. herba-alba* are involved in its autotoxicity. The *A. herba-alba* aqueous extract is rich in phenolic compounds, and some of them (*e.g.* phenolic acids) drive its autotoxicity. Although the effects of volatile compounds were apparently stronger, the role of water soluble allelopathic compounds might be more important in arid and semiarid environments than previously thought.

6. The allelopathic activity of *A. herba-alba* constitutes a biological filter that determines which species are able or not to coexist near the allelopathic plants and, in general, is more relevant during the germination stage than it is during the seedling growth. Nevertheless, some species that coexist with *A. herba-alba* in the same plant communities (*e.g. Lygeum spartum* L.) show a high tolerance to the allelopathic compounds of *A. herba-alba*, due likely to a long co-evolutionary history.

7. Under natural conditions, the allelopathic interference of *A. herba-alba* is a relevant interaction inhibiting seed germination and decreasing plant vitality. However, while in laboratory bioassays it has been found that the chemical compounds produced and released by *A. herba-alba* affect the mortality and the growth of seedlings, such effects have not been observed under natural conditions. This highlights that results obtained in studies of allelopathy under controlled conditions should be interpreted with caution, while more field experiments are necessary to obtain realistic evidences about the significance of allelopathy in natural plant communities.

8. The net interaction outcome of the allelopathic shrub *A. herba-alba* is negative, due to the predominance of negative interactions with the rest of species of the plant community. To some extent, allelopathy counterbalances the potential facilitative effects of more benign microclimatic conditions and enhanced soil fertility beneath allelopathic plants in semiarid plant

communities. However, the net interaction balance of *A. herba-alba* is positive for those species that are able to tolerate the presence of allelopathic compounds produced by this species.

9. The net interaction outcome of *A. herba-alba* changes from neutral to positive under conditions of increased environmental stress (*e.g.* increased drought), regardless of whether the production of allelopathic compounds is also increased, or whether species are more sensitive to their action because of they are subject to multiple stressors. This underlines that the relative importance of allelopathic interference decreases with the degree of environmental harshness, in favor of positive interactions.

Referencias

Aguiar, M.R. & Sala, O.E. (1997) Seed distribution constrains the dynamics of the Patagonian steppe. *Ecology*, **78**, 93–100.

Aguiar, M.R. & Sala, O.E. (1999) Patch structure, dynamics and implications for the functioning of arid ecosystems. *Trends in Ecology & Evolution*, **14**, 273–277.

Alados, C.L., Navarro, T., Komac, B., Pascual, V. & Rietkerk, M. (2010) Dispersal abilities and spatial patterns in fragmented landscapes. *Biological Journal of the Linnean Society*, **100**, 935–947.

Alados, C.L., Pueyo, Y., Giner, M.L., Navarro, T., Escos, J., Barroso, F., Cabezudo, B. & Emlen, J.M. (2003) Quantitative characterization of the regressive ecological succession by fractal analysis of plant spatial patterns. *Ecological Modelling*, **163**, 1–17.

Alías, J.C., Sosa, T., Escudero, J.C. & Chaves, N. (2006) Autotoxicity against germination and seedling emergence in Cistus ladanifer L. *Plant and Soil*, **282**, 327–332.

Allington, G.R.H. & Valone, T.J. (2010) Reversal of desertification: The role of physical and chemical soil properties. *Journal of Arid Environments*, **74**, 973–977.

Amb, M.K. & Ahluwalia, A.S. (2016) Allelopathy: Potential Role to Achieve New Milestones in Rice Cultivation. *Rice Science*, **23**, 165–183.

Araniti, F., Sorgonà, A., Lupini, A. & Abenavoli, M.R. (2012) Screening of Mediterranean wild plant species for allelopathic activity and their use as bio-herbicides. *Allelopathy Journal*, **29**, 107–124.

Armas, C. & Pugnaire, F.I. (2005) Plant interactions govern population dynamics in a semi-arid plant community. *Journal of Ecology*, **93**, 978–989.

Armas, C. & Pugnaire, F.I. (2011) Belowground zone of influence in a tussock grass species. *Acta Oecologica*, **37**, 284–289.

Aschehoug, E.T., Brooker, R., Atwater, D.Z., Maron, J.L. & Callaway, R.M. (2016) The Mechanisms and Consequences of Interspecific Competition Among Plants. *Annual Review of Ecology, Evolution, and Systematics*, **47**, 263–281.

Atoum, M., Al-Charchafchi, F. & Modallal, N. (2006) Biological activity and antimutagencity of water soluble phytotoxins from Artemisia herba alba Asso. *Pakistan Journal of Biological Sciences*, **9**, 1774–1778.

Augé, R.M. (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza*, **11**, 3–42.

Austin, A.T., Yahdjian, L., Stark, J.M., Belnap, J., Porporato, A., Norton, U., Ravetta, D.A. & Schaeffer, S.M. (2004) Water pulses and biogeochemical cycles in arid and semiarid ecosystems. *Oecologia*, **141**, 221–235.

Bais, H.P., Vepachedu, R., Gilroy, S., Callaway, R.M. & Vivanco, J.M. (2003) Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science*, **301**, 1377–80.

Bautista, I., Boscaiu, M., Lidón, A., Llinares, J.V., Lull, C., Donat, M.P., Mayoral, O. & Vicente, O. (2016) Environmentally induced changes in antioxidant phenolic compounds levels in wild plants. *Acta Physiologiae Plantarum*, **38**.

Bazzaz, F.A. (1975) Plant Species Diversity in Old-Field Successional Ecosystems in Southern Illinois. *Ecology*, **56**, 485–488.

Belhattab, R., Amor, L., Barroso, J.G., Pedro, L.G. & Cristina Figueiredo, A. (2014) Essential oil from Artemisia herba-alba Asso grown wild in Algeria: variability assessment and comparison with an updated literature survey. *Arabian Journal of Chemistry*, **7**, 243–251.

Belnap, J. (2003) The World at Your Feet: Desert Biological Soil Crusts. *Frontiers in Ecology and the Environment*, **1**, 181.

Ben-Hammouda, M., Kremer, R.J., Minor, H.C. & Sarwar, M. (1995) A chemical basis for differential allelopathic potential of sorghum hybrids on wheat. *Journal of Chemical Ecology*, **21**, 775–786.

Bertness, M.D. & Callaway, R. (1994) Positive interactions in communities. *Trends Ecol Evol*, **9**, 191–3.

Bestelmeyer, B.T., Ward, J.P. & Havstad, K.M. (2006) Soil-geomorphic heterogeneity governs patchy vegetation dynamics at an arid ecotone. *Ecology*, **87**, 963–973.

Blum, U., Shafer, S.R. & Lehman, M.E. (1999) Evidence for Inhibitory Allelopathic Interactions Involving Phenolic Acids in Field Soils: Concepts vs. an Experimental Model. *Critical Reviews in Plant Sciences*, **18**, 673–693.

Blum, U., Staman, K.L., Flint, L.J. & Shafer, S.R. (2000) Induction and/or selection of phenolic acid-utilizing bulk-soil and rhizosphere bacteria and their influence on phenolic acid phytotoxicity. *Journal of Chemical Ecology*, **26**, 2059–2078.

Bourgou, S., Tammar, S., Salem, N., Mkadmini, K. & Msaada, K. (2016) Phenolic composition, essential oil, and antioxidant activity in the aerial part of *Artemisia herba-alba* from several provenances: a comparative study. *International Journal of Food Properties*, **19**, 549–563.

Braun-Blanquet, J. & Bolòs, O. (1957) Les groupements vegetaux du bassin moyen de l´ebre et leur dynamisme. , **5**, 1–266.

Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.L., Cavieres, L.A., Kunstler, G., Liancourt, P., TielbÖRger, K., Travis, J.M.J., Anthelme, F., Armas, C., Coll, L., Corcket, E., Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F., Quiroz, C.L., Saccone, P., Schiffers, K., Seifan, M., Touzard, B. & Michalet, R. (2008) Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology*, **96**, 18–34.

Bruno, J.F., Stachowicz, J.J. & Bertness, M.D. (2003) Inclusion of facilitation into ecological theory. *Trends in Ecology & Evolution*, **18**, 119–125.

Caballero, I., Olano, J.M., Escudero, A. & Loidi, J. (2008a) Seed bank spatial structure in semi-arid environments: beyond the patch-bare area dichotomy. *Plant Ecology*, **195**, 215–223.

Caballero, I., Olano, J.M., Loidi, J. & Escudero, A. (2008b) A model for small-scale seed bank and standing vegetation connection along time. *Oikos*, **117**, 1788–1795.

Callaway, R.M. (2007) *Positive Interactions and Interdependence in Plant Communities*. Springer, Dordrecht, Netherlands.

Callaway, R.M. & Aschehoug, E.T. (2000) Invasive Plants Versus Their New and Old Neighbors: A Mechanism for Exotic Invasion. *Science*, **290**, 521–523.

Callaway, R.M. & Ridenour, W.M. (2004) Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment*, **2**, 436–443.

Callaway, R.M. & Walker, L.R. (1997) Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology*, **78**, 1958–1965.

Castaldi, S., Carfora, A., Fiorentino, A., Natale, A., Messere, A., Miglietta, F. & Cotrufo, M.F. (2009) Inhibition of net nitrification activity in a Mediterranean woodland: possible role of chemicals produced by Arbutus unedo. *Plant and Soil*, **315**, 273–283.

Castellano, M.J. & Valone, T.J. (2007) Livestock, soil compaction and water infiltration rate: Evaluating a potential desertification recovery mechanism. *Journal of Arid Environments*, **71**, 97–108.

Castro, J. (2006) Short delay in timing of emergence determines establishment success in Pinus sylvestris across microhabitats. *Annals of Botany*, **98**, 1233–1240.

Cavieres, L.A., Badano, E.I., Sierra-Almeida, A. & Molina-Montenegro, M.A. (2007) Microclimatic Modifications of Cushion Plants and Their Consequences for Seedling Survival of Native and Non-native Herbaceous Species in the High Andes of Central Chile. *Arctic, Antarctic, and Alpine Research*, **39**, 229–236.

Cavieres, L.A., Brooker, R.W., Butterfield, B.J., Cook, B.J., Kikvidze, Z., Lortie, C.J., Michalet, R., Pugnaire, F.I., Schöb, C., Xiao, S., Anthelme, F., Björk, R.G., Dickinson, K.J.M., Cranston, B.H., Gavilán, R., Gutiérrez-Girón, A., Kanka, R., Maalouf, J.-P., Mark, A.F., Noroozi, J., Parajuli, R., Phoenix, G.K., Reid, A.M., Ridenour, W.M., Rixen, C., Wipf, S., Zhao, L., Escudero, A., Zaitchik, B.F., Lingua, E., Aschehoug, E.T. & Callaway, R.M. (2014) Facilitative plant interactions and climate simultaneously drive alpine plant diversity. *Ecology Letters*, **17**, 193–202.

Cerdà, A. (1997a) Soil erosion after land abandonment in a semiarid environment of southeastern Spain. *Arid Soil Research and Rehabilitation*, **11**, 163–176.

Cerdà, A. (1997b) The effect of patchy distribution of Stipa tenacissima L. on runoff and erosion. *Journal of Arid Environments*, **36**, 37–51.

Chacón-Labella, J., de la Cruz, M. & Escudero, A. (2015) Beyond the classical nurse species effect: diversity assembly in a Mediterranean semi-arid dwarf shrubland. *Journal of Vegetation Science*, n/a-n/a.

Cheng, F. & Cheng, Z. (2015) Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Frontiers in Plant Science*, **6**.

Chesson, P., Gebauer, R.L.E., Schwinning, S., Huntly, N., Wiegand, K., Ernest, M.S.K., Sher, A., Novoplansky, A. & Weltzin, J.F. (2004) Resource pulses, species interactions, and diversity maintenance in arid and semi-arid environments. *Oecologia*, **141**, 236–253.

Chiapusio, G., Sánchez, A.M., Reigosa, M.J., González, L. & Pellissier, F. (1997) Do germination indices adequately reflect allelochemical effects on the germination process? *Journal of Chemical Ecology*, **23**, 2445–2453.

Chomel, M., Guittonny-Larchevêque, M., Fernandez, C., Gallet, C., DesRochers, A., Paré, D., Jackson, B.G. & Baldy, V. (2016) Plant secondary metabolites: a key driver of litter decomposition and soil nutrient cycling (ed R Aerts). *Journal of Ecology*, **104**, 1527–1541.

Chon, S.-U. & Nelson, C.J. (2010) Allelopathy in Compositae plants. A review. *Agronomy for Sustainable Development*, **30**, 349–358.

Chou, C.-H. (1999) Roles of allelopathy in plant biodiversity and sustainable agriculture. *Critical Reviews in Plant Sciences*, **18**, 609–636.

Chung, I.-M., Seigler, D., Miller, D.A. & Kyung, S.-H. (2000) Autotoxic compounds from fresh alfalfa leaf extracts: Identification and biological activity. *Journal of Chemical Ecology*, **26**, 315–327.

Cipollini, D., Rigsby, C.M. & Barto, E.K. (2012) Microbes as Targets and Mediators of Allelopathy in Plants. *Journal of Chemical Ecology*, **38**, 714–727.

Collins, S.L. (1998) Modulation of Diversity by Grazing and Mowing in Native Tallgrass Prairie. *Science*, **280**, 745–747.

Dawson, T.E. (1993) Hydraulic lift and water use by plants: implications for water balance, performance and plant-plant interactions. *Oecologia*, **95**, 565–574.

D'Odorico, P., Bhattachan, A., Davis, K.F., Ravi, S. & Runyan, C.W. (2013) Global desertification: Drivers and feedbacks. *Advances in Water Resources*, **51**, 326–344.

D'Odorico, P., Caylor, K., Okin, G.S. & Scanlon, T.M. (2007) On soil moisture-vegetation feedbacks and their possible effects on the dynamics of dryland ecosystems. *Journal of Geophysical Research: Biogeosciences*, **112**, n/a-n/a.

Dorning, M. & Cipollini, D. (2005) Leaf and root extracts of the invasive shrub, Lonicera maackii, inhibit seed germination of three herbs with no autotoxic effects. *Plant Ecology*, **184**, 287–296.

Drezner, T.D. (2007) An analysis of winter temperature and dew point under the canopy of a common Sonoran Desert nurse and the implications for positive plant interactions. *Journal of Arid Environments*, **69**, 554–568.

Duke, S.O., Cedergreen, N., Velini, E.D. & Belz, R.G. (2006) Hormesis: Is it an important factor in Herbicide use and Allelopathy? *Outlooks on Pest Management*, **17**, 29–33.

Duke, S.O. & Dayan, F.E. (2006) Modes of action of phytotoxins from plants. *Allelopathy: A Physiological Process with Ecological Implications* (eds M.J. Reigosa, N. Pedrol & L. González), pp. 511–536. Springer, Dordrecht, Netherlands.

Ehlers, B.K., Charpentier, A. & Grøndahl, E. (2014) An allelopathic plant facilitates species richness in the Mediterranean garrigue. *Journal of Ecology*, **102**, 176–185.

Einhellig, F.A. (1994) Allelopathy: Current Status and Future Goals. *Allelopathy* (eds Inderjit, K.M.M. Dakshini & F.A. Einhellig), pp. 1–24. American Chemical Society, Washington, DC.

Einhellig, F.A. & Leather, G.R. (1988) Potentials for exploiting allelopathy to enhance crop production. *Journal of Chemical Ecology*, **14**, 1829–1844.

Eldridge, D.J., Poore, A.G.B., Ruiz-Colmenero, M., Letnic, M. & Soliveres, S. (2016) Ecosystem structure, function, and composition in rangelands are negatively affected by livestock grazing. *Ecological Applications*, **26**, 1273–1283.

Escudero, A., Albert, M.J., Pita, J.M. & Pérez-García, F. (2000) Inhibitory effects of Artemisia herba-alba on the germination of the gypsophyte Helianthemum squamatum. *Plant Ecology*, **148**, 71–80.

Escudero, A., Somolinos, R.C., Olano, J.M. & Rubio, A. (1999) Factors controlling the establishment of Helianthemum squamatum, an endemic gypsophile of semi-arid Spain. *Journal of Ecology*, **87**, 290–302.

Fabbro, C.D., Güsewell, S. & Prati, D. (2013) Allelopathic effects of three plant invaders on germination of native species: a field study. *Biological Invasions*, **16**, 1035–1042.

Farooq, M., Jabran, K., Cheema, Z.A., Wahid, A. & Siddique, K.H. (2011) The role of allelopathy in agricultural pest management. *Pest Management Science*, **67**, 493–506.

Fensham, R.J., Silcock, J.L. & Firn, J. (2014) Managed livestock grazing is compatible with the maintenance of plant diversity in semidesert grasslands. *Ecological Applications*, **24**, 503–517.

Fernandez, C., Santonja, M., Gros, R., Monnier, Y., Chomel, M., Baldy, V. & Bousquet-Melou, A. (2013) Allelochemicals of Pinus halepensis as drivers of biodiversity in Mediterranean open mosaic habitats during the colonization stage of secondary succession. *Journal of Chemical Ecology*, **39**, 298–311.

Fernandez, C., Voiriot, S., Mévy, J.-P., Vila, B., Ormeño, E., Dupouyet, S. & Bousquet-Mélou, A. (2008) Regeneration failure of Pinus halepensis Mill.: The role of autotoxicity and some abiotic environmental parameters. *Forest Ecology and Management*, **255**, 2928–2936.

Filep, R., Pal, R.W., Balázs, V.L., Mayer, M., Nagy, D.U., Cook, B.J. & Farkas, Á. (2016) Can seasonal dynamics of allelochemicals play a role in plant invasions? A case study with Helianthus tuberosus L. *Plant Ecology*, **217**, 1489–1501.

Flores, J. & Jurado, E. (2003) Are nurse-protege interactions more common among plants from arid environments? *Journal of Vegetation Science*.

Friedman, J. (1987) Allelopathy in Desert Ecosystems. *Allelochemicals: Role in Agriculture and Forestry* (ed G.R. Waller), pp. 53–68. American Chemical Society, Washington, DC.

Friedman, J. & Orshan, G. (1975) The distribution, emergence and survival of seedlings of Artemisia herba-alba Asso in the Negev desert of Israel in relation to distance from the adult plants. *The Journal of Ecology*, **63**, 627.

Friedman, J., Orshan, G. & Ziger-Cfir, Y. (1977) Suppression of annuals by Artemisia herba-alba in the Negev desert of Israel. *Journal of Ecology*, **65**, 413–426.

Friedman, J. & Waller, G.R. (1985) Allelopathy and autotoxicity. *Trends in Biochemical Sciences*, **10**, 47–50.

Friedman, J., Yaniv, Z., Dafni, A. & Palewitch, D. (1986) A preliminary classification of the healing potential of medicinal plants, based on a rational analysis of an ethnopharmacological field survey among Bedouins in the Negev Desert, Israel. *Journal of Ethnopharmacology*, **16**, 275–287.

Gagliardo, R.W. & Chilton, W.S. (1992) Soil transformation of 2(3H)-benzoxazolone of rye into phytotoxic 2-amino-3H-phenoxazin-3-one. *Journal of Chemical Ecology*, **18**, 1683–1691.

Gant, R.E. & Clebsch, E.E.C. (1975) The allelopathic influences of Sassafras albidum in old-field succession in Tennessee. *Ecology*, **56**, 604–615.

Giladi, I., Segoli, M. & Ungar, E.D. (2013) Shrubs and herbaceous seed flow in a semi-arid landscape: dual functioning of shrubs as trap and barrier. *Journal of Ecology*, **101**, 97–106.

Gniazdowska, A. & Bogatek, R. (2005) Allelopathic interactions between plants. Multi site action of allelochemicals. *Acta Physiologiae Plantarum*, **27**, 395–407.

Gómez-Aparicio, L. (2008) Spatial patterns of recruitment in Mediterranean plant species: linking the fate of seeds, seedlings and saplings in heterogeneous landscapes at different scales. *Journal of Ecology*, **96**, 1128–1140.

Gómez-Aparicio, L. (2009) The role of plant interactions in the restoration of degraded ecosystems: a meta-analysis across life-forms and ecosystems. *Journal of Ecology*, **97**, 1202–1214.

Gómez-Aparicio, L. & Canham, C.D. (2008) Neighbourhood analyses of the allelopathic effects of the invasive tree Ailanthus altissima in temperate forests. *Journal of Ecology*, **96**, 447–458.

Gómez-Aparicio, L., Zamora, R., Gómez, J.M., Hódar, J.A., Castro, J. & Baraza, E. (2004) Applying plant facilitation to forest restoration: a meta-analysis of the use of shrubs as nurse plants. *Ecological Applications*, **14**, 1128–1138.

Graff, P., Aguiar, M.R. & Chaneton, E.J. (2007) Shifts in Positive and Negative Plant Interactions along a Grazing Intensity Gradient. *Ecology*, **88**, 188–199.

Grime, J.P. (1977) Evidence for the Existence of Three Primary Strategies in Plants and Its Relevance to Ecological and Evolutionary Theory. *The American Naturalist*, **111**, 1169–1194.

Grøndahl, E. & Ehlers, B.K. (2008) Local adaptation to biotic factors: reciprocal transplants of four species associated with aromatic Thymus pulegioides and T. serpyllum. *Journal of Ecology*, **96**, 981–992.

Grove, S., Haubensak, K.A. & Parker, I.M. (2012) Direct and indirect effects of allelopathy in the soil legacy of an exotic plant invasion. *Plant Ecology*, **213**, 1869–1882.

Guerrero-Campo, J. (1998) *Respuestas de La Vegetación Y de La Morfología de Las Plantas a La Erosión Del Suelo. PhD Thesis*. Universidad de Barcelona, Barcelona, Spain.

Guo, Q., Rundel, P.W. & Goodall, D.W. (1998) Horizontal and vertical distribution of desert seed banks: patterns, causes, and implications. *Journal of Arid Environments*, **38**, 465–478.

Harper, J.L. (1975) Allelopathy (a review). *The Quarterly Review of Biology*, **50**, 493–495.

Hättenschwiler, S. & Vitousek, P.M. (2000) The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in Ecology & Evolution*, **15**, 238–243.

He, Q. & Bertness, M.D. (2014) Extreme stresses, niches, and positive species interactions along stress gradients. *Ecology*, **95**, 1437–1443.

Herranz, J.M., Ferrandis, P., Copete, M.A., Duro, E.M. & Zalacaín, A. (2006) Effect of allelopathic compounds produced by Cistus ladanifer on germination of 20 Mediterranean taxa. *Plant Ecology*, **184**, 259–272.

Hille, M. & den Ouden, J. (2005) Charcoal and activated carbon as adsorbate of phytotoxic compounds - a comparative study. *Oikos*, **108**, 202–207.

Holmgren, M. & Scheffer, M. (2010) Strong facilitation in mild environments: the stress gradient hypothesis revisited. *Journal of Ecology*, **98**, 1269–1275.

Holmgren, M., Scheffer, M. & Huston, M.A. (1997) The interplay of facilitation and competition in plant communities. *Ecology*.

Holzapfel, C. & Mahall, B.E. (1999) Bidirectional facilitation and interference between shrubs and annuals in the Mojave Desert. *Ecology*.

Holzapfel, C., Tielbörger, K., Parag, H.A., Kigel, J. & Sternberg, M. (2006) Annual plant–shrub interactions along an aridity gradient. *Basic and Applied Ecology*, **7**, 268–279.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J. & Wardle, D.A. (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs*, **75**, 3–35.

Hooper, D.U. & Johnson, L. (1999) Nitrogen limitation in dryland ecosystems: Responses to geographical and temporal variation in precipitation. *New Perspectives on Nitrogen Cycling* *in the Temperate and Tropical Americas* (ed A.R. Townsend), pp. 247–293. Springer Netherlands, Dordrecht.

Hortal, S., Bastida, F., Moreno, J.L., Armas, C., García, C. & Pugnaire, F.I. (2015) Benefactor and allelopathic shrub species have different effects on the soil microbial community along an environmental severity gradient. *Soil Biology and Biochemistry*, **88**, 48–57.

Huang, J., Yu, H., Guan, X., Wang, G. & Guo, R. (2015) Accelerated dryland expansion under climate change. *Nature Climate Change*.

Inderjit & Callaway, R.M. (2003) Experimental designs for the study of allelopathy. *Plant and Soil*, **256**, 1–11.

Inderjit & Duke, S.O. (2003) Ecophysiological aspects of allelopathy. *Planta*, **217**, 529–539.

Inderjit & del Moral, R. (1997) Is separating resource competition from allelopathy realistic? *The Botanical Review*, **63**, 221–230.

Inderjit, Wardle, D.A., Karban, R. & Callaway, R.M. (2011) The ecosystem and evolutionary contexts of allelopathy. *Trends Ecol Evol*, **26**, 655–62.

Inderjit & Weston, L.A. (2000) Are Laboratory Bioassays for Allelopathy Suitable for Prediction of Field Responses? *Journal of Chemical Ecology*, **26**, 2111–2118.

Inderjit, Weston, L.A. & Duke, S.O. (2005) Challenges, achievements and opportunities in allelopathy research. *Journal of Plant Interactions*, **1**, 69–81.

IPCC. (2014) *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II, and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. IPCC, Geneva, Switzerland.

Jackson, R.B., Canadell, J., Ehleringer, J.R., Mooney, H.A., Sala, O.E. & Schulze, E.D. (1996) A global analysis of root distributions for terrestrial biomes. *Oecologia*, **108**, 389–411.

James, J.J., Tiller, R.L. & Richards, J.H. (2005) Multiple resources limit plant growth and function in a saline-alkaline desert community. *Journal of Ecology*, **93**, 113–126.

Jankju, M. (2013) Role of nurse shrubs in restoration of an arid rangeland: Effects of microclimate on grass establishment. *Journal of Arid Environments*, **89**, 103–109.

Jensen, C.G. & Ehlers, B.K. (2010) Genetic variation for sensitivity to a thyme monoterpene in associated plant species. *Oecologia*, **162**, 1017–25.

Karban, R. (2007) Experimental clipping of sagebrush inhibits seed germination of neighbours. *Ecol Lett*, **10**, 791–7.

Kato-Noguchi, H., Nakamura, K., Ohno, O., Suenaga, K. & Okuda, N. (2017) Asparagus decline: Autotoxicity and autotoxic compounds in asparagus rhizomes. *Journal of Plant Physiology*, **213**, 23–29.

Kaur, H., Kaur, R., Kaur, S., Baldwin, I.T. & Inderjit. (2009) Taking Ecological Function Seriously: Soil Microbial Communities Can Obviate Allelopathic Effects of Released Metabolites (ed FM Ausubel). *PLoS ONE*, **4**, e4700.

Kefi, S., Rietkerk, M., Alados, C.L., Pueyo, Y., Papanastasis, V.P., Elaich, A. & de Ruiter, P.C. (2007a) Spatial vegetation patterns and imminent desertification in Mediterranean arid ecosystems. *Nature*, **449**, 213–7.

Kefi, S., Rietkerk, M., van Baalen, M. & Loreau, M. (2007b) Local facilitation, bistability and transitions in arid ecosystems. *Theoretical Population Biology*, **71**, 367–379.

Khlifi, D., Sghaier, R.M., Amouri, S., Laouini, D., Hamdi, M. & Bouajila, J. (2013) Composition and anti-oxidant, anti-cancer and anti-inflammatory activities of Artemisia herba-alba, Ruta chalpensis L. and Peganum harmala L. *Food and Chemical Toxicology*, **55**, 202–208.

Kikvidze, Z., Pugnaire, F.I., Brooker, R.W., Choler, P., Lortie, C.J., Michalet, R. & Callaway, R.M. (2005) Linking patterns and processes in alpine plant communities: a global study. *Ecology*, **86**, 1395–1400.

Kobayashi, K. (2004) Factors affecting phytotoxic activity of allelochemicals in soil. *Weed Biology and Management*, **4**, 1–7.

Kong, C., Hu, F. & Xu, X. (2002) Allelopathic potential and chemical constituents of volatiles from Ageratum conyzoides under stress. *Journal of Chemical Ecology*, **28**, 1173–1182.

Koocheki, A., Lalegani, B. & Hosseini, S.A. (2013) Ecological Consequences of Allelopathy. *Allelopathy* (eds Z.A. Cheema, M. Farooq & A. Wahid), pp. 23–38. Springer Berlin Heidelberg, Berlin, Heidelberg.

Kulmatiski, A. (2011) Changing Soils to Manage Plant Communities: Activated Carbon as a Restoration Tool in Ex-arable Fields. *Restoration Ecology*, **19**, 102–110.

Kulmatiski, A. & Beard, K.H. (2006) Activated carbon as a restoration tool: potential for control of invasive plants in abandoned agricultural fields. *Restoration Ecology*.

Lau, J.A., Puliafico, K.P., Kopshever, J.A., Steltzer, H., Jarvis, E.P., Schwarzlander, M., Strauss, S.Y. & Hufbauer, R.A. (2008) Inference of allelopathy is complicated by effects of activated carbon on plant growth. *New Phytol*, **178**, 412–23.

Le Bagousse-Pinguet, Y., Xiao, S., Brooker, R.W., Gross, N., Liancourt, P., Straile, D. & Michalet, R. (2014) Facilitation displaces hotspots of diversity and allows communities to persist in heavily stressed and disturbed environments. *Journal of Vegetation Science*, **25**, 66–76.

Ledger, K.J., Pal, R.W., Murphy, P., Nagy, D.U., Filep, R. & Callaway, R.M. (2015) Impact of an invader on species diversity is stronger in the non-native range than in the native range. *Plant Ecology*, **216**, 1285–1295.

Levitt, J. (1980) *Responses of Plants to Environmental Stresses. Water, Radiation, Salt, and Other Stresses*, 2nd ed. Academic Press, New York, NY.

Li, S., Nie, Z. & Zhang, D. (2016) Competition between cocksfoot (Dactylis glomerata L.) and companion species: Evidence for allelopathy. *Field Crops Research*, **196**, 452–462.

Li, X.F., Wang, J., Huang, D., Wang, L.X. & Wang, K. (2011) Allelopathic potential of Artemisia frigida and successional changes of plant communities in the northern China steppe. *Plant and Soil*, **341**, 383–398.

Li, Z.H., Wang, Q., Ruan, X., Pan, C.-D. & Jiang, D.-A. (2010) Phenolics and plant allelopathy. *Molecules*, **15**, 8933–8952.

Li, J., Zhang, Q., Hu, W., Yang, X. & He, H. (2015) Stability of phenolic acids and the effect on weed control activity. *Journal of the Korean Society for Applied Biological Chemistry*, **58**, 919–926.

Liancourt, P., Callaway, R.M. & Michalet, R. (2005) Stress tolerance and competitive-response ability determine the outcome of biotic interactions. *Ecology*, **86**, 1611–1618.

Linhart, Y.B., Gauthier, P., Keefover-Ring, K. & Thompson, J.D. (2015) Variable phytotoxic effects of Thymus vulgaris (Lamiaceae) terpenes on associated species. *International Journal of Plant Sciences*, **176**, 20–30.

Liu, Z., Zhu, J. & Deng, X. (2012) Arrival vs. retention of seeds in bare patches in the semi-arid desertified grassland of Inner Mongolia, northeastern China. *Ecological Engineering*, **49**, 153–159.

Longares, L.A. (1997) *El Paisaje Vegetal En El Entorno de La Reserva ornitológica "El Planeron" (Belchite-Zaragoza)*. Consejo de Protección de la Naturaleza de Aragón y Sociedad Española de Ornitología (SEO/BirdLife), Zaragoza.

López, R.P., Larrea-Alcázar, D.M. & Teresa, O. (2009) Positive effects of shrubs on herbaceous species richness across several spatial scales: evidence from the semiarid Andean subtropics. *Journal of Vegetation Science*, **20**, 728–734.

López, R.P., Squeo, F.A., Armas, C., Kelt, D.A. & Gutiérrez, J.R. (2016) Enhanced facilitation at the extreme end of the aridity gradient in the Atacama Desert: a community-level approach. *Ecology*, **97**, 1593–1604.

Lorenzo, P., Rodríguez, J., González, L. & Rodríguez-Echeverría, S. (2016) Changes in microhabitat, but not allelopathy, affect plant establishment after *Acacia dealbata* invasion. *Journal of Plant Ecology*, rtw061.

Ludwig, J.A., Tongway, D.J. & Marsden, S.G. (1999) Stripes, strands or stipples: modelling the influence of three landscape banding patterns on resource capture and productivity in semi-arid woodlands, Australia. *CATENA*, **37**, 257–273.

Ludwig, J.A., Wilcox, B.P., Breshears, D.D., Tongway, D.J. & Imeson, A.C. (2005) Vegetation patches and runoff-erosion as interacting ecohydrological process in semiarid landscapes. *Ecology*, **86**, 288–297.

Macek, P., Prieto, I., Macková, J., Pistón, N. & Pugnaire, F.I. (2016) Functional Plant Types Drive Plant Interactions in a Mediterranean Mountain Range. *Frontiers in Plant Science*, **7**.

Macias, F.A., Molinillo, J.M., Varela, R.M. & Galindo, J.C. (2007) Allelopathy--a natural alternative for weed control. *Pest Manag Sci*, **63**, 327–48.

Maestre, F.T., Bowker, M.A., Cantón, Y., Castillo-Monroy, A.P., Cortina, J., Escolar, C., Escudero, A., Lázaro, R. & Martínez, I. (2011) Ecology and functional roles of biological soil crusts in semi-arid ecosystems of Spain. *Journal of Arid Environments*, **75**, 1282–1291.

Maestre, F.T., Callaway, R.M., Valladares, F. & Lortie, C.J. (2009) Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology*, **97**, 199–205.

Maestre, F.T., Castillo-Monroy, A.P., Bowker, M.A. & Ochoa-Hueso, R. (2012a) Species richness effects on ecosystem multifunctionality depend on evenness, composition and spatial pattern. *Journal of Ecology*, **100**, 317–330.

Maestre, F.T., Eldridge, D.J., Soliveres, S., Kéfi, S., Delgado-Baquerizo, M., Bowker, M.A., García-Palacios, P., Gaitán, J., Gallardo, A., Lázaro, R. & Berdugo, M. (2016) Structure and Functioning of Dryland Ecosystems in a Changing World. *Annual Review of Ecology, Evolution, and Systematics*, **47**, 215–237.

Maestre, F.T., Quero, J.L., Gotelli, N.J., Escudero, A., Ochoa, V., Delgado-Baquerizo, M., Garcia-Gomez, M., Bowker, M.A., Soliveres, S., Escolar, C., Garcia-Palacios, P., Berdugo, M., Valencia, E., Gozalo, B., Gallardo, A., Aguilera, L., Arredondo, T., Blones, J., Boeken, B., Bran, D., Conceicao, A.A., Cabrera, O., Chaieb, M., Derak, M., Eldridge, D.J., Espinosa, C.I., Florentino, A., Gaitan, J., Gatica, M.G., Ghiloufi, W., Gomez-Gonzalez, S., Gutierrez, J.R., Hernandez, R.M., Huang, X.W., Huber-Sannwald, E., Jankju, M., Miriti, M., Monerris, J., Mau, R.L., Morici, E., Naseri, K., Ospina, A., Polo, V., Prina, A., Pucheta, E., Ramirez-Collantes, D.A., Romao, R., Tighe, M., Torres-Diaz, C., Val, J., Veiga, J.P., Wang, D.L. & Zaady, E. (2012b) Plant Species Richness and Ecosystem Multifunctionality in Global Drylands. *Science*, **335**, 214–218.

MAGRAMA. (2016) *Impactos del cambio climático en los procesos de desertificación en España*. Ministerio de Agricultura, Alimentación y Medio Ambiente.

Mahall, B.E. & Callaway, R.M. (1992) Root communication mechanisms and intracommunity distributions of 2 Mojave desert shrubs. *Ecology*.

Mallik, A.U. (2008) Allelopathy: advances, challenges and opportunities. *Allelopathy in Sustainable Agriculture and Forestry* (eds R.S. Zeng, A.U. Mallik & S.M. Luo), pp. 25–38. Springer New York, New York, NY.

Martinez-Duro, E., Luzuriaga, A.L., Ferrandis, P., Escudero, A. & Herranz, J.M. (2012) Does aboveground vegetation composition resemble soil seed bank during succession in specialized vegetation on gypsum soil? *Ecological Research*, **27**, 43–51.

Mazzoleni, S., Bonanomi, G., Incerti, G., Chiusano, M.L., Termolino, P., Mingo, A., Senatore, M., Giannino, F., Cartenì, F., Rietkerk, M. & Lanzotti, V. (2015) Inhibitory and toxic effects of extracellular self-DNA in litter: a mechanism for negative plant-soil feedbacks? *New Phytologist*, **205**, 1195–1210.

McIntire, E.J.B. & Fajardo, A. (2014) Facilitation as a ubiquitous driver of biodiversity. *New Phytologist*, **201**, 403–416.

Michalet, R., Brooker, R.W., Cavieres, L.A., Kikvidze, Z., Lortie, C.J., Pugnaire, F.I., Valiente-Banuet, A. & Callaway, R.M. (2006) Do biotic interactions shape both sides of the humped-back model of species richness in plant communities? *Ecology Letters*, **9**, 767–773.

Michalet, R., Le Bagousse-Pinguet, Y., Maalouf, J.-P. & Lortie, C.J. (2014) Two alternatives to the stress-gradient hypothesis at the edge of life: the collapse of facilitation and the switch from facilitation to competition. *Journal of Vegetation Science*, **25**, 609–613.

Millennium Ecosystem Assessment. (2005) *Ecosystems and Human Well-Being: Desertification Synthesis*. Word Resources Institute, Washington, DC.

Miriti, M.N. (2006) Ontogenetic shift from facilitation to competition in a desert shrub. *Journal of Ecology*, **94**, 973–979.

Mittelbach, G.G., Steiner, C.F., Scheiner, S.M., Gross, K.L., Reynolds, H.L., Waide, R.B., Willig, M.R., Dodson, S.I. & Gough, L. (2001) What Is the Observed Relationship between Species Richness and Productivity? *Ecology*, **82**, 2381.

Mohamed, A.E.-H.H., El-Sayed, M.A., Hegazy, M.E., Helaly, S.E., Esmail, A.M. & Mohamed, N.S. (2010) Chemical constituents and biological activities of Artemisia herba-alba. *Records of Natural Products*, **4**, 1–25.

Mohsen, H. & Ali, F. (2009) Essential Oil Composition of Artemisia herba-alba from Southern Tunisia. *Molecules*, **14**, 1585–1594.

Molisch, H. (2001) *The Influence of One Plant on Another. Allelopathy* (ed SS Narwal, trans LJ La Fleur and MAB Mallik). Scientific Publishers, Jodhpur.

Moral, R.D. & Cates, R.G. (1971) Allelopathic potential of the dominant vegetation of western Washington. *Ecology*, **52**, 1030–1037.

Morris, C., Grossl, P.R. & Call, C.A. (2008) Elemental allelopathy: processes, progress, and pitfalls. *Plant Ecology*, **202**, 1–11.

Muldavin, E.H., Moore, D.I., Collins, S.L., Wetherill, K.R. & Lightfoot, D.C. (2008) Aboveground net primary production dynamics in a northern Chihuahuan Desert ecosystem. *Oecologia*, **155**, 123–132.

Muller, C.H. (1966) The Role of Chemical Inhibition (Allelopathy) in Vegetational Composition. *Bulletin of the Torrey Botanical Club*, **93**, 332.

Muller, C.H. (1969) Allelopathy as a factor in ecological process. *Vegetatio*, **18**, 348–357.

Muller, C.H., Muller, W.H. & Haines, B.L. (1964) Volatile growth inhibitors produced by aromatic shrubs. *Science*, **143**, 471–473.

Narwal, S.S. & Haouala, R. (2013) Role of Allelopathy in Weed Management for Sustainable Agriculture. *Allelopathy* (eds Z.A. Cheema, M. Farooq & A. Wahid), pp. 217–249. Springer Berlin Heidelberg, Berlin, Heidelberg.

Nilsen, E.T. (2003) Ecological relevance of allelopathy: some considerations related to Mediterranean, subtropical, temperate, and boreal forest shrubs. *Chemical Ecology of*

Plants: Allelopathy in Aquatic and Terrestrial Ecosystems (eds A.U. Mallik & Inderjit), pp. 109–129. Birkhäuser Basel, Basel.

Nilsson, M.-C. (1994) Separation of allelopathy and resource competition by the boreal dwarf shrub Empetrum hermaphroditum Hagerup. *Oecologia*, **98**, 1–7.

Nilsson, M.-C., Zackrisson, O., Sterner, O. & Wallstedt, A. (2000) Characterisation of the differential interference effects of two boreal dwarf shrub species. *Oecologia*, **123**, 122–128.

Noy-Meir, I. (1973) Desert ecosystems: environment and producers. *Annual Review of Ecology and Systematics*, **4**, 25–51.

Olano, J.M., Caballero, I., Loidi, J. & Escudero, A. (2005) Prediction of plant cover from seed bank analysis in a semi-arid plant community on gypsum. *Journal of Vegetation Science*, **16**, 215.

Olff, H. & Ritchie, M.E. (1998) Effects of herbivores on grassland plant diversity. *Trends in Ecology & Evolution*, **13**, 261–265.

Osem, Y., Perevolotsky, A. & Kigel, J. (2002) Grazing effect on diversity of annual plant communities in a semi-arid rangeland: interactions with small-scale spatial and temporal variation in primary productivity. *Journal of Ecology*, **90**, 936–946.

Padilla, F.M. & Pugnaire, F.I. (2006) The role of nurse plants in the restoration of degraded environments. *Frontiers in Ecology and the Environment*, **4**, 196–202.

Padilla, F.M. & Pugnaire, F.I. (2007) Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. *Functional Ecology*, **21**, 489–495.

Palacio, S., Johnson, D., Escudero, A. & Montserrat-Martí, G. (2012) Root colonisation by AM fungi differs between gypsum specialist and non-specialist plants: Links to the gypsophile behaviour. *Journal of Arid Environments*, **76**, 128–132.

Pedrol, N., González, L. & Reigosa, M.J. (2006) Allelopathy and abiotic stress. *Allelopathy* (eds M.J. Reigosa, N. Pedrol & L. González), pp. 171–209. Springer Netherlands, Dordrecht.

Pescador, D.S., Chacon-Labella, J., de la Cruz, M. & Escudero, A. (2014) Maintaining distances with the engineer: patterns of coexistence in plant communities beyond the patch-bare dichotomy. *New Phytologist*, **204**, 140–148.

Pisula, N.L. & Meiners, S.J. (2010) Allelopathic Effects of Goldenrod Species on Turnover in Successional Communities. *The American Midland Naturalist*, **163**, 161–172.

Preston, C.A., Betts, H. & Baldwin, I.T. (2002) Methyl Jasmonate as an Allelopathic Agent: Sagebrush Inhibits Germination of a Neighboring Tobacco, Nicotiana Attenuata. *Journal of Chemical Ecology*, **28**, 2343–2369.

Pueyo, Y. (2005) *Evaluación de los factores ambientales y del uso antrópico como condicionantes de la conservación de la vegetación del sector central de la depresión del Ebro. PhD thesis*. Universidad de Zaragoza. Departamento de Agricultura y Economía Agraria, Zaragoza, Spain.

Pueyo, Y., Alados, C.L., GarcÃa-Ã•v ila, B., KÃ©fi, S., Maestro, M. & Rietkerk, M. (2009) Comparing Direct Abiotic Amelioration and Facilitation as Tools for Restoration of Semiarid Grasslands. *Restoration Ecology*, **17**, 908–916.

Pueyo, Y., Kefi, S., Alados, C.L. & Rietkerk, M. (2008) Dispersal strategies and spatial organization of vegetation in arid ecosystems. *Oikos*.

Pueyo, Y., Moret-Fernández, D., Saiz, H., Bueno, C.G. & Alados, C.L. (2013) Relationships Between Plant Spatial Patterns, Water Infiltration Capacity, and Plant Community Composition in Semi-arid Mediterranean Ecosystems Along Stress Gradients. *Ecosystems*, **16**, 452–466.

Pugnaire, F.I., Armas, C. & Valladares, F. (2004) Soil as a mediator in plant-plant interactions in a semi-arid community. *Journal of Vegetation Science*, **15**, 85–92.

Pugnaire, F.I., Haase, P. & Puigdefabregas, J. (1996) Facilitation between higher plant species in a semiarid environment. *Ecology*.

Puigdefábregas, J. & Mendizabal, T. (1998) Perspectives on desertification: Western Mediterranean. *Journal of Arid Environments*, **39**, 209–224.

van der Putten, W.H. (2009) A multitrophic perspective on functioning and evolution of facilitation in plant communities. *Journal of Ecology*, **97**, 1131–1138.

Reigosa, M.J., Sánchez-Moreiras, A. & González, L. (1999) Ecophysiological approach in allelopathy. *Critical Reviews in Plant Sciences*, **18**, 577–608.

Renne, I.J., Sinn, B.T., Shook, G.W., Sedlacko, D.M., Dull, J.R., Villarreal, D. & Hierro, J.L. (2014) Eavesdropping in plants: delayed germination via biochemical recognition. *Journal of Ecology*, **102**, 86–94.

Reynolds, J.F., Smith, D.M., Lambin, E.F., Turner, B.L., 2nd, Mortimore, M., Batterbury, S.P., Downing, T.E., Dowlatabadi, H., Fernandez, R.J., Herrick, J.E., Huber-Sannwald, E., Jiang, H., Leemans, R., Lynam, T., Maestre, F.T., Ayarza, M. & Walker, B. (2007) Global desertification: building a science for dryland development. *Science*, **316**, 847–51.

Rice, E.L. (1984) *Allelopathy (Second Edition)*. Academic Press, San Diego.

Rietkerk, M., Dekker, S.C., de Ruiter, P.C. & van de Koppel, J. (2004) Self-organized patchiness and catastrophic shifts in ecosystems. *Science*, **305**, 1926–9.

Rivoal, A., Fernandez, C., Greff, S., Montes, N. & Vila, B. (2011) Does competition stress decrease allelopathic potential? *Biochemical Systematics and Ecology*, **39**, 401–407.

Ruan, X., Li, Z.-H., Wang, Q., Pan, C.-D., Jiang, D.-A. & Wang, G.G. (2011) Autotoxicity and allelopathy of 3,4-dihydroxyacetophenone isolated from Picea schrenkiana needles. *Molecules*, **16**, 8874–8893.

Ruijven, J., De Deyn, G.B. & Berendse, F. (2003) Diversity reduces invasibility in experimental plant communities: the role of plant species. *Ecology Letters*, **6**, 910–918.

Saatkamp, A., Poschlod, P. & Venable, D.L. (2014) The functional role of soil seed banks in natural communities. *Seeds: the ecology of regeneration in plant communities*, 3rd ed (ed R.S. Gallagher), pp. 263–295. CABI, Wallingford.

Safriel, U. & Adeel, Z. (2005) Dryland systems. *Ecosystems and Human Well-Being: Current State and Trends*, Millennium Ecosystem Assessment, pp. 623–62. Washington, DC.

Saleh, N.A.M., El-Negoumy, S.I., Abd-Alla, M.F., Abou-Zaid, M.M., Dellamonica, G. & Chopin, J. (1985) Flavonoid glycosides of Artemisia monosperma and A. herba-alba. *Phytochemistry*, **24**, 201–203.

Salido, S., Valenzuela, L.R., Altarejos, J., Nogueras, M., Sánchez, A. & Cano, E. (2004) Composition and infraspecific variability of Artemisia herba-alba from southern Spain. *Biochemical Systematics and Ecology*, **32**, 265–277.

Santonja, M., Rancon, A., Fromin, N., Baldy, V., Hättenschwiler, S., Fernandez, C., Montès, N. & Mirleau, P. (2017) Plant litter diversity increases microbial abundance, fungal diversity, and carbon and nitrogen cycling in a Mediterranean shrubland. *Soil Biology and Biochemistry*, **111**, 124–134.

Scanlon, T.M., Caylor, K.K., Levin, S.A. & Rodriguez-Iturbe, I. (2007) Positive feedbacks promote power-law clustering of Kalahari vegetation. *Nature*, **449**, 209–212.

Scheffer, M., Carpenter, S., Foley, J.A., Folke, C. & Walker, B. (2001) Catastrophic shifts in ecosystems. *Nature*, **413**, 591–596.

Schenk, H.J., Callaway, R.M. & Mahall, B.E. (1999) Spatial root segregation: Are plants territorial? *Advances in Ecological Research, Vol 28* (eds A.H. Fitter & D. Raffaelli), pp. 145–180. Academic Press Ltd-Elsevier Science Ltd, London.

Schlesinger, W.H., Raikes, J.A., Hartley, A.E. & Cross, A.F. (1995) On the Spatial Pattern of Soil Nutrients in Desert Ecosystems: Ecological Archives E077-002. *Ecology*, **77**, 364–374.

Schlesinger, W.H., Reynolds, J.F., Cunningham, G.L., Huenneke, L.F., Jarrell, W.M., Virginia, R.A. & Whitford, W.G. (1990) Biological Feedbacks in Global Desertification. *Science*, **247**, 1043–1048.

Schöb, C., Prieto, I., Armas, C. & Pugnaire, F.I. (2014) Consequences of facilitation: one plant's benefit is another plant's cost. *Functional Ecology*, **28**, 500–508.

Schroter, D. (2005) Ecosystem Service Supply and Vulnerability to Global Change in Europe. *Science*, **310**, 1333–1337.

Scognamiglio, M., D'Abrosca, B., Esposito, A., Pacifico, S., Monaco, P. & Fiorentino, A. (2013) Plant growth inhibitors: allelopathic role or phytotoxic effects? Focus on Mediterranean biomes. *Phytochemistry Reviews*, **12**, 803–830.

Seddik, K., Nadjet, I., Daoud, B.A.H. & Lekhmici, A. (2010) Antioxidant and antibacterial activities of extracts from Artemisia herba alba Asso. leaves and some phenolic compounds. *Journal of Medicinal Plants Research*, **4**, 1273–1280.

Sikes, B.A., Cottenie, K. & Klironomos, J.N. (2009) Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. *Journal of Ecology*, **97**, 1274–1280.

da Silva, E.R., Ferreira, P.M.A., Overbeck, G.E. & Soares, G.L.G. (2015) Does the phytotoxic shrub Heterothalamus psiadioides affect a plant community through allelopathy? *Plant Ecology*, **216**, 87–97.

Singh, H.P., Batish, D.R. & Kohli, R.K. (1999) Autotoxicity: concept, organisms, and ecological significance. *Critical Reviews in Plant Sciences*, **18**, 757–772.

Smit, C., Vandenberghe, C., Ouden, J. den & Müller-Schärer, H. (2007) Nurse plants, tree saplings and grazing pressure: changes in facilitation along a biotic environmental gradient. *Oecologia*, **152**, 265–273.

Soliveres, S. & Maestre, F.T. (2014) Plant–plant interactions, environmental gradients and plant diversity: A global synthesis of community-level studies. *Perspectives in Plant Ecology, Evolution and Systematics*, **16**, 154–163.

Soliveres, S., Maestre, F.T., Berdugo, M. & Allan, E. (2015) A missing link between facilitation and plant species coexistence: nurses benefit generally rare species more than common ones (ed S Wilson). *Journal of Ecology*, **103**, 1183–1189.

Sotomayor, D.A., Lortie, C.J. & Lamarque, L.J. (2014) Nurse-plant effects on the seed biology and germination of desert annuals. *Austral Ecology*, **39**, 786–794.

Stowe, L.G. (1979) Allelopathy and its influence on the distribution of plants in an Illinois old-field. *Journal of Ecology*.

Tackenberg, O., Poschlod, P. & Bonn, S. (2003) Assessment of wind dispersal potential in plant species. *Ecological Monographs*, **73**, 191–205.

Tahraoui, A., El-Hilaly, J., Israili, Z.H. & Lyoussi, B. (2007) Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in south-eastern Morocco (Errachidia province). *Journal of Ethnopharmacology*, **110**, 105–117.

Tang, C.S., Cai, W.F., Kohl, K. & Nishimoto, R.K. (1995) Plant stress and allelopathy. *Allelopathy: Organisms, Processes, and Applications* (eds A. Inderjit, K.M.M. Dakshini & F.A. Einhellig), pp. 142–157. Amer Chemical Soc, Washington, DC.

Thompson, J.D. (2005) *Plant Evolution in the Mediterranean*. Oxford University Press, Oxford.

Thorpe, A.S., Aschehoug, E.T., Atwater, D.Z. & Callaway, R.M. (2011) Interactions among plants and evolution. *Journal of Ecology*, **99**, 729–740.

Thorpe, A.S., Thelen, G.C., Diaconu, A. & Callaway, R.M. (2009) Root exudate is allelopathic in invaded community but not in native community: field evidence for the novel weapons hypothesis. *Journal of Ecology*, **97**, 641–645.

Tielbörger, K. & Kadmon, R. (2000) Temporal environmental variation tips the balance between facilitation and interference in desert plants. *Ecology*, **81**, 1544–1553.

Tilaoui, M., Ait Mouse, H., Jaafari, A. & Zyad, A. (2015) Comparative phytochemical analysis of essential oils from different biological parts of Artemisia herba alba and their cytotoxic effect on cancer cells. *PLoS ONE*, **10**, e0131799.

Tilman, D. (1982) *Resource Competition and Community Structure*. Princeton University Press, Princeton, New Jersey.

Tirado, R., Bråthen, K.A. & Pugnaire, F.I. (2015) Mutual positive effects between shrubs in an arid ecosystem. *Scientific Reports*, **5**, 14710.

Tirado, R. & Pugnaire, F. (2005) Community structure and positive interactions in constraining environments. *Oikos*, **111**, 437–444.

Tsubo, M., Nishihara, E., Nakamatsu, K., Cheng, Y. & Shinoda, M. (2012) Plant volatiles inhibit restoration of plant species communities in dry grassland. *Basic and Applied Ecology*, **13**, 76–84.

Valiente-Banuet, A. & Ezcurra, E. (1991) Shade as a Cause of the Association Between the Cactus Neobuxbaumia Tetetzo and the Nurse Plant Mimosa Luisana in the Tehuacan Valley, Mexico. *The Journal of Ecology*, **79**, 961.

Valiente-Banuet, A. & Verdú, M. (2008) Temporal shifts from facilitation to competition occur between closely related taxa: Facilitation-competition balance. *Journal of Ecology*, **96**, 489–494.

Varela, M.C., Arslan, I., Reginato, M.A., Cenzano, A.M. & Luna, M.V. (2016) Phenolic compounds as indicators of drought resistance in shrubs from Patagonian shrublands (Argentina). *Plant Physiology and Biochemistry*, **104**, 81–91.

Vivanco, J.M., Bais, H.P., Stermitz, F.R., Thelen, G.C. & Callaway, R.M. (2004) Biogeographical variation in community response to root allelochemistry: novel weapons and exotic invasion. *Ecology Letters*, **7**, 285–292.

Wang, X.-P., Li, X.-R., Xiao, H.-L., Berndtsson, R. & Pan, Y.-X. (2007) Effects of surface characteristics on infiltration patterns in an arid shrub desert. *Hydrological Processes*, **21**, 72–79.

Wang, J., Li, X., Zhang, J., Yao, T., Wei, D., Wang, Y. & Wang, J. (2012) Effect of root exudates on beneficial microorganisms—evidence from a continuous soybean monoculture. *Plant Ecology*, **213**, 1883–1892.

War, A.R., Paulraj, M.G., Ahmad, T., Buhroo, A.A., Hussain, B., Ignacimuthu, S. & Sharma, H.C. (2012) Mechanisms of plant defense against insect herbivores. *Plant Signaling & Behavior*, **7**, 1306–1320.

Ward, D. (2009) *The Biology of Deserts*. Oxford University Press, New York, NY.

Wardle, D.A., Nilsson, M.-C., Gallet, C. & Zackrisson, O. (1998) An ecosystem-level perspective of allelopathy. *Biological Reviews*, **73**, 305–319.

Weidenhamer, J.D. (2008) Allelopathic Mechanisms and Experimental Methodology. *Allelopathy in Sustainable Agriculture and Forestry* (eds R.S. Zeng, A.U. Mallik & S.M. Luo), pp. 119–135. Springer New York, New York, NY.

Weidenhamer, J.D., Hartnett, D.C. & Romeo, J.T. (1989) Density-Dependent Phytotoxicity: Distinguishing Resource Competition and Allelopathic Interference in Plants. *The Journal of Applied Ecology*, **26**, 613.

Weißhuhn, K. & Prati, D. (2009) Activated carbon may have undesired side effects for testing allelopathy in invasive plants. *Basic and Applied Ecology*, **10**, 500–507.

Weston, L.A. & Mathesius, U. (2013) Flavonoids: Their Structure, Biosynthesis and Role in the Rhizosphere, Including Allelopathy. *Journal of Chemical Ecology*, **39**, 283–297.

Whitford, W.G. (2002) *Ecology of Desert Systems*. Academic Press, London, UK.

Willis, R.J. (1985) The historical bases of the concept of allelopathy. *Journal of the History of Biology*, **18**, 71–102.

Willis, R.J. (2007) *The History of Allelopathy*. Springer, Dordrecht, Netherlands.

Younsi, F., Trimech, R., Boulila, A., Ezzine, O., Dhahri, S., Boussaid, M. & Messaoud, C. (2016) Essential oil and phenolic compounds of Artemisia herba-alba (Asso.): composition, antioxidant, antiacetylcholinesterase, and antibacterial activities. *International Journal of Food Properties*, **19**, 1425–1438.

Zavaleta, E.S., Pasari, J.R., Hulvey, K.B. & Tilman, G.D. (2010) Sustaining multiple ecosystem functions in grassland communities requires higher biodiversity. *Proceedings of the National Academy of Sciences*, **107**, 1443–1446.

Zhang, C., Li, X., Chen, Y., Zhao, J., Wan, S., Lin, Y. & Fu, S. (2016) Effects of Eucalyptus litter and roots on the establishment of native tree species in Eucalyptus plantations in South China. *Forest Ecology and Management*, **375**, 76–83.

Zhang, Q., Zhang, Y., Peng, S. & Zobel, K. (2014) Climate warming may facilitate invasion of the exotic shrub Lantana camara. *PLoS ONE*, **9**, e105500.

Zhu, X., Zhang, J. & Ma, K. (2011) Soil Biota Reduce Allelopathic Effects of the Invasive Eupatorium adenophorum (ed M Rillig). *PLoS ONE*, **6**, e25393.

Ziffer-Berger, J., Weisberg, P.J., Cablk, M.E. & Osem, Y. (2014) Spatial patterns provide support for the stress-gradient hypothesis over a range-wide aridity gradient. *Journal of Arid Environments*, **102**, 27–33.