TESIS DE LA UNIVERSIDAD
DE ZARAGOZA

2021

152

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Importancia de las interacciones bióticas entre plantas de comunidades vegetales gipsícolas para la conservación y restauración de ecosistemas yesíferos

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ISSN 2254-7606

Prensas de la Universidad
Universidad Zaragoza

Servicio de Publicaciones

ISSN 2254-7606

Tesis Doctoral

IMPORTANCIA DE LAS INTERACCIONES BIÓTICAS ENTRE PLANTAS DE COMUNIDADES VEGETALES GIPSÍCOLAS PARA LA CONSERVACIÓN Y RESTAURACIÓN DE ECOSISTEMAS YESÍFIEROS

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UNIVERSIDAD DE ZARAGOZA
Escuela de Doctorado

2019

TESIS DOCTORAL / DOCTORAL THESIS

Importancia de las interacciones bióticas entre plantas de comunidades vegetales gipsícolas para la conservación y restauración de ecosistemas yesíferos

Importance of biotic interactions between plants in gypsum communities for the conservation and restoration of gypsum ecosystems

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Memoria para optar al grado de Doctor por la Universidad de Zaragoza

Programa de Doctorado en Ciencias Agrarias y del Medio Natural

Febrero 2019

A mi familia

Jovencita, para avanzar a veces hay que retroceder. Muchas veces, jovencita, parece que no estemos llegando a ningún sitio, cuando en realidad estamos llegando.

El Sabio (Dentro del Laberinto, 1986)

Agradecimientos/Acknowledgements

Muchos dicen que esta es la parte de la memoria de tesis que más les cuesta escribir. En mi caso creo que será fácil, ya que estoy llena de agradecimiento hacia todas aquellas personas que, de una forma o de otra, me han acompañado desde la entrada hasta la salida de este laberinto "manierista" que ha sido la realización de la tesis doctoral. Es difícil olvidar vuestra ayuda en cualquier aspecto del desarrollo de esta tesis, tanto a nivel académico como a nivel personal.

Quiero empezar los agradecimientos por mis directoras Yolanda e Inma, por aventurarse a dirigir esta tesis y por su incansable ayuda y ánimos para que todo saliera bien. Habéis sido una parte indispensable en el desarrollo de nuestra investigación en los yesos zaragozanos y me habéis aportado mucha sabiduría, tanto en lo académico como en lo personal. Muchas gracias a ambas por tener tanta paciencia conmigo. Quiero agradecérselo especialmente a Yolanda. En primer lugar, porque me eligió entre los candidatos interesados en el proyecto (sin conocerme, menudo riesgo) y, en segundo lugar, porque siempre ha estado trabajando duro para ayudarme, incluso estando de fin de semana, de vacaciones o de baja. Gracias también por ponerme los pies en la tierra cuando me iba por las ramas probando mil formas de analizar lo mismo y dándole tantas vueltas a la redacción de los artículos. Te mereces un monumento a la paciencia.

Muchas gracias a los que han colaborado directamente en la elaboración de esta tesis, estando codo con codo conmigo en el campo, en el invernadero y en el laboratorio, diseñando los experimentos y muestreos, dándome consejos para afrontar con más claridad y decisión los análisis de datos y dando las últimas pinceladas a los artículos para que quedaran redondos: Hugo, Ramón Reiné, Bodil Ehlers, José M. Castillejo, Yolanda, Inma, Antonio, Pedro Bravo, Javier Ramos, Pedro María, Carmela Capistrós, Guille Enguita, Sergio Martínez, Itsaso, Maite, Victoria, María Pueyo y Elena Lahoz. Agradezco especialmente a Mariluz y Pedrito su enorme ayuda en el campo porque sin ellos la tesis no podría haber salido hacia adelante. ¡Mil gracias a los dos! Un agradecimiento especial también a mi compi Antoñico, que ha escuchado todas mis dudas en el despacho y me ha ayudado en todo lo que ha podido (campo,

invernadero, datos, etc.), aunque también estuviera hasta arriba con su tesis. Muchas gracias a Hugo por darme consejos sobre cómo venderme bien en mis artículos y por revisar esta tesis. Gracias a Maite por ayudarme con los datos de humedad del suelo. Muchas gracias también a Manuel Pizarro, por su ayuda con algunos de los mapas tan bonitos e informativos que forman parte de esta tesis. Gracias a los geólogos (mi hermano y mi colega Iván), por comprobar que no metiese la pata hablando de los afloramientos de yeso.

Debo mencionar también a las entidades públicas y privadas que han permitido la realización de esta tesis doctoral. Gracias al Ministerio de Economía, Industria y Competitividad (MINECO) por haber financiado los proyectos de investigación en los que he estado adscrita (CGL-2012-37508 y CGL-2016-80783-R) y haberme concedido la ayuda para mi contrato predoctoral en el IPE (BES-2013-063852) y las ayudas para estancias breves en Aarhus Universitet en Dinamarca (EEBB-I-15-10298) y en New Mexico State University en Estados Unidos (EEBB-I-17-12212). Gracias al IPE-CSIC por acogerme y sobre todo a Cecilia y Trini por hacerme un poco más fácil la burocracia asociada a estas ayudas. Agradezco también al Ayuntamiento de Belchite y al INAGA (Gobierno de Aragón) por concederme los permisos para llevar a cabo muestreos en el Refugio de Fauna Silvestre “La Lomaza de Belchite”. A Jesús Cosculluela de los viveros de la DGA por proporcionar las plántulas para los experimentos de plantación y por dar consejos para el pretratamiento de semillas. A ENDESA y PLACO Ibérica por darme permiso para entrar en sus propiedades para realizar los experimentos de plantación y los muestreos de vegetación. Gracias a la Estación Experimental de Aula Dei (EEAD-CSIC) por hacernos un hueco en sus invernaderos para germinar las semillas del banco de semillas.

También agradezco a José Manuel Nicolau que haya sido mi tutor académico y que me haya permitido impartir algunas charlas en su asignatura de Ecología del Grado en Ciencias Ambientales. Gracias a la comisión académica del programa de doctorado de Ciencias Agrarias y del Medio Natural de la Universidad de Zaragoza por aceptarme incluso fuera de los plazos establecidos y por evaluarme favorablemente cada año. Un agradecimiento especial a Pilar Catalán por ser tan paciente y resolverme tantas dudas del nuevo plan de estudios de doctorado.

Gracias a todos mis compañeros del IPE (y EEAD) por estar ahí dando ánimos y consejos, y por ayudarme a desconectar. Es una lista muy larga, ya que todos los que han pasado por aquí me han aportado mucho en estos últimos cinco años. Gracias a los doctorandos que ya terminaron, a los que siguen dándole caña a la tesis (¡mucho ánimo!), a los compañeros de almuerzos y tuppers, a los que se han echado alguna que otra cerveza conmigo, los que han sufrido mis visitas en su despacho, etc. Muchas gracias a Natalie, Elena Granda "sí a todo", Ángela "bellotera", Javi Zabalza, Josu, Kike, Paloma, Cecilia, Esteban, Clara, Fergus, Mocho (y Cristi), Iván L., Leti P., Àngela, David, Leti G., Edu, Gabriel Sangüesa, Miguel Bartolomé, Hugo, María gallega, Iker, Sam, Jesús Villellas, Jesús Revuelto, Fabián, Pili, Marina, Antxon, Miguelico (¡ánimo paisano!), Dani (y Dana), Makki, Paco, Iván "nuevo", Alberto, Merce, Xavi, Héctor, Jana, Juan, Manué, José Luis, Michele, Manolo, Laura, Sergio "rastas", Andreu, Jorge, Mercedes, Ariadna, Lourdes, Itsaso, Nacho, Santiago, Estela, Belinda, Graciela, Antonio Gazol, Penélope, Begoña, Miguel Sevilla... Gracias por todas esas sonrisas, risas, conversaciones agradables y consejos. De entre los anteriores, tengo guardados unos agradecimientos muy especiales para los que me han dado miles de abrazos llenos de energía y me han escuchado tanto, porque han supuesto un gran apoyo para mí. Ellas y ellos ya saben quiénes son ;). Y no puedo olvidar a mis compis "de promoción" Natalia, María, Miquel y Carlos. Nos hemos desahogado juntos, comprendiéndonos en todo porque estábamos casi en la misma etapa, y dándonos ánimos mutuos. Me ha servido mucho apoyarme y fijarme en vosotros para seguir adelante. He ganado mucho al haberos conocido a todos.

Thanks to all those who sweetened me so much during my stays abroad. To Bodil Ehlers, who supported me academically and personally during my stay in Silkeborg (Denmark). To Lise Lauridsen and Stine Slotsbo, who helped me with the analysis of the leaves and roots of *Cistus clusii* in the GC-MS (*mange tak!*). To my international friends, who helped me to relax by playing *Dobble*, having a picnic and swimming at Lake AlmindsØ or riding our bikes along the bunkers route: Amaia, Nerea, Jennifer, André, Pinar, Luis, Jiai, Eugenio, Joaquim, Moritz… Thanks to my new great friend Nicole Pietrasiak, who helped me a lot during my stay in New Mexico, welcomed me into her house as if I were her sister, taught me so much about biocrusts and supported me all the time (*danke schön!*). To Megan, who was my inseparable companion and a great help under the sun of the Chihuahuan Desert; thank you very much for those days in White Sands, Roswell and Yeso Hills (thanks to your parents too for welcoming me at home). To Michael Moore, for teaching me so much about the plants of the Chihuahuan Desert, for driving me on that road trip when I met the deepest of the American South-West, and for not being afraid of my threatening finger. Thanks to Donovan Bailey for trusting me and giving me the opportunity to expose my results at NMSU. To Emily for her help with the English proofreading over my manuscript, for listening to my confessions holding our glass of wine, for taking me country dancing and for her hugs. I miss you all so much.

Muchas gracias a los que me metieron en esto de la investigación y los que siguen confiando en mí para seguir en ello. A Juan, Eva y mi compi de aventuras Miguel “Gypsy King” por introducirme en el mundo de la experimentación ecológica aplicada y, sobre todo, por darme a conocer los maravillosos ecosistemas gipsícolas. Sin el apoyo moral de ellos tres, su enorme ayuda académica (gracias por contar conmigo en las publicaciones) y sus enseñanzas, no habría podido conseguir una beca para hacer el doctorado. También quiero agradecer a mis compañeros de los departamentos de Botánica y Edafología de la UGR y a los del IFAPA por enseñarme tanto de la vida y por su buen rollo y los viernes clásicos, que hicieron que no tuviera ningunas ganas de abandonar la investigación: Paqui, Antonio, Gloria, David, Diego, Marta, Julio, Paco Valle, Paco Martín-Peinado, Emilia, Irene, Pipo, Noelia, Nines y Eduardo. Gracias a Sara Palacio, por transmitir ese amor incondicional a los ecosistemas de yeso, por invitarme a la primera reunión de Gypnet, por querer colaborar conmigo y por animarme a seguir apostando por los yesos (ánimo con GYPWORLD). Gracias también a Gabriel Montserrat por interesarse por mi trabajo, por ayudarme con la identificación de algunas especies y por animarme a seguir investigando, aunque ahora haya que publicar a un ritmo desenfrenado.

Aunque suene raro, gracias a la música. A Ludovico Einaudi, Yann Tiersen, Adrian Von Ziegler, Michael Nyman, Hans Zimmer, etc. por componer esas inspiradoras piezas musicales que me han acompañado durante las largas horas que he pasado delante del ordenador intentando escribir *papers*. A Doro y Dio por acompañarme en mis largos ratos de soledad en el invernadero (en Silkeborg y en Aula Dei). Y a bandas como Eluveitie, Blind Guardian, Masterplan, Faun, Amon Amarth, Iced Earth, Epica (y un larguísimo etcétera), que me transmitían mucha energía para pelear con R. Gracias a DragonForce por su tema “Chemical Interference”, por estar tan relacionado con mi tesis (ver capítulo 3, jaja) y por aquella situación tan inverosímil y a la vez tan "tronchante" que viví con Yolanda.

Gracias a todas mis amistades "de ahora y siempre" por estar ahí porque, aunque a algunos no les veo desde hace tiempo, recibo su calor y apoyo. Muchas gracias a Carlota, Fabián, Esteban, Elena, Ana y Ángela por las terapias huertiles y por entender que a veces no tenía tiempo de ir a regar el huerto. A Cristina y Antonio por distraerme los domingos con juegos de mesa y apadrinar a Niara y Kerrigan (*Cuiidame el gaatoo este mes*). A mis compis de danza oriental, danza tribal y lengua de signos por compartir esos ratos de actividades "extra-escolares" desestresantes conmigo. A las *Biogirls* Bibi, Tere, Bea, Senda y Araceli, y a mis alemanas Lina y Cori por apoyarme en la distancia, hacerme alguna visita en Zaragoza y darme consejos tan útiles por la experiencia que os ha dado hacer vuestras tesis. A mis colegas-hermanos de la Komuna Egao: Martita, Laurita, Ruri, Peio, Menchero, Elenita, Irenita y Mariajo; gracias por estar siempre ahí como si no hubiera pasado el tiempo. A mis amigos "ludópatas" y "borrachos" de Graná por amenizar mis vacaciones: Pepe, Shigeo, Juanlu, Álvaro, Fume, Alex, Sabri, Elisa, Ato, Rubén, etc. Gracias a la Yoli por estar dispuesta a quedar conmigo cuando volvía al pueblo a reencontrarme con mi zona de confort (mi hogar: Cazorla y su sierra).

Infinitas gracias a mis padres por haber apoyado siempre que siguiera la carrera científica (moral y económicamente), por haber confiado siempre en mí y por sentirse tan orgullosos con los "logros" de mi tesis. A mi hermano por sus consejos en mis momentos de bajón y por "apretarme las tuercas" para que siguiera dándole duro a la tesis. Gracias a él y a Bea por acogerme en su casa en mis fines de semana de escape. A mis sobrinos Olivia y Mario por hacer diabluras que me hacían reír en mis momentos de frustración, haciéndome feliz incluso en la distancia. Y, aunque no les enumere (porque son decenas), les doy las gracias a mis tías, tíos, primas y primos por ser tan alegres, alimentarme e hidratarme tan bien y confiar en mí. A mis abuelos, que escuchaban atentos cómo de importante es conservar "un secarral" y, en especial, a mi abuelo Pablo por esperar a que volviera de Dinamarca para darme un achuchón. Gracias a mi familia granaína por su apoyo, por sus gritos de ánimo por teléfono y por entender que en algunas ocasiones tenía que faltar a los planes. Quiero hacer especial mención a Angelillas, que ha elaborado las cianotipias que decoran esta tesis, haciendo un homenaje a las mujeres científicas, en concreto a la botánica Anna Atkins. Millones de gracias, máquina.

Y por último y más importante a Javi por acompañarme a Zaragoza dejando atrás a su familia y a sus amigos. Gracias por haberte quedado a pesar de sufrir mis vaivenes durante estos cinco años. Muchas gracias por haberme ayudado no sólo moralmente (¡vamos hormiguita!), sino también con mis experimentos de invernadero y campo (aun quedándote sin playa y sufriendo el ataque de la mosca negra o el cierzo), con los datos y leyendo algún párrafo que otro para ver si se entendía bien. Gracias por estar ahí porque has hecho posible que hiciera esta tesis.

Afloramientos de yeso en los alrededores de la Sierra de Alcubierre
(Leciñena, Zaragoza, España)

Cianotipia elaborada por Ángeles Muñoz
Fotografía original: Ana Foronda

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Vista general de las comunidades vegetales
gipsícolas del Valle Medio del Ebro
(Leciñena, Zaragoza, España)

Cianotipia elaborada por Ángeles Muñoz
Fotografía original: Ana Foronda

Summary / Resumen

Gypsum soils have physical and chemical limitations for plant development, such as low availability of water and essential macronutrients, high concentrations of sulfate and calcium that can be toxic to plants, or surface crusts that prevent root penetration. However, these soils harbor specialized plant communities, which rare and rich in gypsum-adapted species (gypsophytes), of great importance for the conservation of global biodiversity. Despite their relevance, these habitats are often degraded and fragmented due to anthropogenic activities such as intensive agriculture, overgrazing or mining. The recovery of degraded gypsum ecosystems is challenging due to the harsh environmental conditions that limit vegetation development.

The regeneration and persistence of plant communities in these stressful environments depend on plant establishment and survival, which are critical in these environments. Plants in the early life stages are highly vulnerable to environmental stress (especially less gypsum-adapted species, gipsovags) and often need the presence of nurse plants to germinate and survive to the adult stage. In general, nurse plants are shrubs that improve micro-environmental conditions under their canopy, creating favorable microhabitats for the establishment of less adapted species, thus forming species-rich vegetation patches. Shrubs can have a double positive function in the community, as they also trap and accumulate seeds under their canopies, where micro-environmental conditions will be favorable for their germination. Facilitation can be a key process in the restoration of degraded ecosystems, as it would promote plant succession and thus spontaneous regeneration of the plant community. It is, therefore, necessary to identify the key species that have significant positive effects on the community, either by trapping seeds or by facilitating plant establishment, which should be considered in conservation and restoration plans.

In addition to facilitation, negative plant interactions (known as interference) also influence the composition and structure of plant communities. Once the facilitated seedlings become adults, they can exert an adverse effect on nurse plants through competition for scarce resources. Some species release compounds with adverse effects on the establishment and growth of other plants to eliminate potentially competing nearby plants. This would lead to an impoverishment of species in their vicinity and, as a consequence, these plants may become locally dominant in gypsum communities. The net balance of interactions depends on the species involved and the environmental conditions. There are studies that postulate that while facilitation would be of greater relevance under more environmental stress, interference would dominate under moderate stress. However, others suggest a predominance of interference under higher stress due to increased competition for scarce resources or intensification of phytotoxic effects.

The objective of the thesis was to investigate the role of gypsophyte and gypsovag shrubs in structuring plant diversity in gypsum plant communities in order to identify key species for the conservation and restoration of these ecosystems. Unlike most studies on plant-plant interactions focusing on the effects of one species on another, this doctoral thesis provides insight into the net balance of interactions at the community level. Since plants are sessile organisms and biotic interactions occur between neighboring individuals, spatial patterns of vegetation may be useful indicators for inferring the net balance of interactions at the community level. The study has been approached from an observational perspective evaluating the spatial structure of the community, and from an experimental perspective testing the effect that certain key species have on plant establishment. Experiments complement observational data by helping to unravel the mechanisms by which plants influence the spatial pattern of the community. The study was carried out in the Middle Ebro Valley (NE Spain), which comprises one of the most extensive outcrops of gypsum in Europe. In the study area, there is an aridity gradient from north to south, facilitating the evaluation of interactions under different environmental stress.

The observational data collected in **Chapter 1** served to highlight the relevance of positive interactions in the plant community from both the facilitated and the facilitator plants. It was found that independently of their life strategy (gypsophytes or gipsovags), the plants need to be associated with adult plants to establish in the community. In this chapter, species with a positive role in the richness and abundance of plants in the community were identified. Three gypsophytes (*Gypsophila struthium*, *Ononis tridentata,* and *Helianthemum squamatum*) and three gipsovags (*Cistus clusii*, *Rosmarinus officinalis,* and *Thymus vulgaris*) were selected as target species. Except for *H. squamatum*, the target species have a positive role in the establishment of other plants compared to open areas. It was observed that these shrubs improve micro-environmental conditions under canopy, which could be the underlying mechanism that favors plant establishment on these microsites. However, significant differences in facilitation abilities were found among the species studied. The gypsophytes *G.struthium* and *O. tridentata* harbor more plants under their canopies than other shrubs, structuring species-rich patches in their vicinity.

The abilities of these shrubs as seed sources and sinks and, therefore, as structuring agents of the soil seed bank in these plant communities were studied in **Chapter 2**. Among the target species, the shrub *G. struthium* is the one that accumulates the richest and most abundant seed bank under canopy, because its architecture and size allow it to trap and accumulate more seeds than other plants (seed sink). In addition, this shrub acts as a seed source through the plants harbored under canopy (chapter 1), which provides seeds to the soil in neighboring areas. The significant seed sink and seed source roles of *G. struthium* favors the formation of a soil seed bank structured in patches in its vicinity. Seeds accumulated in the vicinity of *G. struthium* would also find favorable micro-environments for their germination, thus shaping species-rich vegetation patches.

Additionally, in chapter 1 it was observed that, despite having nurse architecture and improving the micro-environmental conditions under their canopies, the gypsovag shrubs *R. officinalis* and *C. clusii* harbor less richness and abundance of plants than the gypsophytes *G. struthium* and *O. tridentata*. Both gypsovag species are locally dominant in gypsum plant communities of the Middle Ebro Valley. These observations suggest that these gypsovags may have adverse effects on certain neighboring species, likely caused by a higher competitive ability or chemical interference. While the chemical interference of *R. officinalis* has been demonstrated in other plant communities, there is no evidence that *C. clusii* exerts adverse chemical effects on vegetation (although there are studies that demonstrate adverse chemical effects of other species of the same genus). In **Chapter 3**, observational field data were combined with data obtained from a greenhouse experiment to test the potential interference mechanism that *C. clusii* could be exerting on co-occurring species in the community. It was observed that the germination and survival of some species were negatively affected by the aqueous extracts of leaves and roots of *C. clusii*, coinciding with what was observed under natural conditions. Complementarily, compounds commonly considered with phytotoxic potential were found in the aqueous extracts. These results

showed that *C. clusii* exerts a species-specific chemical interference in neighboring plants.

Since gypsum ecosystems are often degraded by human activities, it is necessary to elaborate effective restoration plans to return the ecosystem to its initial state. Recently, facilitation is being considered as a crucial mechanism for ecosystem restoration. One of the major contributions of the thesis has been the identification of *G. struthium* as a key species maintaining diversity by its significant role as a nurse plant. **Chapter 4** studied the suitability of this shrub as a nurse plant in the restoration of gypsum ecosystems degraded by mining. On the one hand, an observational study in a gypsum quarry confirmed the appearance of *G. struthium* as a pioneer species in the community, which had been previously demonstrated by other researchers. Other gypsophytes also appeared as pioneers in the plant community. On the other hand, a plantation and sowing experiment under the canopy of *G. struthium* in a gypsum spoil dump confirmed its positive role on the establishment and growth of other plants of interest for the restoration of gypsum plant communities. This experiment revealed that the improvement of micro-environmental conditions could be the underlying mechanism of the facilitation exerted by this shrub. This work valued the use of plants that appear as pioneers in the community as nurse plants for restoration, which would improve natural plant succession and the persistence of the plant community.

This thesis increases the understanding of plant interactions at the community level and highlights the key role played by certain species in structuring diversity in gypsum plant communities of the Middle Ebro Valley. Although species with an adverse effect on the establishment of certain species have been identified (*e.g.*, *C. clusii*), the thesis shows that the net balance of interactions at the community level is positive. On the one hand, plants need to be facilitated to establish under the harsh conditions of gypsum environments. On the other hand, shrubs with a significant nurse role in structuring species-rich vegetation patches around them have been identified. In general, the species that has been shown to play the most decisive role in the net balance of interactions is the gypsophyte *G. struthium* (followed by the gypsophyte *O. tridentata*), for their nurse role and for their ability to form abundant and species-rich seed banks. *Gypsophila struthium* appears as a pioneer in gypsum plant communities, which together with its positive role in plant establishment, is valuable for use in the restoration of gypsum ecosystems.

Despite having identified gypsophyte shrubs as relevant for the conservation and restoration of gypsum plant communities in the Middle Ebro Valley, future work is needed to broaden the knowledge of plant-plant interactions plants in gypsum ecosystems. In order to generalize our conclusions, it is necessary to carry out parallel studies in gypsum plant communities in other regions under different environmental conditions and with other species involved. Also, it would be interesting to develop experimental work under natural conditions at the community level in order to draw firm conclusions about the underlying mechanisms generated by the patterns observed in this thesis. It is also necessary to experimentally prove the suitability of other species that appear as pioneers for use as nurses in the restoration of gypsum ecosystems.

Los suelos de yeso presentan limitaciones físicas y químicas para el desarrollo vegetal, como una baja disponibilidad de agua y macronutrientes esenciales, altas concentraciones de sulfato y calcio que pueden llegar a ser tóxicas para las plantas, o costras superficiales que impiden la penetración de las raíces. Sin embargo, estos suelos albergan comunidades vegetales especializadas, raras y ricas en especies adaptadas a estas condiciones (gipsófitos), que son de gran importancia para la conservación de la biodiversidad mundial. A pesar de su relevancia, estos hábitats suelen estar degradados y/o fragmentados debido a actividades antrópicas como la agricultura intensiva, el sobrepastoreo o la minería. La restauración de ecosistemas gipsícolas degradados es un proceso difícil debido a las duras condiciones ambientales, que limitan la regeneración de la vegetación.

La regeneración y la persistencia de la comunidad vegetal en estos ambientes estresantes dependen de la capacidad de establecimiento y supervivencia de las plantas, que son fases críticas en estos ambientes. Las plantas en las primeras etapas de su vida son altamente vulnerables al estrés ambiental (especialmente las plantas menos adaptadas al yeso, gipsovagos) y a menudo necesitan la presencia de plantas nodrizas para germinar y sobrevivir hasta la etapa adulta. En general, las plantas que actúan como nodrizas son arbustos que mejoran las condiciones micro-ambientales bajo su dosel, creando microhábitats favorables para el establecimiento de especies menos adaptadas al estrés, formando así parches de vegetación ricos en especies. Los arbustos pueden presentar una doble función positiva en la comunidad, ya que también captan y acumulan semillas bajo su dosel, donde las condiciones serán favorables a su germinación. La facilitación puede ser un proceso clave en la restauración de ecosistemas gipsícolas degradados, ya que promovería la sucesión de las plantas y, por tanto, la regeneración espontánea de la comunidad vegetal. Por tanto, es necesario identificar las especies clave que ejercen efectos positivos significativos en la comunidad, ya sea mediante la captura de semillas o facilitando el establecimiento de plantas, y habrían de tenerse en cuenta en planes de conservación y restauración.

Además de la facilitación, las interacciones negativas entre plantas (conocidas como interferencia) también influyen en la composición y estructura de las comunidades vegetales. Una vez que las plántulas facilitadas son adultas, pueden ejercer

un efecto adverso sobre las plantas nodrizas a través de la competencia por los recursos escasos. Para eliminar plantas vecinas potencialmente competidoras, hay algunas especies que liberan compuestos con efectos adversos sobre el establecimiento y crecimiento de otras plantas. Esto conduciría a un empobrecimiento de especies en su vecindad y, como consecuencia, estas plantas pueden llegar a ser localmente dominantes en las comunidades gipsícolas. El balance neto de las interacciones depende de las especies involucradas y de las condiciones ambientales. Hay investigaciones que postulan que mientras que en ambientes con más estrés ambiental la facilitación tomaría mayor relevancia, en ambientes con estrés moderado dominaría la interferencia. Sin embargo, otras sugieren una predominancia de la interferencia en condiciones de elevado estrés, por ejemplo debido a un aumento de la competencia por los recursos escasos o a la intensificación de los efectos fitotóxicos.

El objetivo de la tesis es investigar el papel de los arbustos gipsófitos y gipsovagos en la estructuración de la diversidad vegetal en las comunidades gipsícolas para identificar especies clave para la conservación y restauración de estos ecosistemas. A diferencia de la mayoría de estudios sobre interacciones bióticas entre plantas, centrados en los efectos de una especie sobre otra, esta tesis doctoral aporta conocimiento sobre el balance neto que tienen las interacciones a nivel de comunidad. Dado que las plantas son organismos sésiles y que las interacciones bióticas ocurren entre individuos vecinos, los patrones espaciales de la vegetación pueden ser buenos indicadores para inferir el balance neto de las interacciones entre plantas de la comunidad. El estudio se ha abordado desde distintas perspectivas, tanto a nivel observacional evaluando la estructura espacial de la comunidad, como a nivel experimental testando el efecto que tienen ciertas especies clave en el establecimiento de otras. Los experimentos complementan a los datos observacionales, ya que ayudan a desentrañar los mecanismos por los cuáles las plantas influyen en el patrón espacial de la comunidad. El estudio se realizó en el Valle Medio del Ebro (NE de España), que comprende uno de los mayores afloramientos de yeso de Europa. En el área de estudio existe un gradiente de aridez de norte a sur, facilitando la evaluación de las interacciones bajo distintos niveles de estrés ambiental.

Los datos observacionales recogidos en el **capítulo 1** sirvieron para destacar la relevancia que tienen las interacciones positivas en la comunidad vegetal tanto desde el punto de vista de las plantas facilitadas como de las facilitadoras. Se encontró que independientemente de su estrategia de vida (gipsófitos o gipsovagos), las plantas de las comunidades gipsícolas necesitan estar asociadas a plantas adultas para establecerse. En este capítulo se identificaron especies con un papel positivo sobre la riqueza y abundancia de plantas en la comunidad. Como especies de estudio se seleccionaron los arbustos más abundantes en la comunidad vegetal, siendo tres gipsófitos (*Gypsophila struthium*, *Ononis tridentata* y *Helianthemum squamatum*), y tres gipsovagos (*Cistus clusii*, *Rosmarinus officinalis* y *Thymus vulgaris*). A excepción de *H. squamatum*, los arbustos estudiados tienen un papel positivo en el establecimiento de otras plantas con respecto a áreas abiertas. Se observó que estos arbustos mejoran las condiciones micro-ambientales bajo su dosel, lo que podría ser el mecanismo subyacente que favorece el establecimiento de plantas en esos micrositios. Sin embargo, se encontraron diferencias significativas entre las especies estudiadas en la capacidad de facilitación. Los gipsófitos *G.struthium* y *O. tridentata* albergan más plantas bajo su dosel que los otros arbustos, estructurando parches ricos en especies en su vecindad.

En el **capítulo 2** se estudió la capacidad que tienen estos arbustos como fuentes y sumideros de semillas y, por tanto, como estructuradores del banco de semillas del suelo en estas comunidades vegetales. De entre las especies estudiadas, el arbusto *G. struthium* es el que acumula más riqueza y abundancia de semillas bajo su dosel, debido a que su arquitectura y tamaño le permite captar y acumular más semillas que otras plantas (sumidero de semillas). Además, este arbusto actúa como fuente de semillas a través de las plantas que alberga bajo su dosel (capítulo 1), que aportan semillas al suelo de las áreas vecinas. El papel significativo que *G. struthium* ejerce como sumidero y como fuente de semillas favorece la formación de un banco de semillas estructurado en parches en áreas vecinas. Existiría un mecanismo de retroalimentación positiva, ya que las semillas acumuladas en las cercanías de *G. struthium* también encontrarían condiciones micro-ambientales favorables a su germinación, por tanto, formando parches de vegetación ricos en especies.

Por otro lado, en el **capítulo 1** se observó que, a pesar de tener arquitectura de nodriza y mejorar las condiciones micro-ambientales bajo su dosel de igual manera que los gipsófitos *G. struthium* y *O. tridentata*, los arbustos gipsovagos *R. officinalis* y *C.*

clusii albergan menor riqueza y abundancia de plantas que los anteriores. Ambas especies son localmente dominantes en las comunidades vegetales gipsícolas del Valle Medio del Ebro. Estas observaciones sugieren que estos arbustos gipsovagos pueden tener efectos adversos sobre ciertas especies vecinas, potencialmente causados por una mayor capacidad competitiva o por interferencia química. Mientras que la interferencia química de *R. officinalis* ha sido demostrada en otras comunidades vegetales, no existen evidencias de que *C. clusii* ejerza efectos químicos negativos sobre la vegetación (aunque sí existen trabajos que demuestran efectos químicos negativos de otras especies del mismo género). En el **capítulo 3** de esta tesis se combinaron datos observacionales en campo con datos obtenidos de un experimento de siembra en invernadero para estudiar el posible mecanismo de interferencia que este arbusto podría estar ejerciendo sobre las especies cohabitantes en la comunidad. Se observó que la germinación y la supervivencia de algunas especies se veían afectadas negativamente por los extractos acuosos de hojas y raíces de *C. clusii*, coincidiendo con lo observado bajo condiciones naturales. Complementariamente, en los extractos acuosos se encontraron compuestos comúnmente considerados con potencial fitotóxico. Estos resultados evidenciaron que *C. clusii* ejerce un papel químico negativo en plantas vecinas y que este efecto es especie-específico.

Dado que los ecosistemas gipsícolas se encuentran a menudo degradados por las actividades humanas, se hace necesario elaborar planes de restauración eficientes para devolver el ecosistema al estado inicial. Recientemente se está considerando a la facilitación como un mecanismo crucial para la restauración de ecosistemas. Una de las mayores aportaciones de la tesis ha sido la identificación de *G. struthium* como especie clave el mantenimiento de la diversidad por su papel significativo como nodriza. En el **capítulo 4** se estudió la idoneidad de este arbusto como planta nodriza en la restauración de ecosistemas gipsícolas degradados por la minería. Por un lado, un trabajo observacional en una cantera de yeso confirmó la aparición de *G. struthium* como especie pionera en canteras de yeso, que ya había sido demostrada anteriormente por otros investigadores. Otros especialistas edáficos (gipsófitos) también aparecieron como pioneros en la comunidad vegetal. Por otro lado, un experimento de plantación y siembra bajo el dosel de *G. struthium* en un vertedero de yeso confirmó su papel positivo sobre el establecimiento y crecimiento de otras plantas de interés para la restauración de comunidades vegetales gipsícolas. Este experimento desveló que la mejora de las condiciones micro-ambientales podría ser el mecanismo subyacente de la facilitación que ejerce este arbusto. Este trabajo puso en valor el uso de plantas que aparecen como pioneras en la comunidad como plantas nodrizas para la restauración, lo que mejoraría la sucesión vegetal espontánea y la persistencia de la comunidad vegetal.

Los distintos capítulos que componen esta tesis mejoran el entendimiento de las interacciones entre plantas a nivel de comunidad y destacan el papel clave que tienen ciertas especies que son abundantes en la estructuración de la diversidad en las comunidades vegetales gipsícolas del Valle Medio del Ebro. Aunque se han identificado especies que tienen un efecto negativo en el establecimiento de ciertas especies (por ejemplo *C. clusii*), la tesis pone de manifiesto que el balance neto de las interacciones a nivel de comunidad es positivo. Por un lado, se ha demostrado que las plantas necesitan ser facilitadas para establecerse bajo las condiciones extremas que suponen los ambientes de yeso. Por otro lado, se han identificado arbustos con un claro papel de nodriza, que estructuran la vegetación en parches ricos en especies a su alrededor. En general, el arbusto que ha demostrado tener un papel más positivo en balance neto de las interacciones es el gipsófito *G. struthium* (seguido del gipsófito *O. tridentata*), tanto por su papel de nodriza como por su capacidad de formar bancos de semillas abundantes y ricos en especies. Este arbusto aparece como pionero en las comunidades vegetales gipsícolas, que junto a su papel positivo en el establecimiento de plantas, pone en valor su uso en los planes de restauración de ecosistemas de yeso degradados por la minería.

A pesar de haber identificado que los arbustos gipsófitos son clave en la conservación y restauración de las comunidades vegetales gipsícolas del Valle del Ebro, hacen falta futuros trabajos para ampliar el conocimiento de las interacciones entre plantas en los ecosistemas gipsícolas. Para poder generalizar nuestras conclusiones, se hace necesario realizar trabajos paralelos en comunidades vegetales gipsícolas de otras regiones bajo diversas condiciones ambientales y en las que están implicadas otras especies. Además, sería interesante desarrollar trabajos experimentales bajo condiciones naturales a nivel de comunidad, para permitir sacar conclusiones firmes sobre los mecanismos subyacentes que generan los patrones observados en esta tesis. Asimismo, se hace necesario comprobar experimentalmente la idoneidad de otras especies que

aparecen como pioneras para su uso como nodrizas en la restauración de ecosistemas gipsícolas.

***Yucca elata* en las dunas de yeso de White Sands**
National Monument (New Mexico, Estados Unidos)

Cianotipia elaborada por Ángeles Muñoz
Fotografía original: Ana Foronda

General Introduction

Arid and semi-arid ecosystems in the Earth

Arid and semi-arid areas account for 41 % of the Earth's emerged surface (Prăvălie, 2016). These are regions where rainfall is scarce and unpredictable and where the high solar radiation and extreme temperatures cause strong evapotranspiration (Reynolds *et al.*, 2007). Low precipitation and high evapotranspiration result in water deficit that induces water-stress in plants, affecting plant metabolism, morphology, growth, and establishment (Hsiao, 1973).

Plants living in arid and semi-arid areas have morpho-anatomical and physiological adaptations to overcome water-stress and make efficient use of water (De Micco and Aronne, 2012). First, to enhance water uptake, plants develop deep tap roots to reach the water table and long lateral roots to search for other water sources like capillary water (De Micco and Aronne, 2012). Unlike shoot growth, root growth is generally not inhibited under water deficit conditions (Sharp and Davies, 1989), which is reflected in the root/shoot ratio that increases as the more xeric the environment is (Bray, 1963). Second, to enhance water retention, plants have xeromorphic leaves with a small surface/volume ratio, including succulent leaves that accumulate water in parenchyma tissues, or sclerophyllous leaves with a thick cuticle and waxes that prevent water permeability (De Micco and Aronne 2012; Figure 1A). Furthermore, plants can restrict water losses by stomatal closure under severe drought, high temperature and high light intensity, decreasing photosynthetic rates accordingly (Chaves *et al.*, 2002). In arid and semi-arid ecosystems it is also common the predominance of plants with C4 or CAM metabolism, which reduce water overuse by high photosynthetic efficiency (*e.g.*, grasses and cacti; Noy-Meir 1973; Figure 1B-C). Finally, due to the uncertainty of rainfall in these regions, some plants avoid water deficit by regulating their phenology (De Micco and Aronne, 2012). For example, annuals or geophytes evade the most stressful conditions in a dormant state (*e.g.*, seeds or bulbs), responding their growth and reproduction very quickly to rainfall pulses (Chesson *et al.*, 2004; Noy-Meir, 1973).

Figure 1 Adaptations of plants living in arid and semiarid ecosystems: **A)** *Berberis trifoliata* Torr. has sclerophyllous leaves; **B)** *Sporobolus nealleyi* Vasey, a grass with C4 metabolism; and **C)** *Echinocereus pectinatus* (Scheidw.) Engelm, with CAM metabolism. Photos: Ana Foronda (Chihuahuan Desert, New Mexico, USA).

In arid and semi-arid areas water deficit limits the development of a continuous plant cover, thus vegetation is commonly structured in patches interspersed with open areas almost devoid of vegetation (Aguiar and Sala, 1999). Vegetation patches play an important role in water redistribution in the soil because they act as water sinks by favoring water infiltration and reducing evaporation compared to open areas (Berdugo *et al*., 2014; Pueyo *et al*., 2012; Valiente-Banuet and Ezcurra, 1991). This water redistribution promotes plant establishment and growth in vegetation patches to a greater extent than in open areas due to more extended water availability (Pueyo *et al*., 2016). Open areas are not entirely bare surfaces, but often shelter biological soil crusts (hereafter biocrusts) on top (Bowker 2007; Figure 2). Biocrusts consist of soil aggregates with cyanobacteria, algae, microfungi, lichens and bryophytes in different proportions (Belnap *et al*., 2003). Biocrusts have fundamental ecological roles in arid and semi-arid ecosystems through water retention, soil stabilization or nitrogen and carbon fixation (Belnap and Lange, 2001; Pietrasiak *et al*., 2013). This creates improved environmental conditions below biocrusts, positively influencing the establishment of specific plants by enhancing their germination, growth and nutrients uptake (Zhang and Belnap, 2015). Therefore, the presence of vegetation patches and biocrusts are important triggers for determining plant diversity and composition in arid and semi-arid communities (Bowker *et al*., 2011; López-Peralta *et al*., 2016; Michalet, 2006; Zhang and Belnap, 2015). Maintaining biodiversity has beneficial effects on ecosystem functioning because it sustains trophic interactions, increases nutrient retention, reduces the susceptibility to the invasion by alien species, and stabilizes ecosystems in response to human disturbances and abiotic changes, ensuring a stable supply of ecosystem goods and services (Maestre *et al.*, 2012).

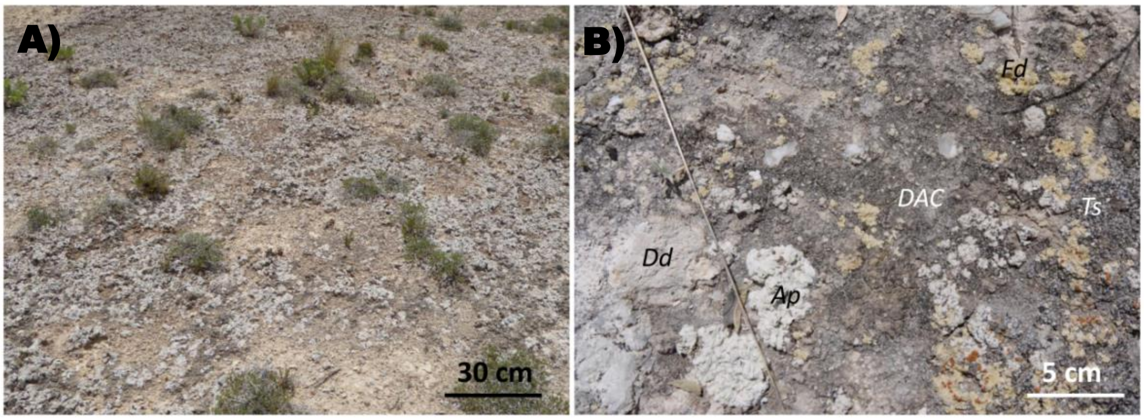


Figure 2 Biocrusts covering open areas in semiarid plant communities: **A)** Overview of open areas covered by biocrusts on gypsum soils in Roswell, New Mexico, USA; and **B)** Detail of the taxa that commonly compose biocrusts in gypsum outcrops in Leciñena, Zaragoza, Spain: cyanobacteria (*DAC*: dark algal crust) and the lichen species *Acarospora placodiiformis* (*Ap*), *Diploschistes diacapsis* (*Dd*), *Fulgensia desertorum* (*Fd*) and *Toninia sedifolia* (*Ts*). Photos: Ana Foronda.

Arid and semi-arid areas are highly vulnerable to degradation (*i.e.*, desertification) by climatic factors and human activities such as intensive farming or overgrazing (Geist and Lambin, 2004; Maestre *et al.*, 2016). Climate predictions corrected from CMIP5 (Fifth Coupled Model Intercomparison Project of the World Climate Research Programme) forecast an increment in global aridity (Huang *et al.*, 2016), thus increasing the risk of desertification in arid and semi-arid areas, which in many cases is irreversible (*e.g.*, Allington and Valone 2010). Recent studies estimate that about 25 % of arid and semi-arid areas are affected by desertification, with major environmental effects on soil erosion, reduction of vegetation cover, changes in plant community composition, and biodiversity and land productivity loss (D'Odorico *et al.*, 2013). Arid and semi-arid areas are inhabited by more than 38 % of the world's population (Reynolds *et al.*, 2007) whose well-being depends on the ecosystem services they provide, such as pastures for livestock (Mortimore *et al.*, 2009). Therefore, it is necessary to preserve the productivity and diversity of arid and semi-arid areas to avoid the rural exodus and global migrations driven by desertification (Mélanie, 2008). Huang *et al.* (2016) also predicted that the percentage of the Earth's area occupied by arid and semi-arid areas will rise to approximately 55 % by the end of this century. It is important to acquire a solid knowledge on the functioning of the ecosystems in arid and semi-arid areas for their proper management and conservation and for dealing more effectively with future changes (Maestre *et al.*, 2016).

Gypsum soils: a special substrate within arid and semi-arid areas

Gypsum soils are distributed in almost all the continents, occupying an area that exceeds 100 million ha (Boyadgiev and Verheye, 1996). Although belowground gypsum facies can occur in humid climates (Bąbel, 2012), gypsum soils persist almost exclusively in arid and semi-arid regions (Boyadgiev and Verheye, 1996). In these regions, the scarce rainfall prevents the leaching of the gypsum, $CaSO_4 \cdot 2H_2O$ (Porta, 1998), and the strong evaporative uplift mechanisms favor its accumulation in the upper soil horizons (Herrero *et al.*, 2009). Specifically, gypsum soils are mainly located in interior deposits of the North, Central and East Africa, South-central and North-central Asia, the Middle East, the Mediterranean Basin, South and West Australia and the Chihuahuan Desert region of North America (Boyadgiev and Verheye 1996; Figure 3).

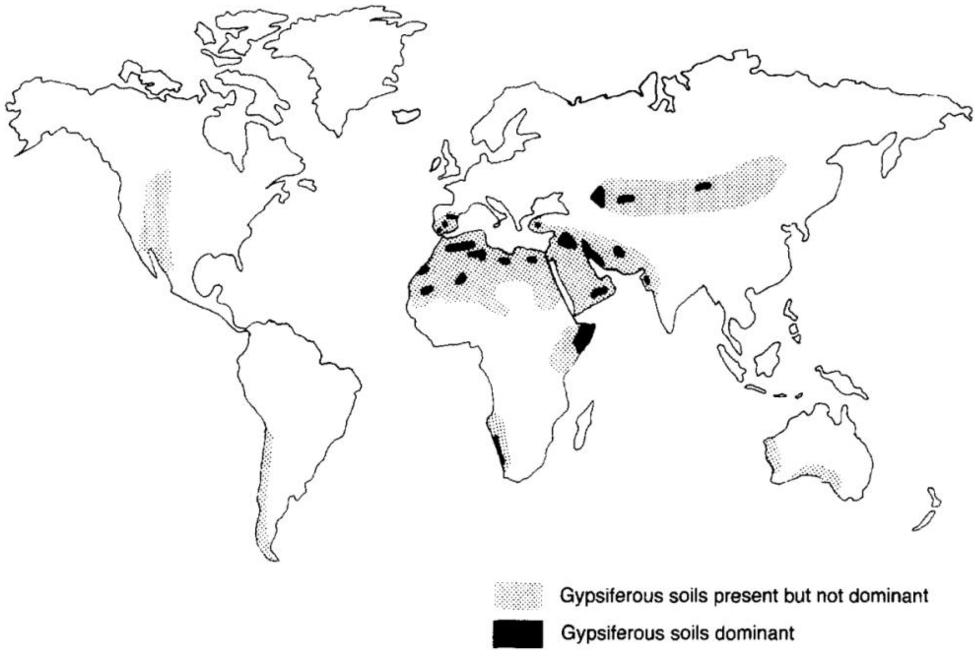


Figure 3 Occurrence of gypsum soils in the world. Source: Boyadgiev and Verheye (1996).

In Europe, surface gypsum outcrops are predominantly placed in semi-arid areas of Spain (0.3 % of the global surface gypsum outcrops; Casby-Horton *et al.* 2015), explicitly extending to the eastern half of the country and occupying approximately 4.2 % of the country area (Escavy *et al.* 2012; Figure 4). These outcrops appeared from

Triassic to Quaternary ages, being the most massive and abundant originated in endorheic basins or coast deposits during the Messinian Salinity Crisis (Escavy *et al.*, 2012; Rouchy and Caruso, 2006). Gypsum is not widespread in a continuum, but dots the territory in island-like outcrops surrounded by a matrix dominated by limestones, marls or clays (Escudero *et al.*, 2015).

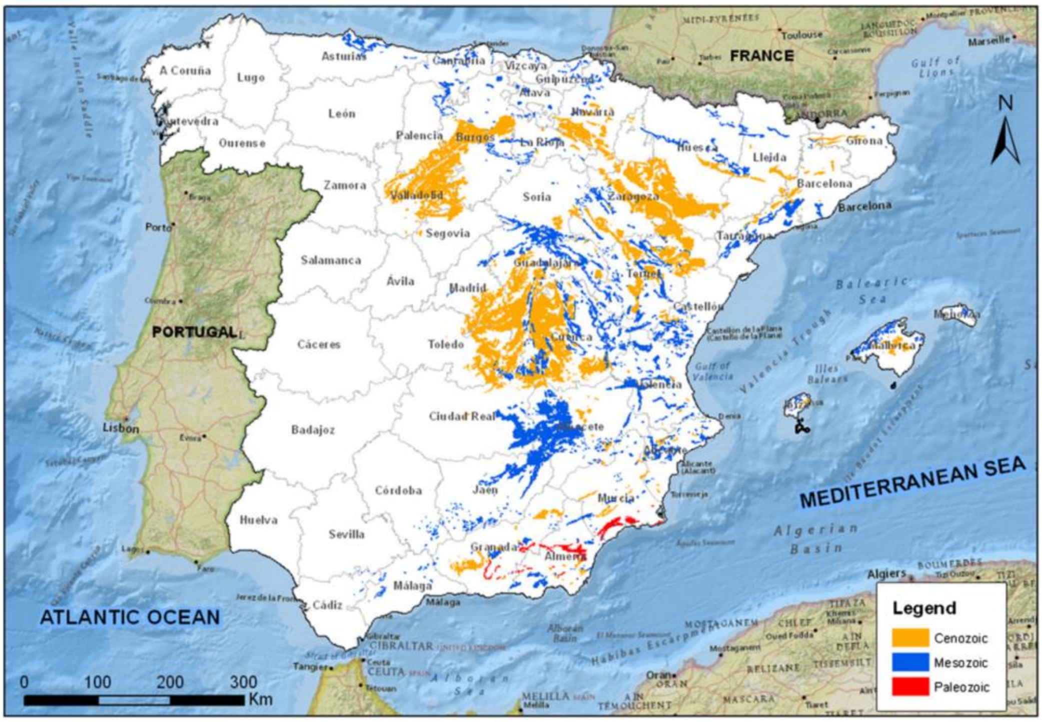


Figure 4 Occurrence of gypsum outcrops in Spain classified by age: Cenozoic in yellow, Mesozoic in blue and Paleozoic in red. Source: Escavy *et al.* (2012).

Gypsum can occur as geologic deposits mainly originated by chemical precipitation of sulfate calcium either from shallow hypersaline lagoons and inland seas (Schreiber and Tabakh, 2000) or from hot springs (Herrero *et al.*, 2009). Due to its high solubility, gypsum is also present as traces or dominating in soils either through pedogenic processes in gypsum bedrocks, or by the intermixing with the surrounding substrates (Eswaran and Gong, 1991; Porta, 1998). Gypsum soils (*i.e.*, gypsisols) are characterized by gypsum contents higher than 5% and the presence of a gypsic or petrogypsic horizon (FAO, 2006). The content of gypsum in soils is highly variable and may reach up to 99 % in massive gypsum outcrops (Herrero and Porta, 2000).

The presence of gypsum in the soil determines a stressful and extreme ecological environment for plant life (Escudero *et al.*, 2015). There are studies supporting that soil physical properties are the main limiting factors for plant development in gypsum substrates (*e.g.*, Parsons 1976). Gypsum soils are mechanically unstable due to the lack of plasticity, cohesion and aggregation of soil particles, hence being highly erodible (Casby-Horton *et al.*, 2015). Soil erosion affects plant establishment by removing seeds from the surface (García-Fayos *et al.*, 2010). In addition, gypsum soils have less defined microstructure than non-gypsum soils, presenting lower water retention capacity (Moret-Fernández and Herrero, 2015) that limits water availability for plants in the upper layers of the soil. Moreover, these soils encompass horizons with low porosity that restricts the penetration of plant roots (Guerrero-Campo *et al.* 1999). In addition, a typical process in gypsum substrates is surface physical crusting (Figure 5). Physical crusts are the consequence of uplift water movements that bring the dissolved gypsum to the surface, which recrystallizes as water evaporates (Badía-Villas and del Moral, 2016). These surface crusts are hard physical barriers that prevent or hinder seedling establishment and root penetration in the soil, resulting in adverse effects on vegetation (Romão and Escudero, 2005). However, physical surface crusts, together with the biocrusts frequent in arid and semi-arid areas (Bowker, 2007), can lead to the preservation of high moisture in low layers of the soil during drought (Badía-Villas and del Moral, 2016; Belnap and Lange, 2001; Meyer and Garcia-Moya, 1989), allowing plants to survive under extreme environmental conditions.

Other studies support that gypsum soils bear chemical limitations that hinder plant development (*e.g.*, Merlo *et al.* 1998). These soils have exceedingly high sulfate and calcium contents (Herrero and Porta, 2000), which can be toxic for plants at high concentrations in the cytoplasm (Hawkesford *et al.*, 2012; Ruiz *et al.*, 2003). Saturation of calcium in soils derives in low macronutrients availability, such as nitrogen or phosphorus (Merlo *et al.*, 1998; Pueyo *et al.*, 2007) due to their intensive substitution by calcium in the soil complex (Guerrero-Campo *et al.* 1999; Badía-Villas and del Moral 2016). Deficiencies of essential macronutrients can have consequences on the growth and morphology of the plants (Hermans *et al.*, 2006). However, given that some researchers experimentally found that gypsophilous and calcicolous plants germinated and grew equally with and without the addition of gypsum (Boukhris and Lossaint, 1975; Cañadas *et al*., 2014), physical limitations are likely to be the strongest determinants of plant development in gypsum soils.

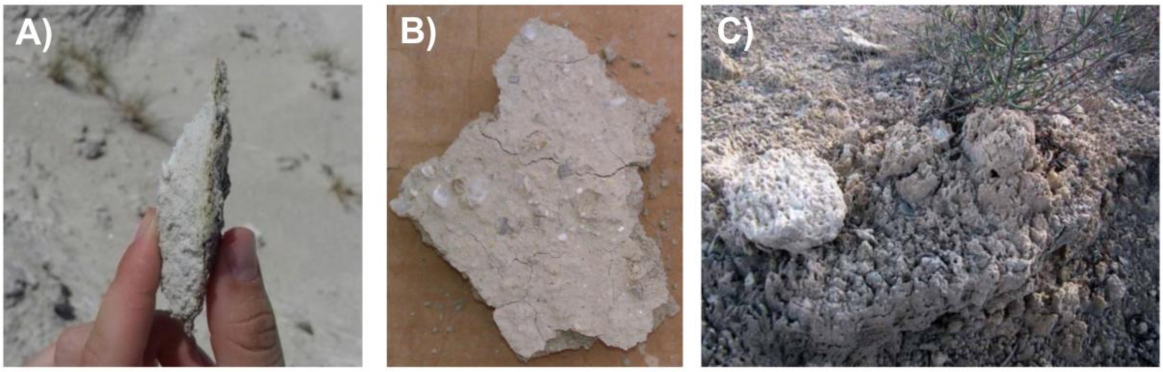


Figure 5 Surface crusting in gypsum substrates: **A)** Fragment of the surface physical crust formed in gypsum dunes of White Sands, New Mexico, USA (Photo: Ana Foronda); **B)** Fragment of the surface physical crust formed in gypsum spoils in a mine in Escúzar, Granada, Spain (Photo: Miguel Ballesteros); and **C)** Micro-reliefs on the surface crust caused by dissolution of the gypsum by the action of rains (Source: Mota *et al*., 2011).

Plants living on gypsum soils in arid and semi-arid areas

Gypsum outcrops are particular ecological environments because of both chemical and physical substrate limitations, but also due to the xeric climate inherent in these areas. Such stressful conditions make gypsum areas considerably unsuitable for plant growth, especially for trees, which are scarce on gypsum soils (Rivas-Martínez and Costa, 1970). Nevertheless, there are stress-tolerant plants that live on gypsum soils, being mostly subshrubs, short-lived perennials and annuals (Parsons, 1976). Following Meyer (1986), plants can be classified depending on the different degrees of linkage to gypsum in a) taxa that only live on gypsum (*i.e.*, gypsophytes or gypsum endemics), b) taxa that grow preferentially but not exclusively on gypsum (*i.e.*, gypsoclines), c) substrate generalist taxa that grow on and off gypsum (i.e., gypsovags), d) taxa that are rare on gypsum (*i.e.*, waif plants), and e) plants that never grow on gypsum (*i.e.*, gypsophobes). In this thesis, plants living on gypsum are classified as gypsophytes and gypsovags, the latter encompassing gypsoclines and gypsovags.

Plants living on gypsum soils have a range of strategies to survive under these harsh substrate and climatic conditions (Escudero *et al*., 2015). Gypsum-tolerant plants show physiological mechanisms to overcome the chemical limitations imposed by the exceedingly high concentrations of calcium and sulfate in the soil (Palacio *et al.* 2014a). The accumulator plants, which often present foliar succulence, sequester calcium and sulfate within cells in chemically unavailable forms, via calcium oxalate crystallization or gypsum recrystallization (Palacio *et al.* 2014a). Moreover, accumulator plants often gather nitrogen and phosphorus in the succulent leaves, hence making efficient use of these scarce macronutrients (Palacio *et al.*, 2007). Some other plants, primarily from halophilic lineages, can excrete these accumulated crystals through salt secretory glands in the leaves (Grigore *et al.*, 2011). There are assimilator plants that produce sulfur-rich secondary metabolites as glucosinolates or phytoalexins, hence detoxifying plant tissues from active sulfur compounds such as sulfates (Rausch and Wachter, 2005). Finally, some plants avoid the effect of gypsum by controlling the ionic acquisition by roots through the presence of arbuscular mycorrhizal fungi (Alguacil *et al.*, 2012). Arbuscular mycorrhizal fungi can induce the accumulation of sulfate and nutrient uptake in roots by enhancing the activity of soil enzymes in the rhizosphere, which additionally allows their survival on nutritionally impoverished soils (Khabou *et al.*, 2014; Rao and Tak, 2001).

Plant mechanisms to cope with chemical restrictions in gypsum soils might include ancestor adaptations to other stressors, rather than traits that have evolved specifically in plants living on gypsum soils (Meyer, 1986). For example, the accumulation of calcium oxalate crystals or secondary sulfur-rich compounds in leaves may be a defense against herbivory or pathogens held by the ancestors (Rausch and Wachter, 2005; Ruiz *et al.*, 2002). Plants living on gypsum soils could take advantage of these pre-adaptations of their lineages to manage the excess of calcium and sulfate in the soil, becoming gypsum-adapted species or gypsum tolerant species. In general, widely distributed gypsophytes show significant adaptations to gypsum (specialist model *sensu* Palacio *et al.*, 2007) by an evident ability to accumulate gypsum crystals (Palacio *et al.*, 2014a). However, narrowly distributed gypsophytes and gypsovags generally behave in a different way concerning the avoidance of gypsum toxicity, which is manifested in a regulation of gypsum uptake (Palacio *et al.*, 2014a) rather than substantial adaptations to gypsum soils (refuge model *sensu* Palacio *et al.* 2007).

Likewise, plants living on gypsum soils show traits typical of plants adapted to water deficit, such as the aforementioned foliar succulence (Figure 6) or the development of deep-rooted tap roots (Grigore *et al.*, 2011; Guerrero-Campo *et al.*, 1999a; Parsons, 1976). These are common adaptations to gypsophytes and gypsovags; however, a highly specialized mechanism to use an alternative water source during severe summer drought (*i.e.*, gypsum crystallization water) has been found in the gypsophyte *Helianthemum squamatum* (L.) Pers. (Palacio *et al.* 2014b). Furthermore, and particularly in dry Mediterranean gypsum areas, plants show adaptations to the seasonality and uncertainty of the climate. These plants present a marked seasonal dimorphism in leaves of the same individual (Palacio *et al.*, 2006), the presence of naked sprouts that enables opportunistic growth under unpredictable favorable conditions (Montserrat-Martí *et al.*, 2011), variability in seed dormancy (Escudero *et al.*, 1997), and a delay in the reproductive peak, which occurs in early summer (Aragón *et al.*, 2008). The high environmental stochasticity of arid and semi-arid areas (Noy-Meir, 1973) leads to the presence of short-lived plants, whose success relies on a high reproductive output at the expense of survival (Aragón *et al.*, 2009). In addition, short-lived plants often endure in the community in a dormant state and so the dynamics and persistence of gypsum plant communities through time often rely on the formation of large seed banks (Aragón *et al.*, 2008; Caballero *et al.*, 2003).

Although both gypsophytes and gypsovags are tolerant to gypsum conditions, gypsophytes are generally better able to overcome the physical limitations inherent in gypsum soils, especially at germination and establishment stages (Escudero *et al.*, 2015, 2005, 2000, 1999). For example, seeds of the gypsophyte shrub *H. squamatum* have a mucilaginous coating that is hygroscopic, therefore helping their anchorage in gypsum surface crusts and generating a more favorable environment for germination (Escudero *et al.*, 1999). Moreover, roots from seedlings of the gypsophytes *H. squamatum* and *Lepidium subulatum* L. can penetrate the hard surface crust enabling their clinging to the substrate (Romão and Escudero, 2005). These abilities may allow gypsophytes take selective advantages over gypsovags, which generally establish only in microsites where physical crusts are weaker (Romão and Escudero, 2005).

Figure 6 Foliar succulence of plants living on gypsum soils: **A)** *Nerisyrenia linearifolia* (S. Wats.) Greene from the Chihuahuan Desert, New Mexico; **B)** *Acleisanthes lanceolata* (Wooton) R.A. Levin from White Sands, New Mexico; **C)** *Ononis tridentata* L. subsp. *crassifolia* (Dufour ex Boiss.) Nyman from El Temple county, SE Spain (Photos: Ana Foronda); and **D)** *Coris hispanica* Lange from Almería province (Source: Mota *et al.*, 2011).

Arid and semi-arid gypsum ecosystems as biodiversity hotspots

In general, gypsum environments present the typical vegetation structure of arid and semi-arid areas, where the stressful conditions result in low plant productivity and coverage, shaping a two-phase community structured in vegetation patches dominated by shrubs interspersed with open areas devoid of perennial vegetation (Maestre and Cortina, 2005). Given their steppe-like appearance (Figure 7), arid and semi-arid gypsum ecosystems have historically been considered barren areas (Mota *et al.*, 2011; Pedrocchi Renault, 1998). However, gypsum environments are species-rich systems with active plant speciation (Merlo *et al.*, 1998; Rivas-Martínez and Costa, 1970), probably driven by the selection of specific traits under stressful substrate and climatic conditions, together with the island-like distribution of gypsum outcrops (Kruckeberg, 1986; Rajakaruna, 2004). Indeed, gypsum environments often harbor a highly diverse and unique flora, including numerous endemic taxa that largely contribute to local or regional floristic diversity (Cerrillo *et al.*, 2002; Dominguez Lozano *et al.*, 2005; Martínez-Hernández *et al.*, 2011; Mota *et al.*, 2011). Mainly, species-rich gypsum floras exist in the Chihuahuan Desert, Somalia, Ethiopia, Turkey, and Spain, with fewer gypsum species in Iran, North Africa, Australia, the Mojave Desert, Cyprus, and Yemen (Moore *et al*., 2014). Despite the highly diverse floras in gypsum areas, gypsum vegetation has been poorly studied in most regions of the world, especially compared to serpentine and halophilic vegetation (Escudero *et al*., 2015).

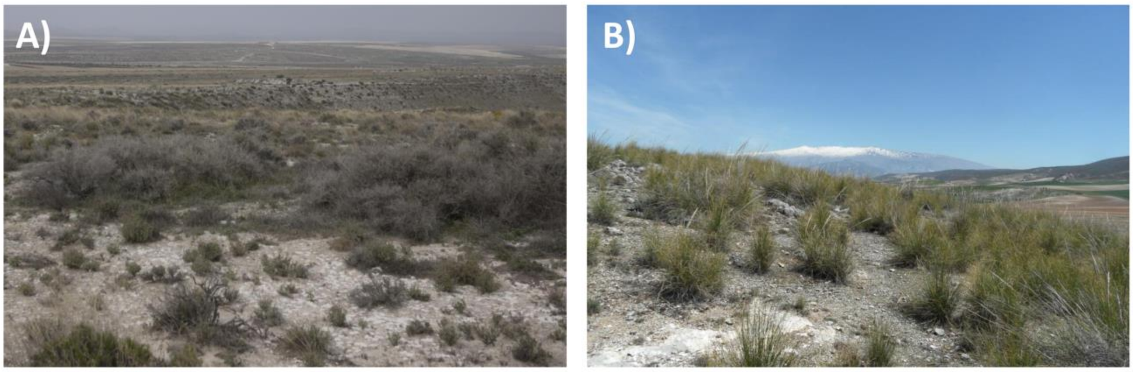


Figure 7 Overview of gypsum plant communities in Spain: **A)** Mediana de Aragón in Zaragoza province (Photo: Ana Foronda); and **B)** Escúzar in Granada province (Photo: Eva Cañadas).

Particularly in Spain, 140 taxa live on gypsum soils (Escudero *et al*., 2015), being 77 gypsum endemics, many of them threatened (50 %; Mota *et al*. 2011). Some of these species have been considered in conservation policies at the national and regional scale (*e.g*., *Senecio auricula* Bourgeau ex Cosson; Moreno 2010; Gobierno de Aragón RD 181/2005). Further, Martínez-Hernández *et al*. (2011) have made an attempt to use gypsum floras as a criterion to set wildlife reserves. Specifically, Iberian gypsum ecosystems (denoted as *Gypsophiletalia*) are considered as a priority for conservation in the European Directive for Conservation of Habitats and Wild Fauna and Flora (92/43/CEE; European Commission 1992). The European Directive defines these ecosystems as open scrubs developed on gypsum-rich soils from the Iberian Peninsula and characterized by the presence of gypsum-specialist plants, phytosociologically corresponding to the syntaxa *Lepidion subulati*, *Gypsophilion hispanicae* and *Thymo-Teucrion verticillati* (Escudero 2009; Mota *et al*. 2011). There are approximately 30 taxa indicative of *Gypsophiletali*a, among which the most common species are the gypsophyte shrubs *Gypsophila struthium* Loefl. (comprising two subspecies: *G. struthium* Loefl. subsp. *hispanica* (Willk.) G. López and *G. struthium* L. subsp. *struthium*), *Ononis tridentata* L. (comprising three subspecies: *O. tridentata* L. subsp.

tridentata, *O. tridentata* subsp. *angustifolia* (Lange) Devesa et G. López, and *O. tridentata* subsp. *crassifolia* (Dufur ex Boiss.) Nyman), *Helianthemum squamatum* (L.) Pers., *Lepidium subulatum* L., *Herniaria fruticosa* L., *Launea fragilis* (Asso) Pau, *Launaea pumila* (Cav.) O. Kuntze, and *Frankenia thymifolia* Desf. (Loidi and Costa, 1997; Mota *et al*., 2011). Plant species belonging to *Gypsophiletalia* coexist with species non-exclusive to gypsum soils (*i.e.*, gypsovags), which are frequently basophilic plants (Escudero, 2009). Widespread Mediterranean shrubs such as *Rosmarinus officinalis* L. or *T. vulgaris* L., and perennial grasses such as *Stipa parviflora* Desf., *S. tenacissima* L. or *Brachypodium retusum* (Pers.) Beauv. are some of the most common gypsovags that live on gypsum soils of the Iberian Peninsula. Standing with these scrublands, there are communities of annuals with numerous gypsum specialists such as *Campanula fastigiata* Dufour ex A. DC, or *Reseda stricta* Pers., and cryptogamic communities with a great diversity of bryophytes and lichens (Mota *et al*., 2011).

Biotic modulators of semi-arid gypsum plant communities: plant-plant interactions

Plant-plant interactions are key ecosystem components (Brooker, 2006). The effect that one individual plant has on neighboring plants, being of the same species or from different species, strongly influences plant and functional diversity, community structure and dynamics, and ecosystem productivity and resilience (Hooper *et al*., 2005; Michalet, 2006; Volaire *et al*., 2014). Both positive and negative interactions play important roles in the net balance of interactions among plants at the community level (Callaway and Walker, 1997).

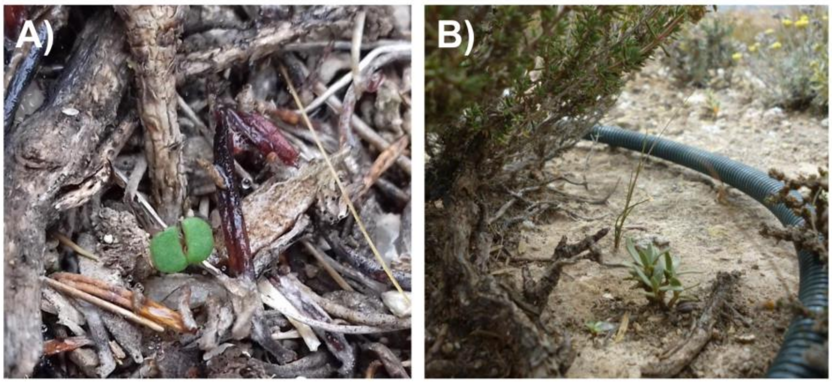


Figure 8 Seedling establishment in gypsum plant communities: **A)** Seedling of *Rosmarinus officinalis* emerging among the leaf litter beneath a shrub canopy; and **B)** Juveniles of *Helianthemum squamatum* and *Stipa sp*. established under the canopy of *Thymus vulgaris*. Photos: Ana Foronda.

In arid and semi-arid environments, where plants are submitted to high environmental stress, positive interactions are essential for plants to persist (Bertness and Callaway, 1994). Positive interactions (*i.e.*, facilitation) consist of beneficial effects of one plant on the establishment, survival or growth of a neighboring plant (Callaway, 2007a). Facilitation is especially relevant for plants in early life stages, when they are highly vulnerable to environmental stress, benefiting of the presence of well-established plants to germinate and survive (Escudero *et al.* 2005; Figure 8). Facilitator plants, which are also known as nurse plants, are often stress-tolerant species (in particular shrubs) that act as ecosystem engineers, modifying the microenvironment in their vicinity (Jones *et al.*, 1994). For example, shrubs provide shade, diminishing solar radiation, attenuating extreme temperatures and decreasing water evaporation (Callaway, 2007a). Besides, shrubs release leaf litter whose decomposition provides nutrients and organic matter that, in turn, improves soil structure (Callaway, 2007a). Soil structure improvement leads to lower surface compaction and higher water infiltration that, together with the lower evaporation and plant hydraulic lift, increase water availability in the local vicinity of shrubs (Dawson 1993; Callaway 2007a; Figure 9). The effect of nurse shrubs as ecosystem engineers are influenced by the morpho-anatomical attributes of these shrubs (Tewksbury and Lloyd, 2001). Ecosystem engineers can reduce environmental stress under their canopy and thus expand the niche of less stress-tolerant species, which establish in the local vicinity thus increasing species richness in the community (Gómez-Aparicio *et al.*, 2005; Michalet, 2006; Soliveres *et al.*, 2011). Together with the enhancement of the microenvironment, nurses can increase plant survival by protecting seedlings, juveniles and even adults from grazing and trampling, especially unpalatable nurse plants (Callaway *et al.*, 2005; Callaway, 2007b; Smit *et al.*, 2006). Additionally, nurses can shape rich seed banks by physically obstructing seeds, protecting them from predators (*e.g.*, granivorous birds), acting as perches for seed-dispersal birds or acting as pollinator magnets (Bullock and Moy, 2004; Caballero *et al.*, 2008; Molina-Montenegro *et al.*, 2008; Pausas *et al.*, 2006; Smit *et al.*, 2008).

Figure 9 Soil water accumulation (brown halo) in vegetation patches in the gypsum dunes of White Sands, New Mexico, USA. Photo: Ana Foronda.

In gypsum environments, gypsophytes can establish and survive under gypsum stressful conditions with no need for a nurse plant (Escudero *et al.*, 2000; Romão and Escudero, 2005), thus appearing as pioneer plants in gypsum plant communities (Dana and Mota, 2006). Contrarily, gypsovags are more vulnerable to gypsum stressful conditions than gypsophytes in early life stages, and often benefit from the presence of nurse plants to establish and survive in gypsum plant communities (de la Cruz *et al.*, 2008a). Regarding the role of facilitator plants, some studies observed that similarly to other stress-adapted species (*e.g.*, to serpentines; Oviedo *et al.*, 2014), well-established individuals of the gypsophytes O. tridentata and G. struthium acted as ecosystem engineers and provided favorable microsites for the establishment of less stress-adapted species (Navarro-Cano *et al.*, 2016, 2015, 2014). As a consequence, gypsophyte shrubs may play a fundamental role in maintaining plant diversity in the community.

Together with positive interactions, negative interactions (*i.e.*, interference) are widely acknowledged as decisive in the relative dominance of species in plant communities (Brooker, 2006). Competition is also of great importance in arid and semi-arid areas, where water and nutrients are scarce resources, strongly affecting ecosystem functioning, the relative abundance of species and plant distribution patterns (Fowler, 1986; Goldberg and Barton, 1992). Both facilitation and interference operate simultaneously and bidirectionally between pairs of neighboring species (Holzapfel and Mahall, 1999). The role that each interaction play in arid and semi-arid ecosystems is disputed in the literature because both take part in plant communities in complex combinations that depend on the species involved and the abiotic conditions (Bertness and Callaway, 1994; Brooker, 2006; Liancourt *et al.*, 2005; Maestre *et al.*, 2005; Michalet, 2006). As mentioned, plants in arid and semi-arid communities take advantage of the presence of nurse plants at the first life stages (de la Cruz *et al.*, 2008a; Escudero *et al.*, 2005; Romão and Escudero, 2005), leading to positive interaction outcomes between nurse and facilitated plants. However, once the facilitated seedlings become adults, they may no longer depend on nurse plants to survive, and even could gain a competitive advantage over the latter (Armas and Pugnaire, 2009; Miriti, 2006). Facilitated plants can reduce the fitness of nurse plants, and can derive in their exclusion, especially in resource-scarce environments (Armas and Pugnaire, 2009; Miriti, 2006; Schöb *et al.*, 2014). This exclusion may be caused by competition via the shortage of resources like water, nutrients or light, the decline in pollinator availability, or the physical obstruction by space occupancy (Schöb *et al.*, 2014; Weiner, 1990). In addition, facilitated plants that successfully reach adult stage might eventually reduce the survival of other facilitated plants, reversing the benefits of the nurse plant (Paterno *et al.*, 2016; Soliveres *et al.*, 2010) thus leading to negative interaction outcomes. Moreover, nurse plants might harm the facilitated plants as they become larger due to the overlapping of their requirements and tolerances, which can result in a competitive advantage of the nurses (Miriti, 2006; Tielbörger and Kadmon, 2000).

Competition is not the only negative interaction that determines interference, but allelopathy also has a conspicuous role in interaction outcomes at the community level (Arroyo *et al.*, 2015). This interaction consists of the release by a plant of phytotoxic compounds that negatively influence the establishment and growth of neighboring plants (Rice, 1984). The most common phytotoxic compounds are aromatics such as terpenes and phenolic compounds (Inderjit, 1996; Langenheim, 1994) that are released through various mechanisms: volatilization, leaching, root exudates and decomposition of plant residues as leaf litter (Bertin *et al.*, 2003; Inderjit and Duke, 2003; Zhang and Fu, 2010). Although this chemically mediated interference does not involve direct competition for limited resources, it is considered an indirect competition by affecting the performance of potential competitive neighboring plants (Novoplansky 2009). In arid and semi-arid areas, chemical interference plays a prominent role in interactions outcome due to the great abundance of plants releasing aromatic compounds (Barney *et al.*, 2008; Tarayre *et al.*, 1995; Vilà and Sardans, 1999). Environmental stress, such as increased aridity or nutrients scarcity, induces the production of phytotoxic compounds and intensifies plant sensitivity to these compounds (Pedrol *et al.*, 2006). Moreover, since rainfall is low, these compounds are accumulated in the soil and therefore the effect on plant development exacerbates due to more prolonged exposure (Chaves *et al.*, 2003). It is difficult to discern the effects of competition for resources from the effect of chemical interference under field conditions (Inderjit and Callaway, 2003). The phytotoxic effect of certain plant species has been widely studied under controlled conditions, but few studies have been conducted taking into account the simultaneity with other biotic interactions in natural communities (Ridenour and Callaway, 2001).

Although plant-plant interactions have widely been evaluated considering the effect of a single species or the interaction between a pair of species (*e.g.*, Armas and Pugnaire 2005), the study of interactions among all the species constituting the community is limited and in recent years is acquiring more attention (*e.g.*, Maestre *et al.* 2008). The effect of a single species on the development of other species can be easily assessed experimentally, but conducting field experiments to explore interactions at the community level is logistically laborious (Schöb *et al.*, 2012). Since biotic interactions in plant communities occur between individuals closely located in space, plant spatial patterns can be a good proxy to infer plant-plant interactions outcome in the whole community (Alados *et al.*, 2006; Arroyo *et al.*, 2015; Gross *et al.*, 2013; Saiz and Alados, 2012; Tirado and Pugnaire, 2005). Given that plants are sessile organisms, when species aggregate in space more often than expected by chance, a dominance of positive biotic interactions among them can be assumed (Tirado and Pugnaire, 2003) and would be reflected in a patchy spatial pattern (Figure 10A). Conversely, when species co-occur in space less often than expected by chance, it can be interpreted as dominance of negative interactions (Tirado and Pugnaire, 2003). Then, competition for resources or allelopathy would be reflected in a more segregated spatial pattern in the community (Figure 10B). Finally, the presence of haphazardly plant distribution (neither aggregated nor segregated plant distributions) can represent either the absence of interactions among species or, most probably, a neutral outcome between positive and negative interactions (Figure 10C).

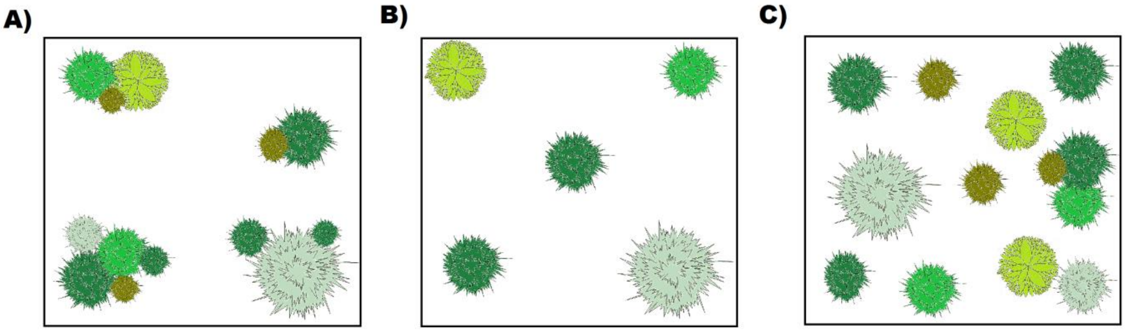


Figure 10 Sketch of the plant spatial patterns resulting from plant-plant interactions: **A)** Multi-specific patchy structure driven by positive interaction outcomes; **B)** Segregated structure driven by negative interaction outcomes; and **C)** Neutral interaction outcomes (haphazardly structured).

Nevertheless, inferring the interaction outcomes at the community level based on observational data is controversial because plant spatial patterns are the result not only of biotic interactions but also of other biotic and abiotic factors acting simultaneously (Ramón *et al*., 2018; Rubio and Escudero, 2000). Other mechanisms capable of generating a patchy spatial pattern can be the patched distribution of favorable resources, the micro-topography, or seed dispersal and trapping by physical attributes (Aguiar and Sala 1997; Caballero *et al.* 2008). These mechanisms, together with positive interactions, promote feedback processes that reinforce the island-like spatial distribution of vegetation in arid and semi-arid ecosystems (Aguiar and Sala, 1999).

Interactions outcome in plant communities may change depending on the species involved and also along environmental gradients (Bertness and Callaway, 1994; Liancourt *et al*., 2005). In the framework of a future increase in aridity (Huang *et al*., 2016), it is important to evaluate the effects of changes in environmental stress on plant interactions that, in turn, will affect ecosystem structure and dynamics (Brooker, 2006). The stress gradient hypothesis (SGH) predicts an increase in facilitation along an increase in stressful environmental conditions and the dominance of competition for resources under moderate stress conditions (Bertness and Callaway, 1994). However, the response of biotic interactions at the extreme of the aridity gradient it is not clear yet, with some studies finding support for SGH (López *et al*., 2016) and others suggesting a shift towards interference (Maestre *et al.*, 2005). For example, some studies predict that competition for below-ground resources would simultaneously increase under more stressful conditions (Pugnaire and Luque, 2001), and others predict more intense allelopathic effects associated with increased aridity (Pedrol *et al.*, 2006).

Threats to Iberian gypsum ecosystems

Similarly to other arid and semi-arid ecosystems, the future of gypsum ecosystems is under the pressure of global change (Escudero *et al.*, 2015). Global temperature increases and rainfall decreases in arid and semi-arid areas can exert adverse effects on the functioning of these ecosystems (Maestre *et al.*, 2013). Specifically, Vicente-Serrano *et al.* (2012) observed in a semi-arid Mediterranean gypsum area that the degradation processes derived from the past overexploitation of the ecosystems were accelerated under higher water-stress. Together with climate change, anthropogenic activities such as intensive agriculture, livestock overgrazing, urban development, communication networks, afforestation, and mining represent threats to gypsum ecosystems (Figure 11). These anthropogenic disturbances cause the fragmentation, loss, and degradation of gypsum habitats (Ballesteros *et al.*, 2013; Cañadas *et al.*, 2010; Lázaro-Nogal *et al.*, 2012; Luzuriaga *et al.*, 2018; Mota *et al.*, 2011; Pueyo *et al.*, 2008). Since gypsum habitats have historically been interpreted as degraded areas (Mota *et al.*, 2011), there are very few studies on the sensitivity of these ecosystems to both environmental and anthropogenic threats.

Socio-economic activities in gypsum areas of Spain have traditionally been related to agro-pastoral land uses. Intermediate levels of grazing (typically consisting of sheep livestock; *e.g.*, Pueyo and Alados 2007) can have an indispensable role in the maintenance of pasture productivity and plant diversity because animals mostly feed on the dominant plant species, diminishing competitive exclusion (Smith and Rushton, 1994) and break the surface soil crust, reducing surface sealing (Pueyo *et al.*, 2012). However, overgrazing can imply severe degradation of pastures, shown in the diminishing of plant cover and species diversity and the threat over some rare gypsophytes (Pueyo *et al.*, 2008, 2006). Plowing activities to grow crops in these areas also degrade natural gypsum vegetation, having adverse effects on the conservation of gypsum endemics (*e.g.*, *Ononis tridentata* subsp. *crassifolia*; Ballesteros *et al.* 2013). Moreover, plowing in such gypsum soils promotes rapid soil erosion (Faulkner *et al.*, 2003). Inappropriate farming practices, such as traditional irrigation (*i.e.*, overwatering), endanger the quality of the substrate resulting in soil salinization (Caballero *et al.*, 2001), which determines a low crop efficiency and the subsequent land abandonment (Cañadas *et al.*, 2010). Although crop abandonment leads to the recovery of the former community through secondary succession, it is a prolonged process due to the low productivity related to the edaphic particularities of gypsum together with the salinization and erosion (Cañadas *et al.*, 2010; Lasanta *et al.*, 2000).

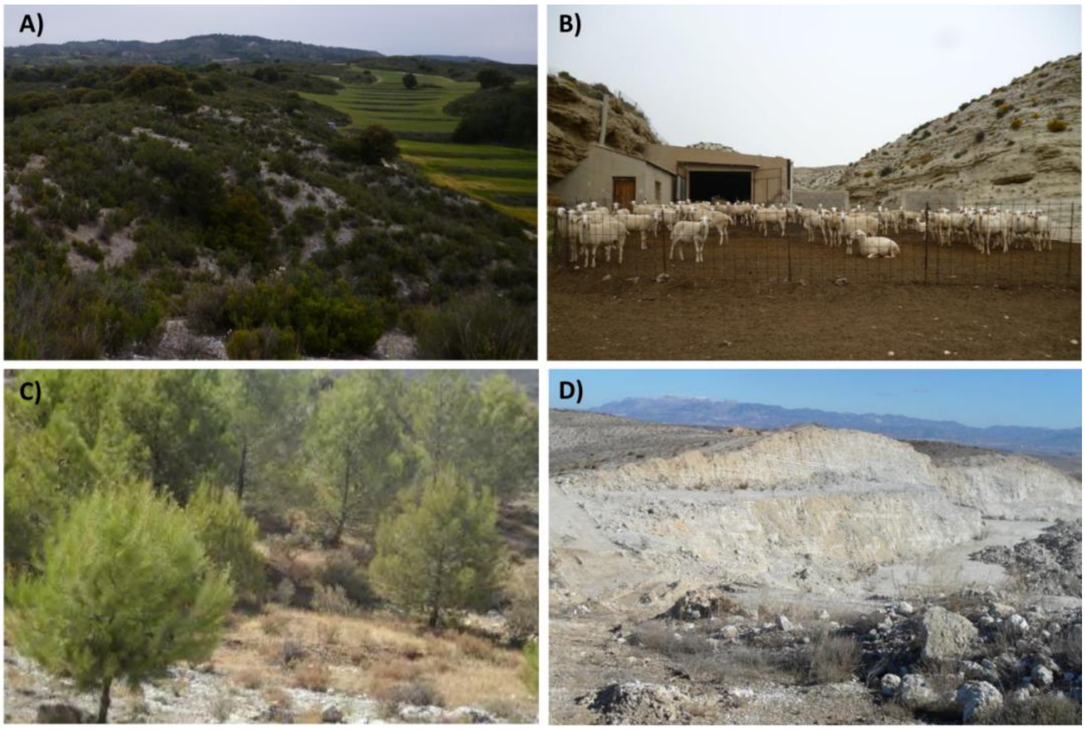


Figure 11 Human activities that disturb gypsum plant communities: **A)** Cereal crops in bottom valleys in Leciñena, Zaragoza, NE Spain (Photo: Ana Foronda); **B)** Sheep livestock farm in Mediana de Aragón, Zaragoza, NE Spain (Photo: Ana Foronda); **C)** Afforestation with *Pinus halepensis* Mill. in Ventas de Huelma, Granada, SE Spain (Photo: Miguel Ballesteros); and D) Gypsum mineral quarrying in Escúzar, Granada, SE Spain (Photo: Miguel Ballesteros).

One of the most severe disturbances to gypsum ecosystems is mining (Ballesteros *et al.*, 2013; Mota *et al.*, 2004). The extraction of the gypsum mineral is relevant in Spain, one of the six greatest producers of gypsum in the world according to the British Geological Survey (2012-2016). Mining often inflicts severe impacts on gypsum ecosystems by removing the vegetation, altering the topography and generating barren infertile substrates due to the topsoil removal (Bradshaw, 1997). Gypsum mines also have a substantial visual impact because they consist of opencast mining that produces long-lasting alterations in the landscape (Mota *et al*., 2004). Additionally, gypsum mining seriously endangers plant diversity because of direct destruction of the vegetation that in some cases includes rare and endemic plants (Ballesteros *et al*., 2013; Mota *et al*., 2004). Natural vegetation recovery in arid and semi-arid areas may require several decades to reach the pre-disturbance state (Dana and Mota, 2006) due to the harsh climatic conditions, the degraded substrate, and the fragmented landscape (Bradshaw, 1997; Kirmer *et al*., 2008; Pueyo and Alados, 2007). Natural plant succession depends on seeds arrival from well-conserved habitat fragments (Kirmer *et al*., 2008), which is often limited in gypsum areas due to the usual small size and low connectivity of habitat fragments (Pueyo and Alados 2007; Matesanz *et al*. 2009; Figure 12) and the low dispersal abilities of gypsophytes (Martínez-Duro *et al*., 2010). Together with the limited seed arrival from habitat fragments, post-mining substrates present resource-scarce conditions and physical instability that prevent plant development (Bradshaw, 1997). Therefore, spontaneous vegetation recovery driven by natural plant succession is critical in gypsum mines.

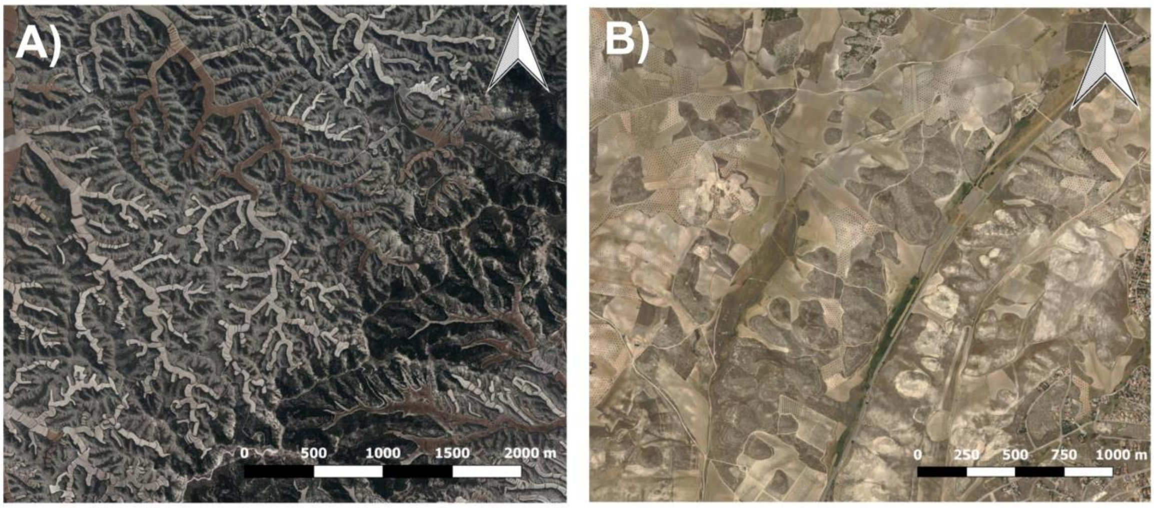


Figure 12 Orthophotos showing the fragmentation of gypsum habitat by agriculture in **A)** Valmadrid, Zaragoza, NE Spain; and **B)** Villaconejos, Madrid, central Spain. Source: PNOA- Imagery from Instituto Geográfico Nacional.

Gypsum extraction remains a primary economic resource, and hence there is a conflict of interest between the conservation of these ecosystems and socio-economic benefits (Mota *et al*., 2004). Impacts of mining on gypsum ecosystems can be repaid by identifying areas with well-conserved communities and then design wildlife reserves for the conservation of the species potentially endangered by this human activity (Mota *et* *al*., 2011). In addition, regarding already disturbed gypsum areas, ecological restoration plans are needed to reach the pre-disturbance state. To provide technical solutions for the ecological restoration of degraded gypsum plant communities, a better understanding of the biotic and abiotic factors that structure diversity in these communities is fundamental. However, few studies have addressed the ecological restoration of gypsum environments (Ballesteros *et al*., 2017, 2012; Dana and Mota, 2006; Martínez-Duro *et al*., 2009; Matesanz and Valladares, 2007). Active restoration strategies such as plantations have been demonstrated to be effective in the medium-short term in these ecosystems (Ballesteros *et al*., 2014); however, promoting plant succession might reduce the time and economic costs of vegetation recovery (Byers *et al*., 2006). Considering nurse plants in restoration plans has proven to be a valuable tool for improving plant establishment, and hence for promoting vegetation recovery in semi-arid plant communities (Gómez-Aparicio *et al*., 2004; Padilla and Pugnaire, 2006).

Justification for the research

Despite the relevance of gypsum outcrops for biodiversity conservation due to the highly diverse flora harbored (Mota *et al*., 2011), gypsum vegetation is under-represented in the scientific literature compared to other edaphic specialists (Escudero *et al*., 2015). Most scientific efforts on gypsum outcrops have been focused on studying diversity, phytosociology and distribution of gypsicolous flora (Braun-Blanquet and Bolòs, 1958; Martínez-Hernández *et al*., 2011; Rivas-Martínez and Costa, 1970). Physiological studies to determine gypsophily and the mechanisms behind the strategies of gypsum tolerance have also been extensively developed (*e.g*., Parsons 1976; Meyer 1986; Merlo *et al*. 1998; Palacio *et al*. 2007). Nevertheless, there is a recent research interest in other features of gypsum vegetation, such as the ecology of the community (*e.g*., Escudero *et al*. 1999; Romão and Escudero 2005; Pueyo *et al*. 2007; Caballero *et al*. 2008; de la Cruz *et al*. 2008a; Saiz *et al*. 2014), the evolution (*e.g*., Matesanz *et al*., 2018; Moore *et al*., 2014) or the restoration of degraded areas (*e.g*., Dana and Mota 2006; Matesanz and Valladares 2007; Martínez-Duro *et al*. 2009; Ballesteros *et al*. 2014; Ballesteros *et al*. 2017).

Although gypsum ecosystems are unique and are of great importance for global biodiversity conservation, anthropogenic activities together with climatic variations cause their loss and degradation (Escudero *et al.*, 2015; Vicente-Serrano *et al.*, 2012). It is necessary to acquire a solid knowledge of the factors that affect composition and structure in gypsum ecosystems to develop effective conservation and restoration plans, anticipating future changes in ecosystems (Maestre *et al.*, 2016). Plant-plant interactions are important for the functioning of arid and semi-arid gypsum ecosystems by increasing diversity, productivity, and resilience of plant communities (Michalet, 2006; Volaire *et al.*, 2014). Although there are studies about biotic interactions occurring in gypsum outcrops at the species level (*e.g.*, Escudero *et al.* 2000; de la Cruz *et al.* 2008b), biotic interactions at the community level remain largely unknown. It is necessary to identify key species that exert positive interactions in the community (for example by trapping seeds or by enhancing micro-environmental conditions for plant establishment) to protect them and employ them in restoration plans.

Objectives and hypotheses of the thesis

The general objective of the thesis was to investigate the role of gypsophyte and gypsovag shrubs in structuring plant diversity in gypsum plant communities to identify key species for the conservation and restoration of gypsum ecosystems. This general objective can be subdivided into more specific objectives:

Objective 1: To understand the patterns of plant establishment and to identify key nurse species in gypsum plant communities. Specifically, assessing whether or not plants need to be facilitated and evaluating the role of gypsophyte and gypsovag shrubs in facilitating the establishment and development of other plants and thus in structuring plant diversity in these communities.

> **Hypothesis 1.1:** While gypsophytes might establish in open areas because they overcome harsh conditions inherent in gypsum soils, gypsovags would need to be spatially associated with standing adult shrubs in early life stages to establish and survive to an adult stage.
>
> **Hypothesis 1.2:** Shrubs may act as ecosystem engineers by ameliorating micro-environmental conditions under their canopy compared to open areas, facilitating plant establishment underneath. Gypsophyte shrubs could facilitate plant establishment to a greater extent than gypsovag shrubs because they are better-adapted species that establish as the pioneer, then promoting new

vegetation patches, and do not need to interfere with potential competing plants to persist in gypsum plant communities.

Hypothesis 1.3: The hypothesized importance of positive interactions at the community level would be reflected in the spatial structure of gypsum plant communities. The notable facilitation mediated by gypsophytes might result in diversity aggregation in species-rich patches around them.

Objective 2: To assess the potential role of different shrub species with diverse physiognomy in determining the spatial structure of the soil seed bank in gypsum plant communities. The aim was focused on the seed sink and seed source roles of gypsophyte and gypsovag shrubs with different size and architecture.

Hypothesis 2.1: Independently of the degree of linkage to gypsum, shrubs may intercept and accumulate seeds in their local vicinity, acting as seed sinks and hence spatially structuring abundant soil seed banks around them. Tall shrubs may intercept more wind-dispersal seeds than short shrubs, and cushion-like shrubs may accumulate more seeds through crawling branches than erect shrubs.

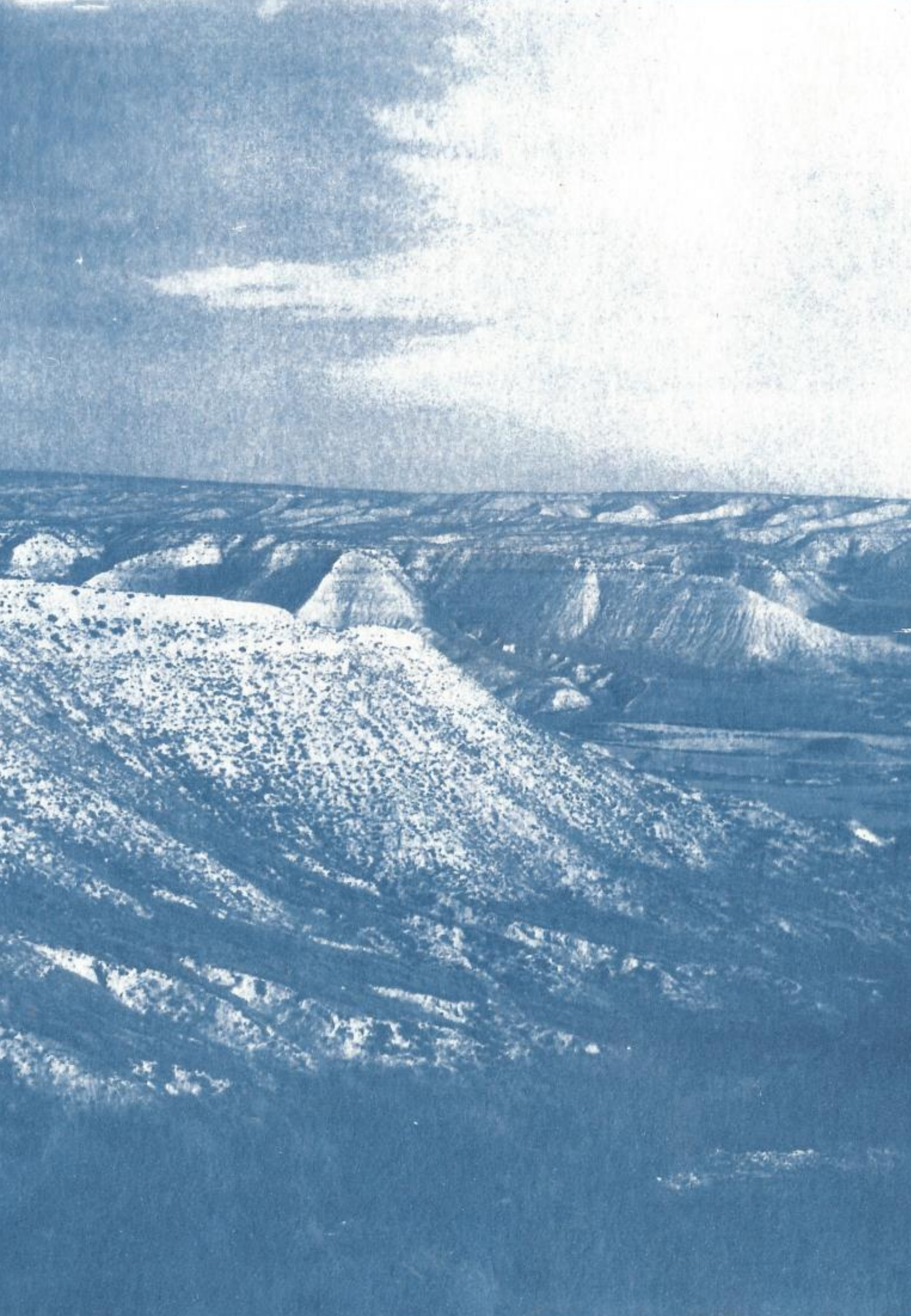
Hypothesis 2.2: Species-rich patches harbored under the canopy of nurse shrubs may provide seeds to the local vicinity, acting as seed sources and hence spatially structuring rich soil seed banks around them.

Objective 3: To identify the strategies making a non-specialist species (*i.e.*, the gypsovag *Cistus clusii*) locally dominant in gypsum plant communities. Particularly, testing whether or not this shrub interferes with neighboring species through chemical mechanisms of interference.

Hypothesis 3.1: Gypsovag shrubs may be less adapted to the limiting gypsum environment than gypsophytes and could develop mechanisms to exclude potential competitors from their local vicinity. The gypsovag shrub *C. clusii* is locally dominant in the Middle Ebro Valley, and could exert adverse effects on the establishment and growth of other plants by the release of phytotoxic compounds through leaves and roots, leading to species-poor local vicinities.

Objective 4: To test the role of the gypsophyte shrub *G. struthium* as a nurse plant, assessing its suitability for the ecological restoration of degraded gypsum ecosystems. One specific aim was to study how plant communities reorganize at different stages of plant succession, focusing on the potential pioneering role of *G. struthium.* The other specific aim was to test the effect that this gypsophyte shrub has on the establishment and development of other species of interest for the restoration of gypsum plant communities, therefore structuring species-rich areas in its vicinity.

> **Hypothesis 4.1:** As gypsophytes easily establish in open areas because they overcome harsh conditions inherent in gypsum soils, *G. struthium* could appear as a pioneer species in the community, spontaneously recovering in bare soils in degraded gypsum ecosystems.
>
> **Hypothesis 4.2:** Well-established individuals of the gypsophyte shrub *G. struthium* might ameliorate micro-environmental conditions underneath, subsequently facilitating the germination, survival and growth of other species of interest in gypsum plant communities. The nurse role of *G. struthium* would promote plant establishment and thus vegetation recovery and would result in species-rich areas in its vicinity in the restored plant communities.



Vista general de las comunidades vegetales gipsícolas del Valle Medio del Ebro
Reserva de Fauna Silvestre "La Lomaza"
(Belchite, Zaragoza, España)

Cianotipia elaborada por Ángeles Muñoz
Fotografía original: Ana Foronda

General Methodology

In order to achieve the objectives and answer the hypotheses proposed, this thesis combines observational field data and experimental data (from both field and greenhouse experiments). This thesis is arranged in four chapters that deal with the different objectives proposed. Each chapter corresponds to an original research article published or in revision in international scientific journals. For this reason, each one has the appropriate structure comprising the sections introduction, methods, results and discussion. Since each chapter includes an exhaustive description of the methods and the statistical analyses used, only the description of the study area and the target species are detailed in this section.

Study area: The Middle Ebro Valley

Delimitation of the study area

The study was conducted in the central sector of the Ebro River Basin (hereafter Middle Ebro Valley), where the highest aridity in this basin occurs (López-Moreno *et al.*, 2010). The Middle Ebro Valley consists of a depression bounded by mountain ranges, with the Pyrenees to the north, the Iberian Mountain Range to the southwest and the Catalan Mediterranean System to the southeast (Elorza and Santolalla, 1998; Figure 13).

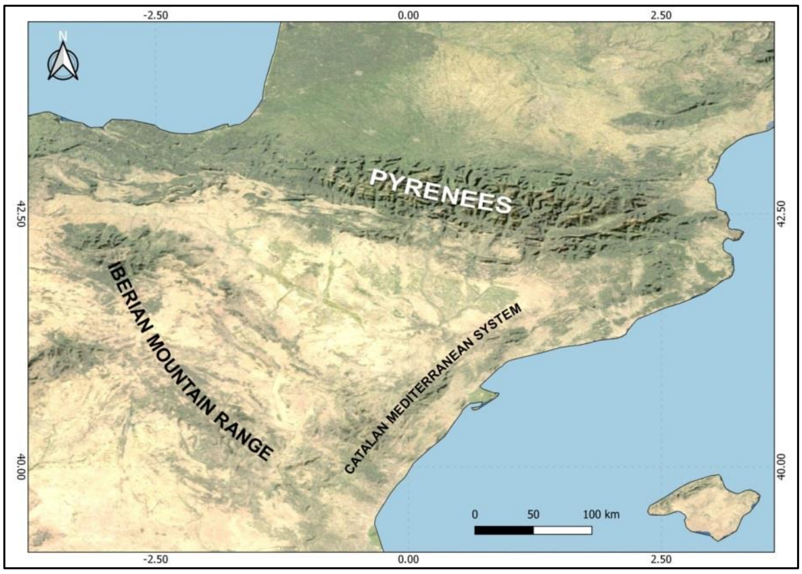


Figure 13 Delimitation of the Middle Ebro Valley in NE Spain (map by courtesy of Manuel Pizarro).

The landscape in this depression mainly consists of low hills and flat-bottomed valleys, with an average height of 300 m a.s.l. (Mota *et al*., 2011), and only a few mountains crossing diagonally from northwest to southeast (Sierra de Alcubierre, maximum height 834 m.a.s.l.).

Lithology and soils

The lithology in the study area is mainly gypsum alternating with marls, limestones, and clays (Quirantes, 1978). The Middle Ebro Valley encompasses the most prominent gypsum outcrop in the northern Iberian Peninsula and one of the most massive gypsum outcrops in Europe, with an occupancy area of 4685 km2 (Escavy *et al*., 2012; Machín and Navas, 1998). These gypsum outcrops have a lacustrine origin, and date from the transition from Oligocene to Miocene (Tertiary), during the period in which the Ebro Basin did not communicate with the Mediterranean Sea (Escavy *et al*., 2012). The most notable gypsum-bearing geological units in this depression are in their majority in the Aragonese territory (Figure 14), being the most massive in Zaragoza province, but also southern Huesca province (Monegros county) and northern Teruel province (Bajo Martín county).

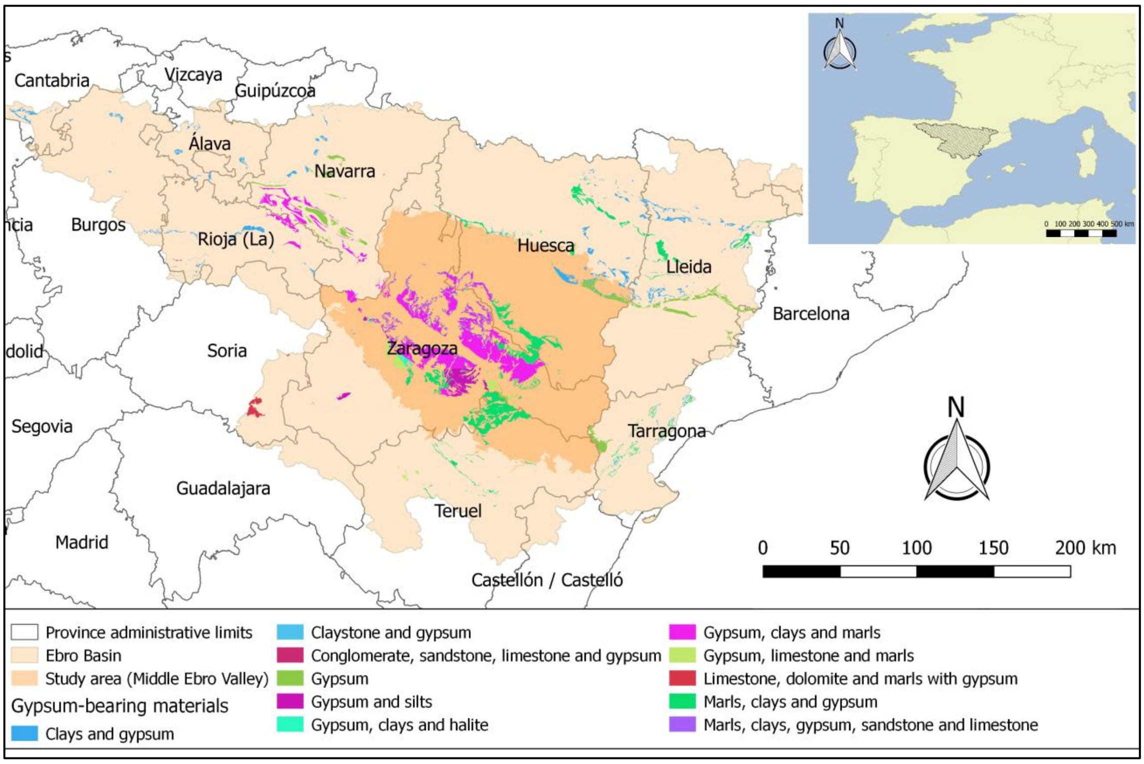


Figure 14 Gypsum-bearing materials in the Ebro Basin. Source: Confederación Hidrográfica del Ebro (iber.chebro.es/sitebro/).

The gypsiferous soils in the study area are Lithic and Eutric Leptosols, Petric, Calcic and Haplic Gypsisols, Gypsic Regosols, and Gypsic Solonchaks (Machín and Navas, 1998). Haplic Calcisols are also abundant, and despite not being conventional gypsiferous soils, they have a significant gypsum content interbedded with marls, sand, and clays (Machín and Navas, 1998). The soil types that are mostly developed in the study area are Leptosols in the hills and Gypsisols in the flat-bottomed valleys (Navas, 1999). Following Navas (1991), these are poorly developed soils very sensitive to erosion and characterized by a high content of gypsum (> 60%), low content of organic matter (< 1.5 %), alkaline pH (7.5-8) and moderate salinity (EC 2-3 dS/m).

Climate

The study area has a semi-arid Mediterranean climate with high continental influence (Creus and Ferraz, 1995). Average annual temperature in the central region of the Middle Ebro Valley is 14.9 ºC, and average annual precipitation is 350 mm·yr-1 ("Farlete" meteorological station, 1986-2012 period; source: Gobierno de Aragón, http://opendata.aragon.es). Intra-annual irregularities in rainfall are marked, with two peaks of maximum seasonal rainfall, one recorded in spring and the other in autumn (Figure 15A). The minimum rainfall occurs in summer (July-August), matching with the maximum seasonal temperature recorded (Figure 15A). Precipitation scarcity caused by the rain shadow exerted by the surrounding mountains and high potential evapotranspiration are characteristic features that define the dry climate in this area (Loidi, 2017).

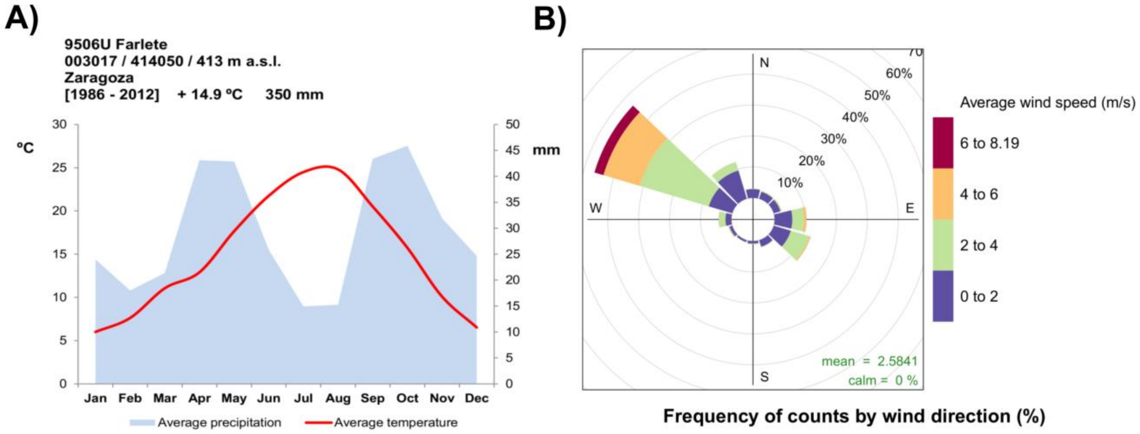


Figure 15 Climatic conditions in the central region of the Middle Ebro Valley: **A)** Climograph representing monthly average precipitation and monthly average temperature in Farlete (1986-2012 period); and **B)** Wind rose representing the direction of the predominant wind in Osera (2004-2018 period). Source: Gobierno de Aragón, http://opendata.aragon.es.

Dryness in this area is accentuated by the effect of the so-called cierzo, a strong wind dominating from NW to SE (Cuadrat *et al.*, 2007; Figure 15B) that causes soil desiccation and the reduction of water in clouds (Herrero and Snyder, 1997). The Middle Ebro Valley is the second driest area in the Iberian Peninsula, after the Iberian Southeast (Mota *et al.*, 2011). This area encompasses a north-south aridity gradient in a relatively short distance, from the Ebro River to the Pyrenees (Cuadrat *et al.*, 2007; Figure 16).

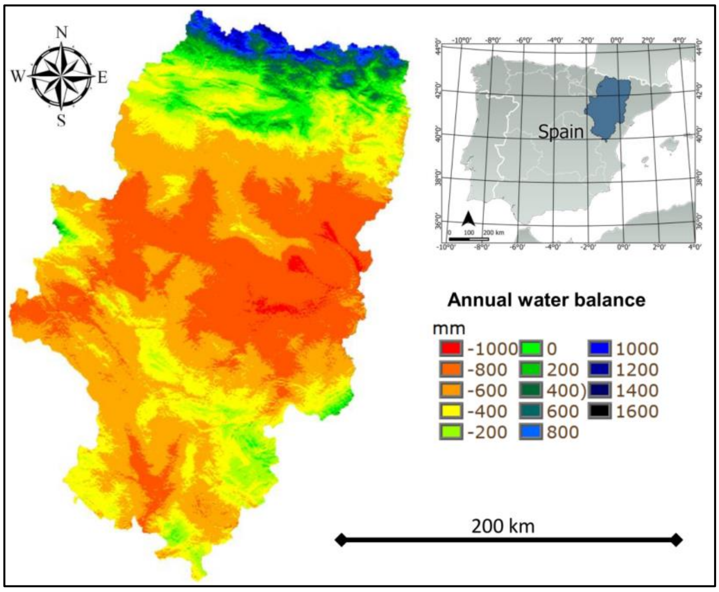


Figure 16 Annual water balance in the Aragonese region. Source: Digital Climate Atlas of Aragón (Cuadrat *et al.*, 2007).

Flora and vegetation

Biogeographically, the Middle Ebro Valley corresponds to the Central Iberian Mediterranean Province, and more specifically to the Bardenero-Monegrino Sector (Loidi, 2017; Figure 17). The Middle Ebro Valley is noteworthy for the singularity of the flora and the species richness, being the second richest gypsum outcrop in the Iberian Peninsula regarding gypsophyte species (Mota *et al.*, 2011). Specifically, the highest richness of gypsophytes has been found in the municipalities of Osera, Villafranca de Ebro, Alfajarín, Nuez de Ebro (17 species), and Bujaraloz (16 species), all in Zaragoza administrative province (Mota *et al.*, 2011). Some of these species are local endemisms such as *Centaurea pinnata* Pau, *Limonium stenophyllum* Erben, *L. viciosoi* (Pau) Erben, and *Thymus loscosii* Willk., and regional endemisms such as *L. hibericum* Erben, L. and *Sideritis spinulosa* Barnades ex Asso. (Mota *et al.*, 2011). But also gypsophyte species more widely distributed in the Iberian Peninsula, or even Iranian-Turanian plants such as the rare *Krascheninnikovia ceratoides* (L.) Gueldenst. coexist in the Middle Ebro Valley (Mota *et al*., 2011).

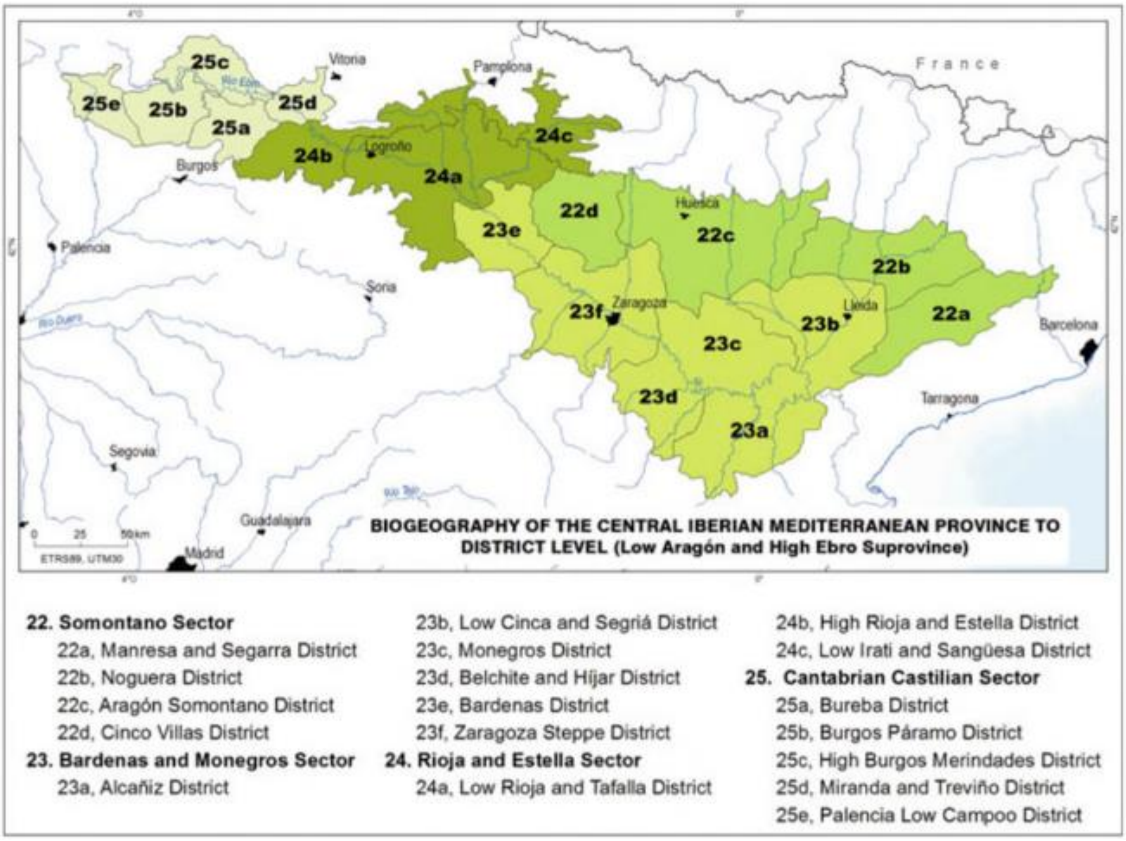


Figure 17 Sectors and districts of the Central Iberian Mediterranean biogeographical province. The Middle Ebro Valley corresponds to the 23-Bardenas and Monegros Sector (bright pistachio green). Source: Loidi, 2017.

Plant communities in gypsum hills are patchy scrublands with large open areas (Figure 18A), composed predominantly of gypsophyte shrubs and Mediterranean widespread basophilic shrubs (*i.e.*, gypsovags) belonging to the orders *Gypsophiletalia* and *Rosmarinetalia* respectively (Braun-Blanquet and Bolòs, 1958; Loidi, 2017). The most abundant gypsophytes in these communities are *Helianthemum squamatum*, *Herniaria fruticosa*, *Ononis tridentata* (all widely distributed in Iberian gypsum communities), and *Gypsophila struthium* subsp. *hispanica* (distributed in gypsum outcrops of NE Spain). Among gypsovags, the most common species are the large shrubs *Cistus clusii* Dunal and *Rosmarinus officinalis*, which are locally dominant in these communities, and the subshrubs *Thymus vulgaris*, *Helichrysum stoechas* and *Helianthemum syriacum*, which are not exclusive but are highly related to gypsum soils

[Table A1.1]. Open woodlands of *Juniperus thurifera* L. and *Pinus halepensis* Mill. occasionally appear in gypsum hills in localities where rainfall is higher than average (Braun-Blanquet and Bolòs, 1958; Figure 18B).

Communities of annuals belonging to the order *Sedo-Cnetopsion gypsophilae* also appear in gypsum hills, sheltered beneath shrubs and in open areas (Mota *et al*., 2011). These communities form low-coverage pastures of small therophytes, which are ephemeral and whose life span is associated with seasonal rains. Species that commonly compose these communities in the Middle Ebro Valley are small grasses such as *Narduroides salzmannii* (Boiss.) Rouy or *Desmazeria rigida* (L.) Tutin., gypsophyte herbs such as *Campanula fastigiata* Dufour ex A. DC or *Chaenorrhinum rubrifolium* (Robill. & Castagne ex DC.) Fourr. (not so exclusive to gypsum), and widely distributed herbs such as *Erodium cicutarium* (L.) L'Hér. or *Neatostema apulum* (L.) I.M. Johnst [Table A1.1].

Plant communities in flat-bottomed valleys are semi-arid grasslands belonging to the order *Lygeo-Stipetalia*, dominated by the perennial grass *Lygeum spartum* L. (Figure 18C). Communities belonging to *Artemisio valentinae-Atriplicetum halimi*, dominated by the shrubs *Artemisia herba-alba* Asso. and *Salsola vermiculata* L., also appear in flat-bottomed valleys and hillsides where there is a transition to substrates richer in clays (Braun-Blanquet and Bolòs, 1958; Loidi, 2017; Mota *et al*., 2011).

Figure 18 Plant communities in the Middle Ebro Valley: **A)** Scrubland dominated by gypsophyte and gypsovag shrubs; **B)** Scrubland dominated by gypsovag shrubs and an isolated specimen of *Juniperus thurifera* remaining from the former open woodland; **C)** Grassland dominated by the perennial grass *Lygeum spartum*. Photos: Ana Foronda.

Land use

Land use in the study area is based on a traditional agro-pastoral system that includes cereal crops and livestock (Pueyo, 2005). Traditionally, agriculture in the area has been based on rainfed crops. Recently, the mechanized extensive agriculture and the expansion of irrigated lands have caused the salinization of the soils and habitat fragmentation, with a consequent reduction of species (Mota *et al.*, 2011). Livestock consist of semi-extensive sheep farming of Rasa Aragonesa breed, sometimes accompanied by a few goats (Pueyo, 2005). In the past, tree felling for fuel and building was one of the main practices in the study area, which caused a decline in open woodlands of *J. thurifera*, whose regeneration was difficult due to the slow growth rate of this species and the past overgrazing (Braun-Blanquet and Bolòs, 1958).

Study sites

Four study sites representative of the study area were selected to carry out the observational field surveys and the field experiments (Figure 19; [Figure A1.1]).

- **Site 1 (Leciñena) and Site 2 (Lomaza)**

These two study sites were selected in gypsum plant communities of the Middle Ebro Valley to carry out the observational studies necessary for understanding biotic interactions in semi-arid gypsum plant communities. Site 1 is located to the north of the Ebro River, in the surroundings of the Alcubierre Mountain Range in Leciñena municipality, and Site 2 is located to the south of this river, in “La Lomaza de Belchite” Wildlife Reserve (Figure 19). Site selection included only natural plant communities deprived of any agro-pastoral activities to avoid disturbances such as grazing and trampling. Both study sites are representative of gypsum plant communities within the study area regarding species composition and soil characteristics (Braun-Blanquet and Bolòs, 1958; Pueyo *et al.*, 2008, 2007; [Table A1.1 and Table A1.2]). However, Leciñena is less arid than Lomaza, with de Martonne’s aridity indices of 16.25 and 12.23 respectively [Table A1.2]. Average annual temperature is 13.5 ºC and annual rainfall is 382 mm·yr-1 in Leciñena, and average annual temperature is 14.7 ºC and annual rainfall is 302 mm·yr-1 in Lomaza (“Lanaja” and “Belchite” meteorological stations; 2004-2017 period; source: Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente).

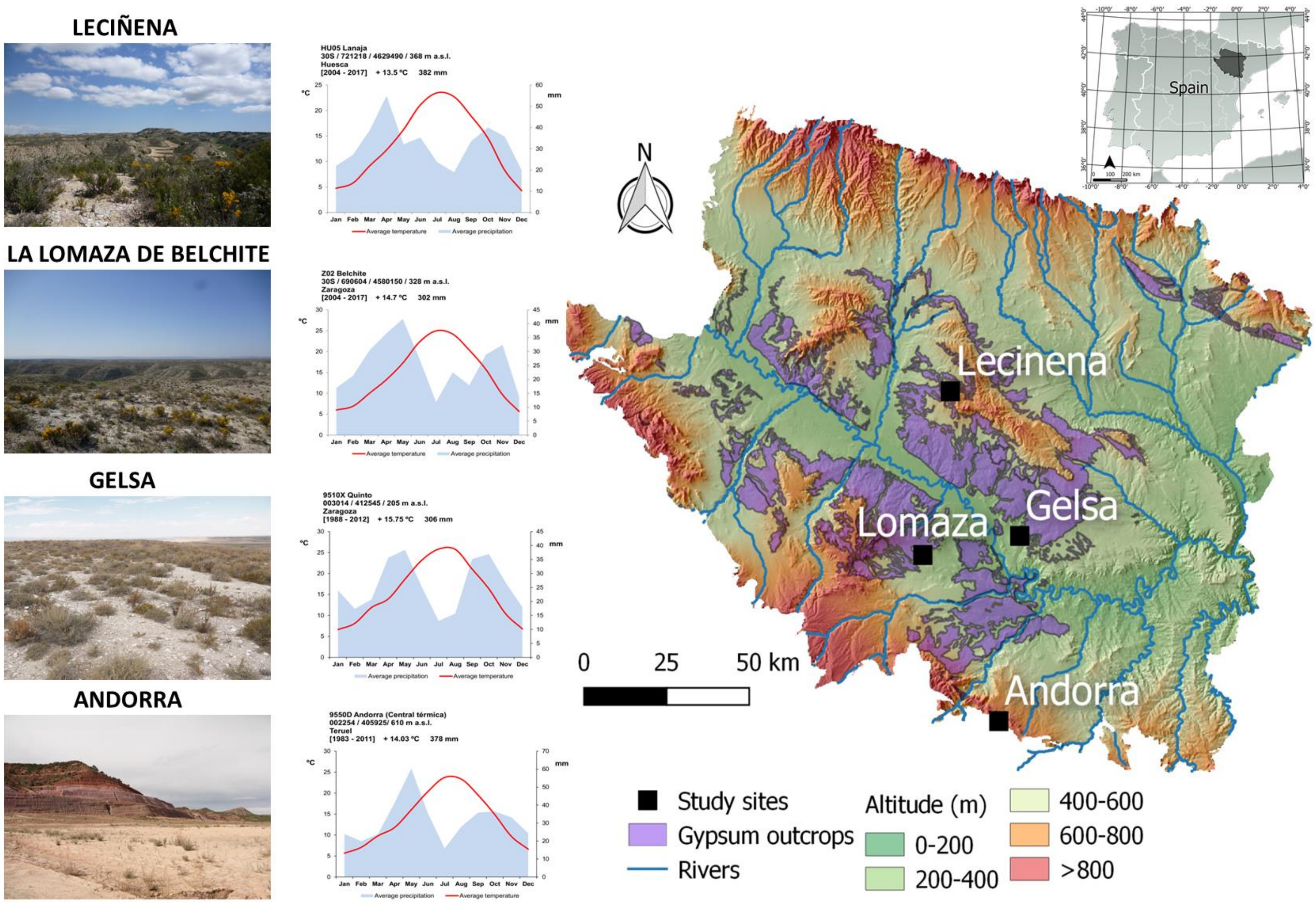


Figure 19 Location, overview and climate of the four study sites.

- **Site 3 (Gelsa)**

This study site was selected to evaluate the role of facilitation in the recovery of degraded gypsum areas, specifically disturbed by mining. This study was conducted in a restored gypsum mine and in the surrounding undisturbed communities, where species composition is representative of the gypsum plant communities of the Middle Ebro Valley [Table A1.1]. Vegetation was spontaneously regenerated inside the gypsum mine, either from the soil seed bank contained in the original topsoil that was used in restoration actions or by the arrival of propagules from the surroundings. Average annual temperature in the study site is 15.8 ºC and annual precipitation is 306 mm·yr-1 ("Quinto" meteorological station, 1988-2012 period; source: Gobierno de Aragón, http://opendata.aragon.es).

- **Site 4 (Andorra)**

This study site was selected to evaluate experimentally the role of a potential nurse shrub on the establishment and growth of other plants of interest for restoration. This study site is in the outer part of the Middle Ebro Valley (Figure 19) and consists of a gypsum spoil dump where experimental plantations of different species (*e.g.*, *G. strutium* or *Atriplex halimus*) were previously performed. Gypsum spoil was formed in a coal-fired power station during the process of desulfurization of lignite by limestone forced oxidation (Srivastava and Jozewicz, 2001). Average annual temperature in the study site is 14.03 ºC, and average annual precipitation is 378 mm·yr-1 ("Andorra-Central térmica" meteorological station, 1983-2011 period; source: Gobierno de Aragón, http://opendata.aragon.es).

Target species: dominant shrubs

All the plant species integrating the community (both perennials and annuals) were taken in consideration for this study at the community level. Nevertheless, the exploration of potential key nurse species in the community was simplified by pre-selecting six target perennial species (shrubs and subshrubs) with a potentially relevant role in the community based on their abundance and traits. Target species were selected among the dominant shrubs in natural communities in gypsum outcrops of the Middle Ebro Valley. The choice of species included shrubs with contrasting size (*i.e.*, tall versus short), architecture (*i.e.*, cushion-like versus erect) and life strategy (*i.e.*, gypsophytes versus gypsovags). Target species were the gypsophytes *G. struthium* subsp. *hispanica*, *O. tridentata* and *H. squamatum*, and the gypsovags *C. clusii*, *R. officinalis* and *T. vulgaris*.

(1) *Gypsophila struthium* Loefl. subsp. *hispanica* (Willk.) G. López (*Caryophyllaceae*) is a wide gypsophile nanophanerophyte (shrub). It is 25-80 (100) cm tall and has a cushion-like architecture, with crawling branches and erect or ascending stems. Leaves are 7-35 x 0.5-1.3 mm, linear and fleshy. It has small white flowers, arranged in cymose inflorescences. Seeds are 1.2-1.7 x 1-1.4 mm, almost kidney-shaped, black or dark brown. It has summer blossoming, occurring from June to October, and autochorous seed dispersal mechanisms (Castroviejo 1986-2012). It presents specific adaptations to gypsum, since oxalate crystals have been found in its leaves (Palacio *et al*., 2014a). Although *G. struthium* Loefl. is widely distributed on most of the Iberian gypsum outcrops, this subspecies can be found only in NE Spain (Figure 20). It has been highly valued as cut flowers.

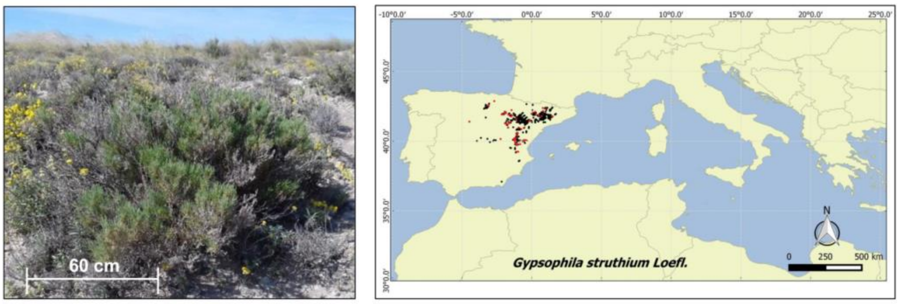


Figure 20 Overview of the shape and size of the gypsophyte shrub *Gypsophila struthium* subsp. *hispanica* (Photo: Ana Foronda) and its distribution (red dots) in the Mediterranean Basin (Source: Global Biodiversity Information Facility, https://www.gbif.org/).

(2) *Ononis tridentata* L. (*Fabaceae*) is a wide gypsophilous nanophanerophyte. It is a shrub up to 150 cm tall, and has an erect architecture, with highly branched stems. Leaves are 2-30 x 1-12 mm, linear-lanceolate and fleshy-succulent, with dentate ends. Inflorescences are formed by 1- 3 papilion flowers of 8-14 mm, pink-coloured with purple and white veins. The flowering period takes place from May to September. The fruit is a legume of 10-20 mm, containing 1-2 seeds that are kidney-shaped and brown (Castroviejo 1986-2012). Seeds are often predated by insects (Ballesteros *et al*., 2013). O. tridentata has specific adaptations to gypsum, since small traces of oxalate crystals have been found in its leaves (Palacio *et al*., 2014a). It is widely distributed on most of the Iberian gypsum outcrops (Figure 21), but presents subspecies that are confined to small outcrops (*e.g.*, *O. tridentata* subsp. *crassifolia* in Granada province, South Spain).

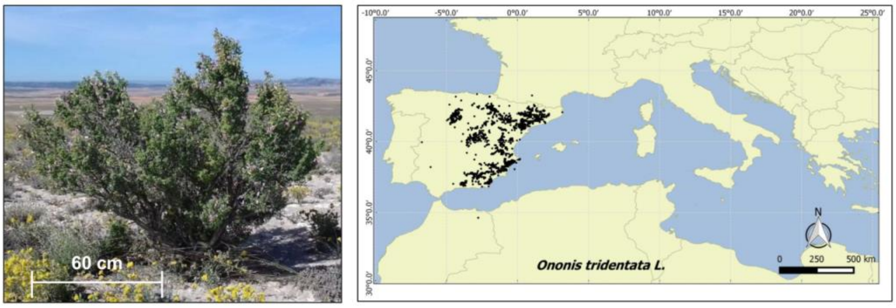


Figure 21 Overview of the shape and size of the gypsophyte shrub *Ononis tridentata* (Photo: Ana Foronda) and its distribution in the Mediterranean Basin (Source: Global Biodiversity Information Facility, https://www.gbif.org/).

(3) *Helianthemum squamatum* (L.) Pers. (*Cistaceae*) is a wide gypsophilous chamaephyte (subshrub). It is 10-40 cm tall and has a cushion-like architecture, with sparsely dense branches. Leaves are 8-25 x 3-10 mm, lanceolate, sometimes a little fleshy. Flowers are small and yellow-coloured, and are arranged in dense inflorescences at the end of new branches (Castroviejo 1986-2012). The flowering period takes place from May to August (Aragón *et al.*, 2007). Fruits are small (3 mm) multi-seeded capsules with small (1.3 mm) light brown seeds (Castroviejo 1986-2012). Similar to other Cistaceae, this species has hard seed-coats, and also present a mucilaginous coating that is hygroscopic (Escudero *et al.*, 1999). Small traces of oxalate crystals have also been found in its leaves (Palacio *et al.*, 2014a). It is one of the most abundant gypsophytes in Spain (Figure 22).

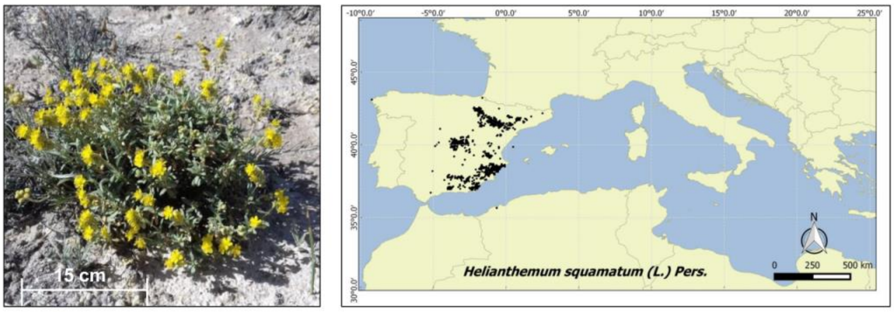


Figure 22 Overview of the shape and size of the gypsophyte subshrub *Helianthemum squamatum* (Photo: Ana Foronda) and its distribution in the Mediterranean Basin (Source: Global Biodiversity Information Facility, https://www.gbif.org/).

(4) *Cistus clusii* Dunal (*Cistaceae*) is a gypsovag nanophanerophyte. It is a 40-100 cm tall shrub and has a multi-branched architecture. Leaves are linear with a convex side and revolute margins, and present a sticky and glossy surface. Inflorescences appear on the top branches, with 2-3 flowers. Flowers have big petals that are white-coloured with a yellow area in the centre of the flower. Flowering occurs from March to June. Fruits are subglobose capsules (4-8 mm), dehiscent in five leaflets, and containing numerous seeds. Seeds are small (1 mm), brown-coloured, and have hard seed-coats (Castroviejo 1986-2012). It is distributed throughout the western Mediterranean region (predominantly in Iberia, Figure 23) on alkaline soils, including gypsum, marls and limestones (Demoly and Montserrat, 1990), and is very tolerant to dry environments (Munné-Bosch *et al.*, 2003).

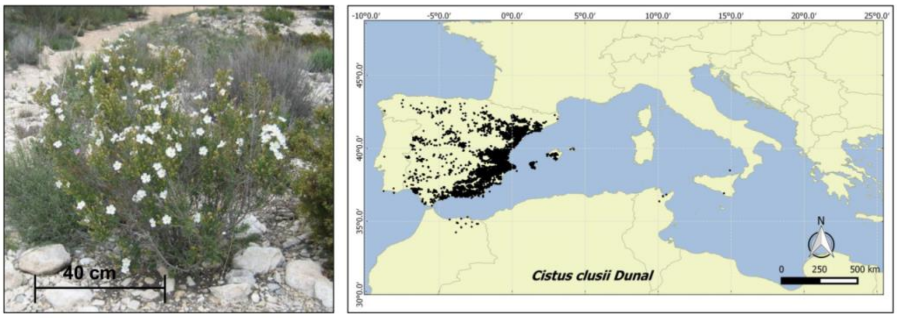


Figure 23 Overview of the shape and size of the gypsovag shrub *Cistus clusii* (Photo: Santiago González Torregrosa, http://www.apatita.com) and its distribution in the Mediterranean Basin (Source: Global Biodiversity Information Facility, https://www.gbif.org/).

(5) *Rosmarinus officinalis* L. (*Labiatae*) is a gypsovag nanophanerophyte. It is a multi-branched erect shrub that grows maximum 1.8 m tall (usually 1 m). Leaves are 10-41 x 1-3 mm, linear-lanceolate, with revolute leaf margin and woolly on the back side, and present a pungent fragrance. It has zygomorphic flowers in the upper branches, with lavender-coloured petals that form a tubular corolla that is two-lipped (the lower lip is trifoliate), and with prominent stamens. Flowering occurs during a long period, from January to June-July. Fruits are schizocarp with four ovoid seeds with a bulging end (Castroviejo 1986-2012). It is edaphic indifferent and grows in wild in the Mediterranean Basin (Figure 24), but nowadays it is cultivated worldwide due to culinary, antioxidant and medicinal uses.

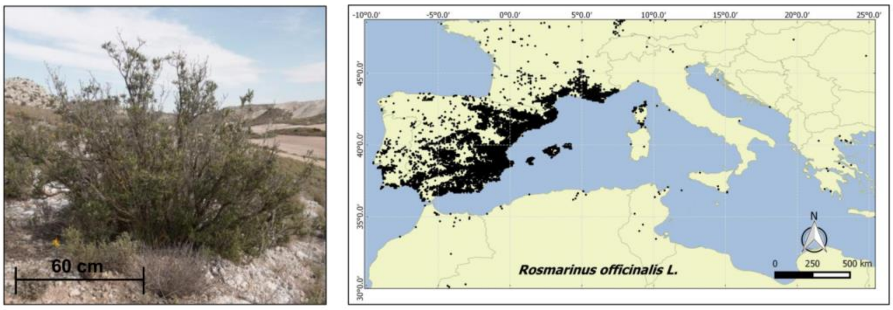


Figure 24 Overview of the shape and size of the gypsovag shrub *Rosmarinus officinalis* (Photo: Ana Foronda) and its distribution in the Mediterranean Basin (Source: Global Biodiversity Information Facility, https://www.gbif.org/).

(6) *Thymus vulgaris* L. (*Labiatae*) is a gypsovag chamaephyte. It is a 10-40 cm tall erect subshrub with woody stems (reddish) growing upward to form a mound. Leaves are linear to elliptic, gray-green, pulverulent, with distinctively revolute margin, and highly aromatic. Inflorescences are disposed in whorls on the stem ends, composed of tiny, tubular, and lilac or pink flowers, blooming from March to August. Fruits have four one-seeded nutlets, which contain small globose (0.5-0.8 mm) and dark brown-coloured seeds (Castroviejo 1986-2012). It grows on alkaline substrates and is indigenous to the Mediterranean Basin, where is widely distributed (Figure 25). It is used as a culinary and medicinal herb.

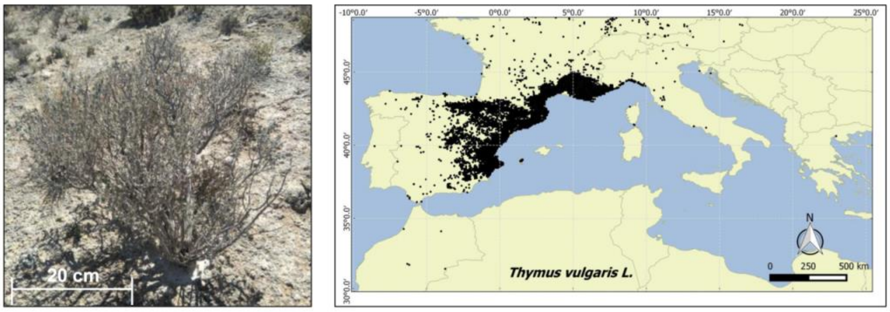


Figure 25 Overview of the shape and size of the gypsovag subshrub *Thymus vulgaris* (Photo: Ana Foronda) and its distribution in the Mediterranean Basin (Source: Global Biodiversity Information Facility, https://www.gbif.org/).

Outline of the thesis

Chapter 1 *The role of nurse plants on the spatial patterning of plant establishment in semi-arid gypsum plant communities.*

This chapter consisted of an observational study that aimed to highlight the importance of positive interactions for plant establishment in gypsum plant communities from the standpoint of both the facilitated and the facilitator plants. Specifically, the microsites where gypsophyte and gypsovag plants established at both the seedling and the adult stages were studied to identify differences in the requirements of being facilitated depending on the life strategy of plants (*i.e.*, the level of linkage to gypsum). The abilities of the six target shrubs for facilitating the establishment of other species were also assessed in this chapter to identify key nurse species in structuring plant diversity in the community. The assessment of richness and abundance of plants sheltered under the canopy of the target shrubs and the diversity at distances up to four meters from these shrubs (*i.e.*, individual species-area relationships) were used as proxies of plant-plant interactions. Moreover, micro-environmental conditions were assessed under shrub canopies to identify the underlying mechanisms that may drive these plant interactions. This observational study was carried out in Leciñena (Site 1) and Lomaza (Site 2), allowing the assessment of plant interactions under different aridity conditions. In this chapter, the evaluation of the facilitative role of gypsophyte and gypsovag shrubs in spatially structuring plant diversity included in objective (1) was achieved.

Chapter 2 *Implications of the seed sink and seed source roles of shrubs for the spatial structure of the soil seed bank in a semi-arid gypsum plant community.*

This chapter aimed to highlight the role of shrubs in spatially structuring the soil seed bank in gypsum plant communities. Specifically, the aim was to assess how different target shrubs with diverse physiognomies and facilitative abilities contribute to the spatial structure of the soil seed bank, trying to unravel their seed sink and seed source roles. Richness and abundance of seeds were counted in three microsites associated to each of the shrubs (under canopy, at the edge of the canopy and in open areas) to study the spatial heterogeneity of the soil seed bank. To discern between the seed sink and seed source roles of the shrubs, similarities in species composition between the soil seed bank and the standing vegetation harbored under the shrub canopy were evaluated, considering that a higher similarity means a source role by plants harbored. The study was focused on one of the study sites (Lomaza, Site 2) to work with affordable sample sizes. Plant cover in this site is lower than in Site 1, therefore making easier the evaluation of the effect of a single shrub on the spatial structure of the soil seed bank. In this chapter, the objective (2) of assessing the seed sink and seed source role of gypsophyte and gypsovag shrubs and their contribution to the spatial structure of the soil seed bank was achieved.

Chapter 3 *Species-specific interference exerted by the shrub Cistus clusii Dunal in a semi-arid Mediterranean gypsum plant community.*

This chapter combined observational field data and a greenhouse experiment to investigate whether the locally dominant gypsovag shrub *C. clusii* exerts chemically mediated interference on neighboring species in gypsum plant communities. Richness and abundance of plants under the canopy of this shrub and *G. struthium* (a nurse shrub of similar architecture) were compared to evaluate whether C. clusii exerts an adverse effect on the establishment of other plants. Additionally, the potential chemically mediated interference that *C. clusii* may exert on neighboring plants was tested experimentally in a greenhouse. Particularly, by testing the effect of leaf and root aqueous extracts from *C. clusii* on the germination, survival and growth of nine coexisting species, including *C. clusii* itself to test for potential autotoxicity. The study was conducted only in Site 1 (Leciñena) because *C. clusii* is absent in Site 2 (Lomaza). In this chapter, the objective (3) of identifying the strategies making some non-specialist species locally dominant in gypsum plant communities was achieved.

Chapter 4 *Substrate-specialist plants enhance vegetation recovery effectiveness in post-mining gypsum substrates.*

This chapter combined observational field data and a field experiment to assess the suitability of the gypsophyte *Gypsophila struthium* Loefl. as a nurse plant in vegetation recovery of gypsum mines. The observational study was conducted in Gelsa, in a gypsum mine where the original topsoil had been reinserted in different years and where vegetation was spontaneously emerging. Vegetation surveys (line-point intercept transects) were set to study community reorganization along a successional gradient after the disturbance to identify pioneer species and to evaluate their role in spatially structuring the community. Complementary, a sowing and planting experiment was set in a gypsum spoil dump (Andorra) to assess how *G. struthium* may positively affect the germination and growth of plants under its canopy. Soil and micro-environmental conditions were measured under the shrub canopy to evaluate the potential role of this shrub as an ecosystem engineer, which may explain the underlying mechanisms making shrubs act as nurse plants. This chapter entails the application of the knowledge generated in the previous chapters in the ecological restoration of gypsum systems, thus achieving the objective (4). In addition, the objective (1) of evaluating the role of a gypsophyte shrub in the establishment and development of other plants was achieved in the field experiment.

There is a **General discussion** section where most important results obtained in these chapters are discussed together with the hypotheses postulated in this thesis. In addition, this section includes recommendations for using nurse shrubs in the restoration of gypsum plant communities, the limitations of the study and future research lines to broaden the knowledge in interactions between plants at the community level in gypsum environments. The main **conclusions** obtained are exposed at the end of the thesis.

Plántula del gipsófito *Herniaria fruticosa*

Cianotipia elaborada por Ángeles Muñoz
Fotografía original: Ana Foronda

Chapter 1

The role of nurse shrubs on the spatial patterning of plant establishment in semi-arid gypsum plant communities

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Journal of Arid Environments 160 (2019) 82-90

https://doi.org/10.1016/j.jaridenv.2018.09.003

Abstract

Plants may depend on nurse plants to establish and survive in gypsum environments, which are stressful for plant life. Shrubs may act as nurse plants by ameliorating micro-environmental conditions in their local vicinity. Despite its importance, the role of nurse shrubs on the spatial patterning of plant establishment remains largely unknown in gypsum communities. We aimed to understand the patterns of plant establishment and to identify key nurse species in facilitating plant establishment and structuring plant diversity at the community level in these environments. We set an observational study in two gypsum plant communities of NE Spain to assess the microsites where seedlings germinate. Also, we assessed the role of six dominant shrubs (gypsophytes and gypsovags), in spatially structuring plant diversity by assessing species-area relationships in their local vicinity to identify key nurse species. To assess the potential amelioration of micro-environmental conditions associated with shrubs, we analyzed microenvironmental and physical-chemical soil conditions under shrub canopies compared to open areas. Most plants were spatially associated with adult shrubs. Gypsophytes accumulated more diversity than gypsovags, despite both showing ameliorated conditions under canopy compared to open areas. In conclusion, gypsophyte shrubs play key roles in the structure of gypsum plant communities by facilitating plant establishment.

Keywords: diversity accumulator; diversity repeller; gypsophytes; gypsovags; nurse plants; seedling establishment.

Introduction

Facilitation is a well-known biotic interaction occurring in arid and semi-arid ecosystems, where harsh environmental conditions prevail (Bertness and Callaway, 1994). Since plants in early life stages are highly vulnerable to environmental stress (Escudero *et al.*, 2005), the likelihood of plant establishment and survival to the adult stage in these environments may increase with the presence of nurse plants *(Escudero et al.*, 2005; Flores and Jurado, 2003). Specifically in semi-arid gypsum plant communities, along with water scarcity and extreme temperatures, soils present physical and chemical constraints for plant development (Escudero *et al.*, 2015). Nevertheless, there are gypsum specialist plants, known as gypsophytes, which can overcome these limitations at establishment stage (Escudero *et al.*, 2015). Gypsophytes coexist with the so-called gypsovags, which might be less adapted to gypsum soil conditions than gypsophytes and may require assistance by nurse plants to become established in the community (Romão and Escudero, 2005). Stress-adapted shrubs provide shade under their canopy at the adult stage, then reducing stress conditions (Gómez-Aparicio *et al.*, 2005) and usually acting as nurse plants (Navarro-Cano *et al.*, 2016, 2014). As a consequence, less adapted species may establish in these favorable microsites, allowing species-rich areas in the vicinity of nurse plants (Soliveres *et al.*, 2011).

Despite the relevance of gypsum outcrops for biodiversity conservation, the research interest in gypsophily (Escudero *et al.*, 2015), and the studies about biotic interactions occurring in gypsum outcrops at species level (*e.g.*, Escudero *et al.*, 2000), the role of biotic interactions on the patterns of plant establishment in these ecosystems remain largely unknown at community level. Moreover, for ecosystem conservation and restoration purposes, it is important to identify key nurse species which may influence gypsum plant community dynamics and structure, and therefore increase diversity, productivity, and resilience of these communities (Michalet, 2006). This study aimed to understand the patterns of plant establishment in gypsum plant communities and to identify key nurse species, assessing their role in facilitating plant establishment and structuring plant diversity at the community level. Specifically, addressing the following questions: Q1) do plants need to be facilitated in gypsum plant communities?; Q2) are there key nurse species facilitating plant establishment?; and consequently Q3) which is the role of key nurse species in structuring plant diversity in gypsum plant communities?

To address these questions, we carried out an observational study in two gypsum plant communities of the Middle Ebro Valley (NE Spain). We first determined the microsites where seedlings and adults of perennial plants preferentially establish (*i.e.*, spatially associated to adult shrubs or in open areas) to assess whether these plants need to be facilitated (Q1). We hypothesized that H1) while gypsophytes might be able to establish in open areas, becoming pioneer species in the community (Martínez-Duro *et al.*, 2010; Mota *et al.*, 2003a), gypsovags may need to establish close to the canopy of adult shrubs. Further, to identify key nurse species in the community (Q2), we surveyed richness and abundance of plants under the canopy of six dominant shrubs (both gypsophytes and gypsovags). Additionally, we evaluated soil properties under the shrub canopies compared to open areas to examine for potential micro-environmental conditions amelioration that would drive facilitation mechanisms (Callaway, 2007). We expected that H2) gypsophyte shrubs would act as key nurse species in the community, finding a high richness and abundance of plants under shrub canopy. Accordingly, we expected to find ameliorated conditions under nurse species compared to open areas (soil humidity and nutrients increasing, surface mechanical resistance decreasing and temperatures softening; Callaway, 2007). Given that pairwise biotic interactions are ultimately reflected in the spatial structure of the community (Arroyo *et al.*, 2015; Saiz *et al.*, 2014), we also surveyed the spatial distribution of plant diversity in the vicinity of nurse species (beyond their canopies) compared to other shrubs (Q3). We predicted that H3) nurse-facilitated positive interactions would lead to species-rich areas in the vicinity of nurse gypsophytes [Figure A2.1]. In this observational study, we considered plant spatial associations as a commonly used proxy for assessing interactions among plants at the community level (Arroyo *et al.*, 2015; Cavieres *et al.*, 2006; Saiz and Alados, 2012; Soliveres and Maestre, 2014).

Methods

Study area

The study was conducted in the Middle Ebro Valley (NE Spain), which is one of the largest gypsum outcrops in Europe (Machín and Navas, 1998). The lithology is mainly gypsum alternating with marls, limestones and clays (Quirantes, 1978). This area has a semi-arid Mediterranean climate with high continental influence and encompasses a north-south aridity gradient (Cuadrat *et al.*, 2007) . Average annual temperature in the study area is 14.9 ºC and average annual precipitation is 350 mm·yr^{-1} ("Farlete" meteorological station, 1986-2012 period; source: Gobierno de Aragón, http://opendata.aragon.es). The landscape mainly consists of low hills (480 m.a.s.l. average) and flat-bottomed valleys, which are usually cultivated. Plant communities in gypsum hills are patchy scrublands composed predominantly of the gypsophytes *Gypsophila struthium* Loefl. ssp. *hispanica* (Willk.) G. López*, Helianthemum squamatum* (L.) Pers., *Herniaria fruticosa* L., *Ononis tridentata* L., and *Lepidium subulatum* L., and Mediterranean widespread gypsovags such as *Cistus clusii* Dunal, *Rosmarinus officinalis* L., *Helianthemum syriacum* (Jacq.) Dum. Cours., *Teucrium capitatum* L., and *Thymus vulgaris* L. (Braun-Blanquet and Bolòs, 1958).

We selected two gypsum plant communities (*i.e.*, study sites) in the Middle Ebro Valley, one to the north of the Ebro river (Site 1, "Leciñena" municipality), and one to the south of this river (Site 2, "La Lomaza de Belchite" Wildlife Reserve). These study sites are representative of gypsum plant communities within the study area regarding species composition and soil characteristics (Braun-Blanquet and Bolòs, 1958; Pueyo et al., 2008, 2007; [Table A1.1 and Table A1.2). Climatic conditions differ between study sites, being Site 2 more arid than Site 1 [Table A1.2]. At each study site, we selected an area of 6 Km^2 where we performed the vegetation surveys.

Vegetation surveys

We conducted a vegetation survey in spring 2014, concurring with the plants growing season, to determine the microsites where seedlings and adults of perennial plants preferentially are. At each study site, we randomly set sixteen plots (5 x 1 m) at least 250 m distant from each other (n=32 plots). We recorded every perennial plant occurring inside the plots, identifying them to species level and differentiating by ontogenetic stages (<1-year-old seedlings, and adults including juveniles and reproductive individuals). We categorized plants depending on the microsite in which they were found [Figure A2.2]: in open areas (OA microsite), on the edge of the canopy of adult shrubs (ES microsite) or under the canopy of adult shrubs (UC microsite). OA microsite consisted of open areas not covered by perennial plants, but occasionally occupied by biological soil crusts (*i.e.*, lichens and mosses); ES microsite consisted of a torus placed in the outer part of the shrub canopy whose width was the 10% of the shrub radius; UC microsite consisted of the area covered by the shrub canopy minus ES microsite area. In each plot, we measured the area covered by each microsite using a grid and counting the number of 10 x 10 cm cells occupied per microsite.

We simultaneously performed an additional vegetation survey to identify key nurse species for plant establishment in these communities. We selected six dominant shrubs in the community, accounting for approximately half of the total abundance of shrubs [Table A3.1]. The choice of species included shrubs with contrasting sizes and life strategies, since we selected four nanophanerophytes and two large chamaephytes (at least 20 cm tall; [Table A3.1]), being three of them gypsophytes (*Gypsophila struthium*, *Ononis tridentata,* and *Helianthemum squamatum*) and three gypsovags (*Cistus clusii*, *Rosmarinus officinalis,* and *Thymus vulgaris*). All selected species occurred at both study sites except *C. clusii*; however, we included it as a target species due to its large abundance in Site 1. We randomly selected 25 individual shrubs per target species and paired adjacent open areas of equal size (Cavieres *et al*., 2014) placed in a random direction at least 50 cm away from any shrub to avoid the shade cast by shrubs [Table A3.2]. We recorded richness (number of species) and abundance (number of individuals of total species) of annuals, perennial seedlings, and perennial adults occurring under the canopy of the target species and in the paired adjacent open areas. For each shrub and the adjacent open area, we determined the sampled area size by measuring the diameter of a ring matching the canopy of the shrub (n=550 rings; 25 pairs plant-open area of each of six target species at Site 1 and 25 pairs plant-open area of each of five target species at Site 2; [Figure A2.2]).

We further investigated whether the roles of shrubs in structuring diversity in gypsum plant communities exceeded the area under canopy, by analyzing the spatial distribution of plant diversity in the vicinity of all shrubs occurring in the community. We randomly arranged six paralleled 250-m linear transects at each study site. Following the line-point intercept sampling method (Goodall, 1952), we recorded all annual and perennial plant species in contact with the transect line at 20 cm intervals (n=7506 points per study site; [Figure A2.2]). We analyzed plant diversity in the vicinity of shrubs with the ISAR (individual species-area relationships) proposed by Wiegand et al. (2007). The $\mathrm{ISAR}_t(d)$ of a target species t is defined as the number of different species present within a distance d from all of the individuals of the target species t along the transect (Arroyo *et al.*, 2015; Wiegand *et al.*, 2007).

$$\mathrm{ISAR}_t(d) = \sum_{j=1}^{N} [1 - P_{tj}(0, d)]$$

where $1 - P_{tj}(0, d)$ is the probability that species j was present within a distance d of individuals of target species t. The $\mathrm{ISAR}_t(d)$ value will be the sum of the probabilities of all the species N present (Wiegand *et al.* 2007). The spatial resolution and thus the minimum spatial scale of the ISAR was 20 cm (equivalent to the spatial intervals in transects). We set a maximum distance d of 4 m (maximum spatial scale), considered an adequate detection range of plant-plant interactions in semi-arid ecosystems (Arroyo *et al.*, 2015; Rayburn and Wiegand, 2012). In order to assess appropriate sample sizes, we computed ISAR only for shrub species having more than 20 individuals per study site.

Microenvironment and soil conditions

We measured surface mechanical resistance ($\mathrm{kg \cdot cm^{-2}}$) as a proxy of soil compaction, soil humidity (volumetric water content, %), and soil temperature (ºC) to assess the potential amelioration in micro-environmental conditions under target species canopies compared to open areas. At each study site, we measured these physical soil properties under the canopy of 15 random individuals of the same target species previously selected for vegetation surveys (15 points under each of six target species at Site 1 and 15 points under each of five target species at Site 2), and at 15 random points in open areas (n = 195). We measured soil surface mechanical resistance with a force gauge equipped with a compression plate with a diameter of 2 cm (MECMESIN Basic Force Gauge 500N). We measured soil volumetric water content (6 cm maximum depth) with a ML3 Theta-Probe soil moisture sensor (Delta-T Devices). Finally, we measured soil temperature (6 cm deep) with a T-bar digital stem thermometer (ATM Ltd ST-9265A). We measured soil physical properties on a typical spring day with moderate temperatures (average temperature of 15 ºC), three days after a rainfall event [Figure A2.3].

In addition, we analyzed the chemical properties of the soil under the target species canopies to assess the potential enriched nutrients content compared to open areas. We collected soil samples under the canopy of five random individuals of the same target species (5 samples under each of six target species at Site 1 and 5 samples under each of five target species at Site 2) and at five random points in open areas at each study site (n=65). We randomly collected and combined three soil cores (4 x 4 cm surface and 7.5 cm deep) per sample. Soil samples were dried and sieved over a 2 mm mesh sieve and analyzed in the laboratory for available phosphorus, total organic carbon, total carbon, and total nitrogen. We estimated available phosphorus extracted with Bray nº1 reagent (Bray and Kurtz, 1945) using a spectrometer (UNICAM 8625 UV/Vis Spectrometer) with the absorbance at 430 nm. We estimated total organic carbon in samples treated by chromatic acid digestion (Heanes, 1984), using the spectrometer with the absorbance at 590 nm. Finally, we measured total carbon and total nitrogen in samples ground to a fine particle size, using a Vario MAX CN analyzer (Elementar Vario MAX CN).

Data analyses

For every recorded species and ontogenetic stage, we compared the observed frequency per microsite (OA, ES and UC microsites) to the expected frequency per microsite at each study site. We estimated the expected frequency of a species per microsite as the total observed frequency of the species (seedlings and adults separately) multiplied by the proportion of the area covered by each microsite. We performed G-tests to assess whether the observed frequencies of each perennial species (seedlings and adults separately) matched their expected frequencies per microsite at each study site.

$$G = 2\sum_{i}^{N} Oi \ln\frac{Oi}{Ei}$$

where *N* is the total number of observations, *Oi* is the observed frequency for each value *i* and *Ei* is the expected frequency for each given value *i* under the null hypotheses (Sokal and Rohlf, 1995). When significant differences were found among microsites

($p \leq 0.05$), we performed post-hoc pairwise G-tests to compare the observed frequencies with the expected frequencies specifically at each microsite. We analyzed differences in richness and abundance of annuals, perennial seedlings and perennial adults separately (six dependent variables in total) under target species canopies (*G. struthium*, *O. tridentata*, *H. squamatum*, *C. clusii*, *R. officinalis,* and *T. vulgaris*) compared to open areas by fitting Generalized Linear Mixed Models (GLMMs). We fitted one separate GLMM per dependent variable. Target species, study site and the covariate sampled area (*i.e.*, canopy area) were included as fixed factors and the pair plant-open area was included as a random effect to control for local spatial heterogeneities. Study site should be considered as a random effect in the GLMM; however, we set it as a fixed factor because we only have two levels of this factor (Gelman and Hill, 2006). Poisson error distribution and log link function were assumed.

To detect whether the ISAR of a species was significantly different than expected, a confidence envelope was calculated using Monte Carlo simulations of 199 Poisson null models (Wiegand *et al.*, 2007). Then, if a species showed the ISAR value for a given distance *d* greater than the 97.5 percentile of the confidence interval from simulations, the species presented higher species richness at that distance, and it was considered a diversity accumulator. Conversely, if a species showed the ISAR value for a given distance *d* lower than the 2.5 percentile of the confidence interval from simulations, the species presented a lower species richness at that distance, and it was considered a diversity repeller. When a species showed the ISAR value within the confidence envelope at a given distance *d*, it was considered neutral (Arroyo *et al.*, 2015; Chacón-Labella *et al.*, 2016; Perry *et al.*, 2016; Rayburn and Wiegand, 2012; Wiegand *et al.*, 2007). For gypsophyte and gypsovag perennial species separately, we determined the percentage of cases, out of the total of times the species occurred at both study sites, in which they acted as diversity accumulators, diversity repellers and neutrals at each distance *d* (Chacón-Labella *et al.*, 2016; Perry *et al.*, 2016; Wiegand *et al.*, 2007).

Differences in microenvironmental and physical-chemical soil conditions (surface mechanical resistance, humidity, temperature, available phosphorous, total organic carbon, total carbon and total nitrogen) under target species canopies (*G. struthium*, *O. tridentata*, *H. squamatum*, *C. clusii*, *R. officinalis* and *T. vulgaris*) compared to open areas were tested with two-way ANOVAs considering target species and study site as factors.

Statistical analyses were performed in R (R Development Core Team, 2014), except ISAR analyses and Monte Carlo simulations, which were performed in MATLAB R2010b.

Results

Microsites where perennial plants establish

In general, at the seedling stage, gypsophytes appeared less frequently than expected in open areas (OA microsite), especially *Gypsophila struthium* and *Ononis tridentata* (Table 1). In contrast, seedlings of gypsophytes generally appeared more frequently than expected at the edge of adult shrubs (ES microsite). The species-by-species analysis showed that this trend was held for *Helianthemum squamatum*, *Hermiaria fruticosa* and *O. tridentata*, depending on the study site (Table 1, [Table A4.1 and Table A4.2]). Seedlings of gypsophytes appeared under the canopy of shrubs (UC microsite) more frequently than expected in Site 1 and less frequently than expected in Site 2 (Table 1, [Table A4.1 and Table A4.2]). As observed in seedlings, adults of gypsophytes also appeared less frequently than expected at OA microsite (*G. struthium* and *O. tridentata*, Table 1), and more frequently than expected at ES microsite (*G. struthium*, *H. squamatum*, *Lepidium subulatum,* and *O. tridentata*, Table 1). Differently to seedlings, adults of gypsophytes generally appeared less frequently than expected at UC microsite in both study sites (Table 1, [Table A4.1 and A4.2]).

Seedlings of gypsovags showed the same trend than seedlings of gypsophytes, appearing less frequently than expected at OA microsite and more frequently than expected at ES microsite. The species-by-species analysis showed that, in both microsites, this trend was held for 30 % of gypsovag species (Table 1). Seedlings of gypsovags appeared at UC microsite more frequently than expected in Site 1 and as frequently as expected in Site 2 (Table 1, [Table A4.1 and Table A4.2]). Gypsovags at the adult stage also appeared less frequently than expected at OA microsite (67 % of the gypsovag species; Table 1) and more frequently than expected at ES microsite (59% of the gypsovag species; Table 1). Adult gypsovags generally appeared as frequently as expected at UC microsite (59% of the gypsovag species; Table 1).

Richness and abundance under the canopy of the target shrubs

Richness and abundance of annuals under shrub canopies differed significantly from open areas except for *H. squamatum* and *T. vulgaris* (Table 2 and Table 3). We also found significant differences in richness and abundance of perennial seedlings under shrub canopies compared to open areas except for *H. squamatum* (Table 2 and Table 3). Regarding perennial adults, richness and abundance under shrub canopies differed significantly from open areas except for *H. squamatum* concerning richness (Table 2), and except for *H. squamatum* and *T. vulgaris* concerning abundance (Table 3). In summary, target species *G. struthium*, *O. tridentata*, *C. clusii,* and *R. officinalis* had significant effects compared to open areas on richness and abundance for all plant categories, having *G. struthium* and *R. officinalis* the largest and smallest effects respectively (Z scores in GLMM; Table 2 and Table 3).

The richness of annuals under *G. struthium* and *O. tridentata* canopies and richness of perennial seedlings under *R. officinalis* canopy differed between study sites (Table 2), being higher in Site 2 than in Site 1 [Figure A5.1]. The abundance of annuals under *G. struthium*, *O. tridentata* and *T. vulgaris* canopies, perennial seedlings under *O. tridentata*, *H. squamatum* and *T. vulgaris* canopies, and perennial adults under *O. tridentata* canopy also depended on the study site (Table 3). Abundance under these target species was higher in Site 2 than in Site 1, except for annuals under *T. vulgaris* canopy [Figure A5.1].

The richness of annuals under *T. vulgaris* canopy, the richness of perennial seedlings under *G. struthium* canopy, and richness of perennial adults under *G. struthium*, *O. tridentata* and *R. officinalis* canopies depended on the sampled area (Table 2). The sampled area also had significant effects on abundance of annuals under shrub canopies, except for *H. squamatum* and *R. officinalis*, on the abundance of perennial seedlings under *G. struthium* and *C. clusii* canopies, and on the abundance of perennial adults under *G. struthium*, *O. tridentata* and *R. officinalis* canopies (Table 3). In all cases, richness and abundance were higher when the sampled area under these target species was larger [Figure A5.2].

Table 1 Summary of the results of the G-tests implemented at the two study sites to evaluate significant differences between the observed frequencies and the expected frequencies of all perennial species (seedlings and adults separately) at each microsite: in open areas (OA), at the edge of adult shrubs (ES) and under the canopy of adult shrubs (UC).

	Seedlings			**Adults**		
	OA	**ES**	**UC**	**OA**	**ES**	**UC**
Gypsophytes	-	+	~	-	+	-
Gypsophila struthium	-	0	+	-	+	~
Helianthemum squamatum	~	~	~	~	+	~
Herniaria fruticosa	0	~	~	~	~	~
Lepidium subulatum	0	0	0	0	+	0
Ononis tridentata	-	~	~	-	+	0
Gypsovags	-	+	~	-	+	0
Agropyron cristatum	n.a.	n.a.	n.a.	0	0	0
Brachypodium retusum	0	0	0	~	~	~
Carex halleriana	n.a.	n.a.	n.a.	0	0	0
Carduus sp.	0	0	0	0	0	0
Carlina corimbosa	n.a.	n.a.	n.a.	0	0	0
Cistus clusii	-	+	0	-	+	0
Coris monspeliensis	0	+	0	-	0	+
Dactylis glomerata	n.a.	n.a.	n.a.	0	0	0
Dorycnium pentaphyllum	0	0	0	-	+	0
Fumana ericoides	0	0	0	-	+	0
Genista scorpius	0	0	0	~	+	0
Helianthemum marifolium	-	+	+	-	+	+
Helianthemum pilosum	0	0	0	-	+	0
Helianthemum syriacum	-	+	~	-	+	-
Helichrysum stoechas	-	+	~	-	+	~
Juniperus thurifera	0	0	0	n.a.	n.a.	n.a.
Koeleria vallesiana	0	0	-	-	+	-
Linum suffruticosum	-	+	0	-	+	0
Lithodora fruticosa	0	0	0	-	0	0
Lygeum spartum	0	0	0	-	+	0
Plantago albicans	+	0	-	-	+	-
Polygala rupestris	~	0	0	-	~	~
Reseda stricta	0	0	0	0	0	0
Rosmarinus officinalis	~	~	0	-	+	~
Stipa lagascae	~	~	~	-	+	+
Teucrium capitatum	-	~	+	-	+	0
Thymelaea tinctoria	n.a.	n.a.	n.a.	0	0	0
Thymus vulgaris	-	+	~	-	+	~

Results are indicated as "+" when the observed frequencies were significantly higher than the expected frequencies wherever it is present, "-" when the observed frequencies were significantly lower than the expected frequencies wherever it is present, "0" when the observed frequencies were similar to the expected frequencies wherever it is present and "~" when the trend differed among study sites. Not available data (n.a.) is indicated when the species was not found within the plots.

Table 2 Summary of the GLMMs implemented to test significant effects ($p \leq 0.05$) of the target species compared to open areas, the study site, the sampled area and the interaction among these factors on richness, for annuals, perennial seedlings and perennial adults.

	Annuals (R^2=0.82)				**Perennial seedlings (R^2=0.67)**				**Perennial adults (R^2=0.69)**			
	Estim.	**SE**	**Z (t)**	**p**	**Estim.**	**SE**	**Z (t)**	**p**	**Estim.**	**SE**	**Z (t)**	**p**
G	2.97	0.52	5.76	**<0.001**	1.77	0.23	7.79	**<0.001**	2.22	0.29	7.64	**<0.001**
O	1.55	0.55	2.80	**<0.01**	1.12	0.23	4.89	**<0.001**	1.81	0.28	6.41	**<0.001**
H	1.74	0.95	1.83	0.067	0.38	0.36	1.06	0.291	0.05	0.67	0.07	0.941
C	0.97	0.38	2.56	**<0.05**	1.29	0.23	5.58	**<0.001**	1.45	0.37	3.94	**<0.001**
R	1.77	0.66	2.66	**<0.01**	0.58	0.31	1.90	**0.054**	1.41	0.39	3.62	**<0.001**
T	-0.63	0.69	-0.91	0.364	0.56	0.20	2.73	**<0.01**	1.02	0.30	3.40	**<0.001**
Site	1.21	0.31	3.87	**<0.001**	-0.68	0.15	-4.66	**<0.001**	-0.63	0.24	-2.65	**<0.01**
Site x G	-1.47	0.63	-2.33	**<0.05**	-0.54	0.32	-1.68	0.093	-0.36	0.42	-0.85	0.398
Site x O	-1.71	0.86	-1.98	**<0.05**	0.55	0.33	1.68	0.094	0.66	0.41	1.61	0.108
Site x H	-1.03	1.09	-0.95	0.342	0.52	0.55	0.94	0.348	-0.19	0.99	-0.19	0.847
Site x C	-	-	-	-	-	-	-	-	-	-	-	-
Site x R	-0.51	0.82	-0.62	0.534	1.21	0.41	2.99	**<0.01**	0.62	0.53	1.18	0.240
Site x T	1.35	0.78	1.73	0.083	0.65	0.35	1.84	0.066	0.07	0.60	0.12	0.904
Area	1.62	0.52	3.11	**<0.01**	1.51	0.19	8.14	**<0.001**	2.50	0.27	9.41	**<0.001**
Area x G	-1.06	0.89	-1.19	0.233	-1.59	0.42	-3.80	**<0.001**	-2.04	0.51	-3.97	**<0.001**
Area x O	0.13	0.99	0.13	0.893	-0.62	0.43	-1.44	0.150	-1.35	0.51	-2.64	**<0.01**
Area x H	-19.72	19.22	-1.03	0.305	4.68	6.21	0.75	0.451	6.43	11.62	0.55	0.580
Area x C	-1.14	1.72	-0.66	0.507	-1.12	0.93	-1.19	0.233	-1.35	1.47	-0.92	0.359
Area x R	-0.79	1.24	-0.64	0.525	-0.41	0.56	-0.73	0.468	-1.79	0.71	-2.51	**<0.05**
Area x T	11.86	5.47	2.17	**<0.05**	1.44	1.99	0.72	0.471	-0.37	2.95	-0.13	0.901

G = *G. struthium,* O = *O. tridentata,* H = *H. squamatum,* C = *C. clusii*, R = *R. officinalis* and T = *T. vulgaris*

Table 3 Summary of the GLMMs implemented to test significant effects ($p \leq 0.05$) of the target species compared to open areas, the study site, the sampled area and the interaction among these factors on abundance, for annuals, perennial seedlings and perennial adults.

	Annuals (R^2=0.95)				**Perennial seedlings (R^2=0.95)**				**Perennial adults (R^2=0.89)**			
	Estim.	**SE**	**Z (t)**	**p**	**Estim.**	**SE**	**Z (t)**	**p**	**Estim.**	**SE**	**Z (t)**	**p**
G	7.81	0.74	10.55	**<0.001**	2.41	0.14	17.74	**<0.001**	2.31	0.21	11.25	**<0.001**
O	4.04	0.56	7.27	**<0.001**	0.99	0.12	8.31	**<0.001**	1.66	0.21	7.78	**<0.001**
H	1.26	1.04	1.21	0.226	0.09	0.33	0.28	0.776	0.74	0.55	1.34	0.180
C	2.55	0.18	14.20	**<0.001**	2.37	0.24	9.93	**<0.001**	1.74	0.38	4.54	**<0.001**
R	2.00	0.66	3.03	**<0.01**	1.59	0.31	5.17	**<0.001**	2.60	0.39	6.74	**<0.001**
T	-0.92	0.76	-1.22	0.222	0.61	0.21	2.85	**<0.01**	0.56	0.36	1.59	0.113
Site	3.10	0.46	6.73	**<0.001**	-1.63	0.20	-8.07	**<0.001**	-1.21	0.25	-4.82	**<0.001**
Site x G	-5.44	0.75	-7.22	**<0.001**	-0.26	0.27	-0.99	0.321	0.10	0.40	0.25	0.800
Site x O	-1.91	0.64	-3.00	**<0.01**	0.61	0.24	2.56	**<0.05**	0.85	0.54	2.16	**<0.05**
Site x H	-1.35	1.13	-1.19	0.233	1.54	0.60	2.58	**<0.01**	-0.35	0.90	-0.39	0.693
Site x C	-	-	-	-	-	-	-	-	-	-	-	-
Site x R	0.28	0.72	0.39	0.699	0.03	0.40	0.08	0.934	-1.03	0.54	-1.90	0.058
Site x T	2.74	0.82	3.36	**<0.001**	1.03	0.41	2.54	**<0.05**	0.56	0.68	0.83	0.408
Area	3.24	0.77	4.22	**<0.001**	1.33	0.30	9.79	**<0.001**	3.82	0.33	11.49	**<0.001**
Area x G	-6.49	1.02	-6.36	**<0.001**	-2.46	0.23	-10.86	**<0.001**	-1.94	0.34	-5.71	**<0.001**
Area x O	-2.40	0.81	-2.25	**<0.05**	-0.19	0.20	-0.95	0.340	-1.82	0.34	-5.27	**<0.001**
Area x H	-10.38	22.53	-0.46	0.645	10.99	5.87	5.87	0.061	-5.44	9.50	-0.57	0.567
Area x C	-4.40	0.66	-6.67	**<0.001**	-4.31	0.85	-5.09	**<0.001**	-1.81	1.51	-1.19	0.233
Area x R	1.73	1.26	1.38	0.169	-0.75	0.85	-1.37	0.170	-3.01	0.68	-4.40	**<0.001**
Area x T	28.91	7.40	3.91	**<0.001**	0.97	0.55	0.41	0.686	-0.80	4.20	-0.19	0.849

G = *G. struthium,* O = *O. tridentata,* H = *H. squamatum,* C = *C. clusii*, R = *R. officinalis* and T = *T. vulgaris*

Spatial distribution of plant diversity in the vicinity of shrubs

Gypsophytes acted as diversity accumulators in up to 57 % of the cases and acted as diversity repellers in 14% of the cases at distances smaller than 100 cm (Figure 26). All gypsophytes acted as neutrals at distances greater than 180 cm (Figure 26). The diversity-accumulator gypsophytes were *G. struthium* and *O. tridentata* in both study sites, while *H. squamatum* acted as diversity repeller at site 2 (Figure 27).

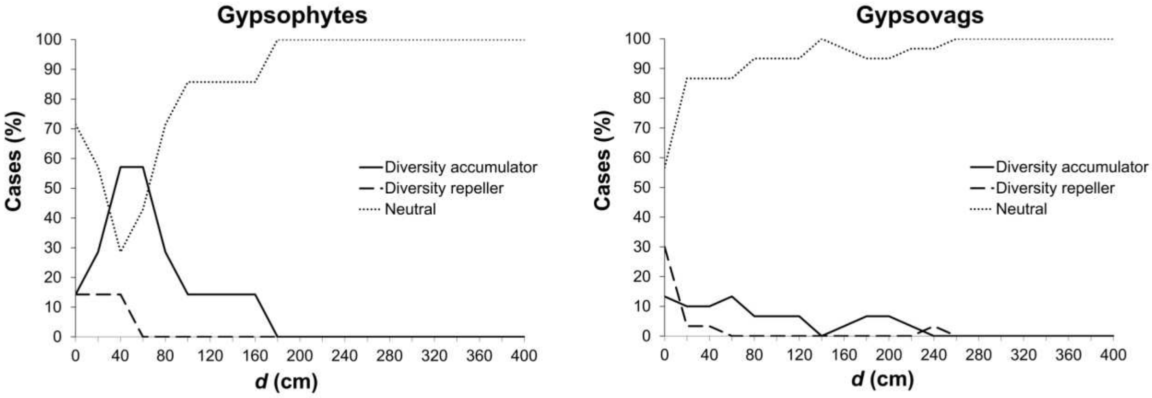

Figure 26 Percentage of cases (out of the total of times they are present at both study sites altogether) in which gypsophyte and gypsovag perennial species acted as diversity repellers, neutrals, and accumulators, at different distances *d* from the target individuals ($d_{\max}$=400 cm). More details in supplementary material [Table A6.1 and Table A6.2].

While diversity-repeller gypsovags overcome diversity-accumulator gypsovags at short distances, being *d* = 0 (the 30 % and the 10% of the gypsovags respectively), the proportion of diversity accumulators overcome diversity repellers at distances greater than 20 cm (Figure 26). Diversity-repeller gypsovags were found at both study sites, but diversity-accumulator gypsovags were found only at Site 1 [Table A6.1 and Table A6.2). The 90% of the gypsovags acted as neutrals at distances greater than 40 cm (Figure 26). Among target species in Site 1, *C. clusii* and *R. officinalis* acted as diversity repellers at short distances, but *T. vulgaris* acted as diversity accumulator at distances between 40 and 120 cm (Figure 27). Only the target gypsovag *T. vulgaris* was occurring in Site 2, where it acted as a diversity repeller at distances smaller than 40 cm (Figure 27).

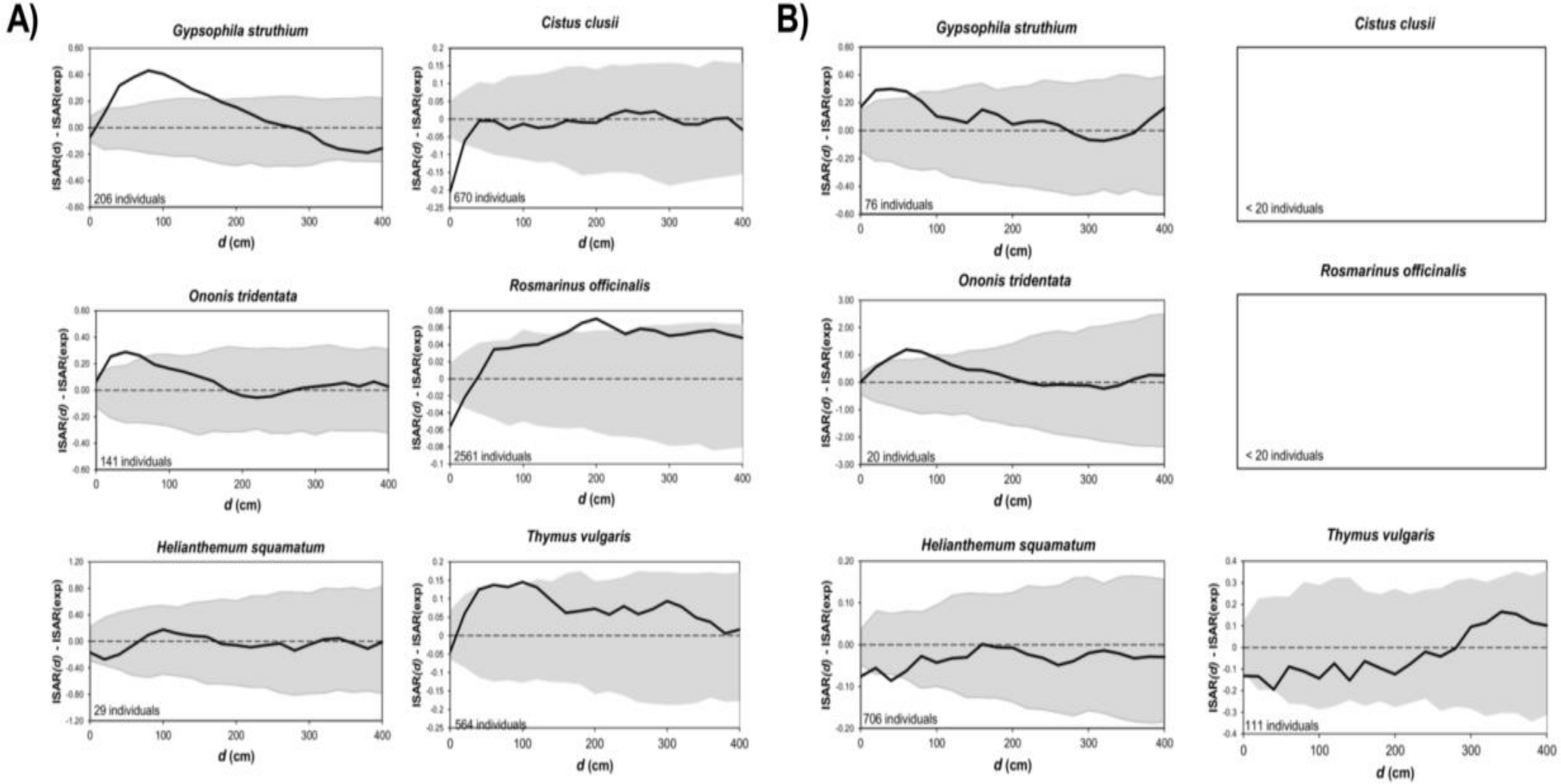

Figure 27 ISAR curves of target shrubs with more than 20 individuals in **A)** Site 1 and **B)** Site 2. When ISAR curve is represented above the confidence envelope (grey shaded), the species act as diversity accumulator, when it is represented below the confidence envelope, the species act as diversity repeller and when it is represented within the confidence envelope, the species act as neutral.

Microenvironmental and soil conditions

We found significant differences between target species and open areas on all the studied microenvironmental and physical-chemical soil conditions, and these differences depended on the study site except for TOC (Table 4 and Table 5). Surface mechanical resistance was lower under all target species canopies than in open areas, being this trend more evident under *O. tridentata* and *R. officinalis* canopies in Site 1 and under *G. struthium* and *R. officinalis* in Site 2 (Table 4 and Figure 28). In general, soil humidity under target species canopies did not differ from that in open areas (Table 4). Soil temperature was lower under shrub canopies than in open areas, especially under *G. struthium*, *O. tridentata, C. clusii* and *R. officinalis* canopies in Site 1 (Table 4 and Figure 28).

Regarding chemical soil properties, available phosphorus was significantly higher under *O. tridentata*, *R. officinalis* and *T. vulgaris* canopies than in open areas (Table 5 and Figure 28). Available phosphorus was higher in Site 2 than in Site 1, especially in open areas and under *G. struthium* and *H. squamatum* canopies (Figure 28). Total organic carbon and total carbon were higher under the canopy of most target species than in open areas (except for total carbon under *H. squamatum* and *T. vulgaris* canopies), being more evident under *G. struthium*, *C. clusii* and *R. officinalis* canopies in Site 1 (Table 5 and Figure 28). Total nitrogen was higher under all target species canopies than in open areas, being more evident under *G. struthium*, *C. clusii* and *R. officinalis* canopies in Site 1 (Table 5 and Figure 28).

We considered micro-environmental amelioration when surface mechanical resistance diminished, humidity increased, temperature decreased, and nutrients increased under target species canopies compared to open areas. Target species that showed amelioration in more soil conditions under canopies compared to open areas were *R. officinalis* and *O. tridentata* [Figure A7.1].

Table 4 Summary of the ANOVAs implemented to test significant effects ($p \leq 0.05$) of the target species compared to open areas, the study site, and the interactions between these factors on surface mechanical resistance, soil humidity and soil temperature.

	Surface mechanical resistance (R^2=0.52)				**Soil humidity (R^2=0.46)**				**Soil temperature (R^2=0.55)**			
	Estim.	**SE**	**t**	**p**	**Estim.**	**SE**	**t**	**p**	**Estim.**	**SE**	**t**	**p**
G	-1.85	0.42	-4.40	**<0.001**	-0.23	0.90	-0.25	0.802	-5.23	0.74	-7.11	**<0.001**
O	-2.60	0.42	-6.19	**<0.001**	-0.94	0.90	-1.04	0.299	-6.07	0.74	-8.25	**<0.001**
H	-1.20	0.50	-2.38	**<0.05**	0.22	1.08	0.21	0.837	-1.74	0.88	-1.97	**<0.05**
C	-2.30	0.42	-5.48	**<0.001**	-0.21	0.90	-0.24	0.813	-4.08	0.74	-5.55	**<0.001**
R	-2.81	0.42	-6.69	**<0.001**	-1.79	0.90	-1.98	**<0.05**	-5.29	0.74	-7.19	**<0.001**
T	-2.38	0.42	-5.67	**<0.001**	1.51	0.90	1.67	0.096	-2.87	0.74	-3.90	**<0.001**
Site	1.59	0.43	3.72	**<0.001**	-2.81	0.81	-3.45	**<0.001**	-7.20	0.64	-11.23	**<0.001**
Site x G	-1.30	0.60	-1.50	**<0.05**	-2.93	1.21	-2.41	**<0.05**	5.72	0.98	5.86	**<0.001**
Site x O	-0.34	0.60	-0.56	0.575	-2.01	1.21	-1.66	0.099	6.45	0.98	6.61	**<0.001**
Site x H	-0.99	0.67	-1.50	0.136	-1.99	1.36	-1.47	0.144	2.69	1.10	2.45	**<0.05**
Site x C	-	-	-	-	-	-	-	-	-	-	-	-
Site x R	-0.55	0.56	-0.98	0.328	1.95	1.13	1.73	0.085	4.81	0.90	5.32	**<0.001**
Site x T	0.62	0.60	1.04	0.299	-2.57	1.21	-2.11	**<0.05**	4.04	0.98	4.14	**<0.001**

G = *G. struthium,* O = *O. tridentata,* H = *H. squamatum,* C = *C. clusii*, R = *R. officinalis* and T = *T. vulgaris*

Table 5 Summary of the ANOVAs implemented to test significant effects (p≤0.05) of the target species compared to open areas, the study site, and the interactions between these factors on chemical soil properties.

	AP **(R^2=0.94)**				**TOC** **(R^2=0.65)**				**TC** **(R^2=0.61)**				**TN** **(R^2=0.59)**			
	Estim.	**SE**	**t**	**p**	**Estim.**	**SE**	**t**	**p**	**Estim.**	**SE**	**t**	**p**	**Estim.**	**SE**	**t**	**p**
G	-4.60	6.65	-0.69	0.492	2.53	0.52	4.90	**<0.001**	5.20	1.22	4.27	**<0.001**	0.28	0.06	4.36	**<0.001**
O	43.36	6.65	6.52	**<0.001**	1.36	0.52	2.64	**<0.05**	2.06	1.22	1.69	**0.097**	0.15	0.06	2.38	**<0.05**
H	-8.75	6.65	-1.32	0.194	1.33	0.52	2.58	**<0.05**	1.99	1.22	1.63	0.109	0.13	0.06	1.99	**0.05**
C	5.17	6.65	0.78	0.440	2.98	0.52	5.79	**<0.001**	5.96	1.22	4.89	**<0.001**	0.32	0.06	4.97	**<0.001**
R	60.64	6.65	9.12	**<0.001**	3.35	0.52	6.50	**<0.001**	7.21	1.22	5.92	**<0.001**	0.37	0.06	5.83	**<0.001**
T	61.52	6.65	9.25	**<0.001**	1.57	0.52	3.05	**<0.01**	2.09	1.22	1.72	0.092	0.13	0.06	2.11	**<0.05**
Site	67.93	6.65	10.22	**<0.001**	-0.24	0.52	-0.47	0.638	-0.36	1.22	-0.29	0.772	-0.01	0.06	-0.15	0.880
Site x G	9.03	9.41	0.96	0.341	-0.59	0.73	-0.81	0.421	-0.76	1.72	-0.44	0.662	-0.03	0.09	-0.35	0.726
Site x O	-25.10	9.41	-2.67	**<0.05**	0.38	0.73	0.52	0.604	1.28	1.72	0.74	0.461	0.06	0.09	0.64	0.526
Site x H	17.45	9.41	1.86	0.069	0.36	0.73	0.49	0.625	1.86	1.72	1.08	0.287	0.07	0.09	0.75	0.455
Site x C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Site x R	-32.08	9.41	-3.41	**<0.01**	-1.52	0.73	-2.09	**<0.05**	-3.64	1.72	-2.11	**<0.05**	-0.22	0.09	-2.45	**<0.05**
Site x T	-44.75	9.41	-4.76	**<0.001**	-0.73	0.73	-1.00	0.320	-0.77	1.72	-0.45	0.658	-0.04	0.09	-0.46	0.648

G = *G. struthium,* O = *O. tridentata,* H = *H. squamatum,* C = *C. clusii*, R = *R. officinalis*, T = *T. vulgaris,* AP = available phosphorus, TOC = total organic carbon, TC = total carbon and TN = total nitrogen.

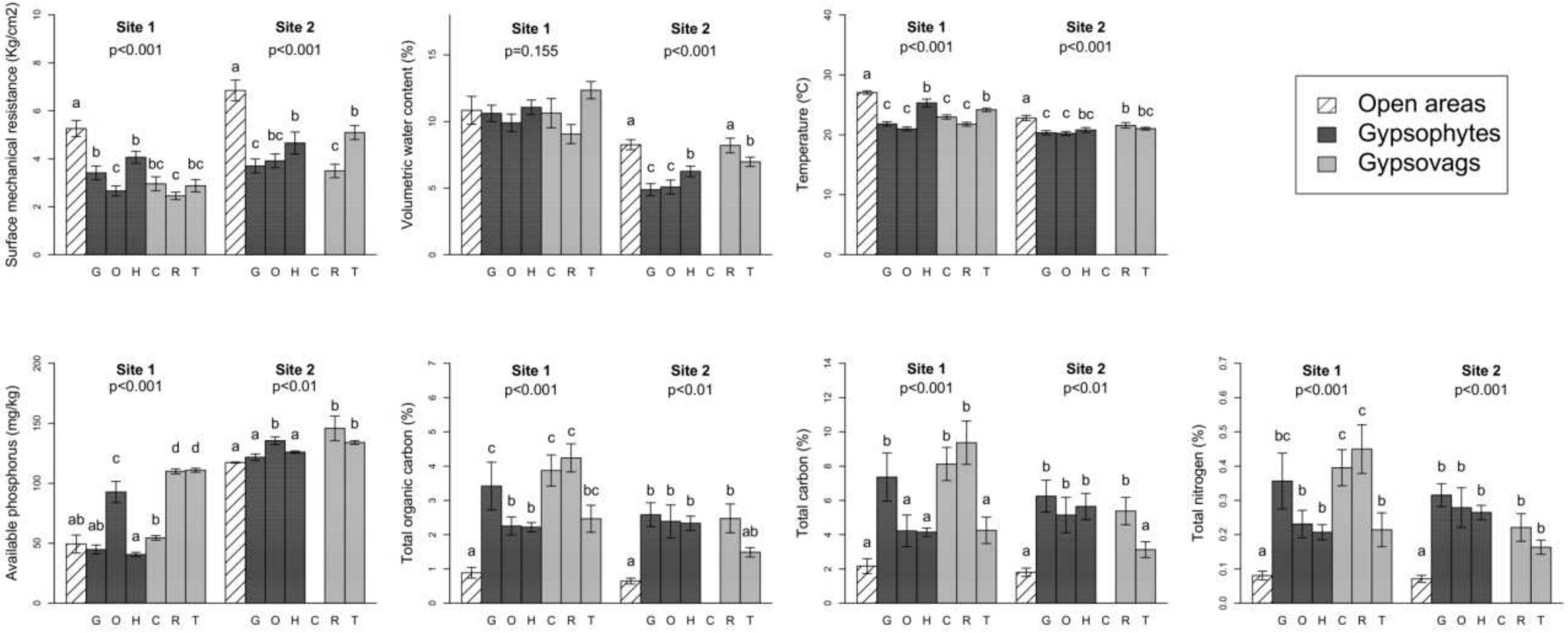

Figure 28 Barplots representing mean values of the microenvironmental and physical-chemical soil conditions (surface mechanical resistance, humidity, temperature, available phosphorus, total organic carbon, total carbon and total nitrogen) in open areas and under the canopy of the target species (G = *G. struthium*, O = *O. tridentata*, H = *H. squamatum*, C = *C. clusii*, R = *R. officinalis*, and T = *T. vulgaris*) at each study site. Different letters indicate significant differences among target species after pairwise Tukey's post-hoc tests when p≤0.05 in ANOVAs fitted per study site.

Discussion

Our results highlight the relevance of being spatially associated to other plants at establishment stage in gypsum plant communities, underlining the role of some key nurse species in structuring plant diversity in their local vicinity by facilitating the establishment of other species. Following other studies, we inferred biotic interactions from spatial patterns of plants in the community (Arroyo *et al.*, 2015; Cavieres *et al.*, 2006; Saiz and Alados, 2012; Soliveres and Maestre, 2014). It is challenging to infer biotic interactions at the community level based on observational data. The reason is that plant spatial patterns are the result of biotic interactions acting together with other biotic and abiotic factors, as seed dispersal patterns or environmental heterogeneity (Ramón *et al.*, 2018; Escudero *et al.*, 2005). However, when species aggregate in space more often than expected by chance, it can be considered that these species benefit from aggregation, assuming positive biotic interactions among them (Saiz and Alados, 2012).

It is well known that facilitative interactions are crucial for seedling establishment, particularly in stressful environments (Soliveres and Maestre, 2014) where harsh conditions hamper plant establishment (Escudero *et al.*, 2015). In arid and semi-arid environments, most seedlings are not able to survive in open areas, as they are often subjected to potentially lethal temperatures and intense dryness (Flores and Jurado, 2003). Our data showed that seedlings mainly established in microsites associated with adult shrubs more often than expected by chance. We expected that the establishment of gypsovags would be facilitated to a greater extent than gypsophytes, which might be able to establish in open areas because of their ability to tolerate the harsh conditions inherent to gypsum environments (Escudero *et al.*, 1999; Escudero *et al.*, 2015; Palacio *et al.*, 2014; Romão and Escudero, 2005). Contrary to our expectations, gypsophytes were also found close to adult shrubs at frequencies higher than expected. These observations further reinforce the importance of facilitation for plant establishment in semi-arid gypsum plant communities, regardless of plant life strategies.

Although proximity to nurse plants may enhance seedling establishment, the positive effects may vary in intensity depending on the relative nurse-facilitated plant position (Reisman-Berman, 2007). Shade provided by shrubs can create favorable microsites for plant establishment (Callaway, 2007), however, limits light availability, which may decrease understory plant performance (Valladares and Pearcy, 2002). Furthermore, facilitated plants development may be at risk due to competition with nurse plants for water or nutrients (Coomes and Grubb, 2000). The results of this study suggest that the most favorable microsite for plant establishment is at the edge of the canopy of adult shrubs, where most gypsophytes and gypsovags were found at frequencies higher than expected. At this microsite, the likely positive effects of nurse shading overcome competition for water, nutrients and light (Reisman-Berman, 2007). The competitive response of a species may be inverse to its ability to tolerate stress (Liancourt *et al.*, 2005; Maestre *et al.*, 2009). In general, gypsophytes (*i.e.*, stress-adapted) appeared at microsites directly under adult shrubs canopies at frequencies lower than expected, suggesting competitive exclusion by nurse plants. However, some gypsovags also seem to avoid this microsite, indicating that these less adapted plants may be excluded by competition as well.

Generally, shrubs are considered to have a positive role in plant communities (Gómez-Aparicio, 2005). To a lesser or a greater extent, most studied shrub species harbored more richness and abundance than open areas. The target species whose understories harbored the highest richness and abundance were the gypsophytes *G. struthium* and *O. tridentata* (especially for perennials). Other studies support the role of *G. struthium* and *O. tridentata* as nurse species in gypsum plant communities (Navarro-Cano *et al.* 2014, 2016). In the case of *O. tridentata*, it belongs to the family *Fabaceae*, which holds the highest number of nurse species recorded in arid and semi-arid environments (Flores and Jurado, 2003), likely due to N fixation in N-limited ecosystems (Sprent and Gehlot, 2010). Indeed, our results showed that the species harboring the highest richness and abundance also have enhanced physical-chemical soil conditions under canopy compared to open areas (*e.g.,* total nitrogen; [Figures A7.2 to A7.13]. Nevertheless, we are aware that observational data provided are not sufficient to detect the abiotic factors driving facilitation, but further investigation is needed.

Gypsovags also harbored more richness and abundance of plants under canopies than open areas, but to a lesser extent than gypsophytes. For example, the gypsovag *R. officinalis* showed a weaker effect on richness and abundance than other shrubs with similar physiognomy, such as the gypsophytes *G. struthium* and *O. tridentata*. Some of the most abundant gypsovag shrubs that are present in gypsum plant communities of the Middle Ebro Valley have demonstrated allelopathic activity (*i.e.*, *R. officinalis* and *T. vulgaris;* Vokou *et al.*, 1993; Thompson *et al.*, 2003). Allelopathy could be a mechanism of less adapted plants (*e.g.*, gypsovags) to avoid competition for scarce resources and succeed in gypsum plant communities. Nevertheless, this idea would need to be tested in other gypsum areas to ascertain whether this phenomenon is either species-specific or relies on life strategies.

Plant interactions play an important role in structuring plant communities in arid and semi-arid environments (Saiz *et al.* 2014, Arroyo *et al.* 2015, Chacón-Labella *et al.* 2016, Perry *et al.* 2016). The presence of key nurse species in the community is reflected in the accumulation of plant species forming species-rich areas (Soliveres et al., 2011), whereas the presence of highly competitive or allelopathic plants is reflected in a decrease of plant diversity in the local vicinity (Arroyo *et al.*, 2015). We found two dominant nurse species in gypsum plant communities of the Middle Ebro Valley (the gypsophytes *G. struthium* and *O. tridentata*), which had an important role in plant spatial structure by accumulating plants in the local vicinity. Similar findings were obtained by Saiz *et al.* (2014) in the same gypsum plant communities following different methodologies. On the other hand, our study showed that 30% of gypsovags acted as diversity repellers at short distances, especially the most abundant gypsovag shrubs in these communities (*i.e.*, *C. clusii* and *R. officinalis*). However, in line with the findings of Perry *et al.* (2016), only a few species acted as either accumulators or repellers in our study site compared to neutrals.

We found that plant spatial associations differed between study sites. Aridity conditions differ between study sites, showing Site 2 higher aridity, and consequently providing more stressful conditions for plant establishment. Taking our findings together, we found greater nurse effects and greater diversity accumulation in the local vicinity of gypsophytes in the least arid site (Site 1), and greater diversity repulsion by gypsovags in the most arid site (Site 2). These findings suggest a shift in biotic interaction outcomes from net facilitative effect towards interference along with stress increment, as reported by numerous studies (Maestre *et al.*, 2009; Saiz *et al.*, 2014; Soliveres and Maestre, 2014). Nevertheless, this result must be taken with caution, as only two study sites along an aridity gradient were considered (Maestre *et al.*, 2006).

In conclusion, this observational study at community level revealed that in such stressful environments most plants were spatially associated with adult shrubs and therefore required being facilitated to become established. The survey of species richness and abundance under the canopy of the most abundant shrubs reported that plant establishment and survival were facilitated mostly by adult gypsophytes, especially by *G. struthium* and *O. tridentata*. Facilitation mediated by gypsophytes led to species enrichment in their vicinities, thus structuring plant diversity in species-rich areas around gypsophytes. Therefore, our study confirmed that gypsophytes play key roles in the spatial patterning of plant establishment in gypsum plant communities of the Middle Ebro Valley. Further research should test the observed patterns of plant establishment and the role of substrate-specialists in different gypsum plant communities to verify the generalization of our findings. Besides, testing our hypotheses by controlled experiments would help to unravel the underlying mechanisms driving the observed patterns.

Detalle de semillas de *Rosmarinus officinalis*

Cianotipia elaborada por Ángeles Muñoz
Fotografía original: Ana Foronda

Chapter 2

Implications of the seed sink and source roles of shrubs for the spatial structure of the soil seed bank in a semi-arid gypsum plant community

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Plant Ecology (in rev.)

Abstract

The soil seed bank is crucial for the stability and regeneration of the specialized gypsum plant communities. The presence of shrubs influences the spatial structure of the soil seed bank by trapping more or fewer seeds depending on their physiognomic attributes and by providing seeds through the plants established under canopies. We aimed to unravel the potential role of different shrub species with diverse physiognomy in determining the spatial structure of the soil seed bank in a semi-arid gypsum plant community of NE Spain, by acting as either seed sinks or seed sources. We examined richness and abundance of the soil seed bank at different microsites associated with four dominant shrubs of different size-type (tall or short), and architecture (cushion-like or erect). Also, we analyzed the similarity in species composition between the soil seed bank and the aboveground vegetation under shrub canopies to unravel the potential roles as seed sinks or seed sources. We found larger richness and abundance of seeds within shrub canopies than in open areas. Specifically, the tall cushion-like shrub *Gypsophila struthium* accumulated the richest and most abundant soil seed bank underneath. This soil seed bank was more similar in species composition with the aboveground vegetation than that found under other shrubs, suggesting a seed source effect through the plants it harbored. Conservation and restoration efforts should focus on *Gypsophila struthium*, which can enhance community stability and regeneration through the formation of a diverse and abundant soil seed bank.

Keywords: complete seed bank; *Gypsophila struthium* Loefl. subsp. *hispanica* (Willk.) G. López; persistent seed bank; seed sink; seed source; species similarity.

Introduction

The soil seed bank is an essential component of plant communities since it constitutes a reservoir of viable propagules in the soil in a dormant state until environmental conditions become favorable to germination (Fenner 2012). It promotes diversity in plant communities acting as a temporary buffer against unfavorable conditions that decrease plant survival and seed production (Venable and Brown, 1988). Thus, the soil seed bank contributes to community regeneration processes (Luzuriaga *et al.*, 2005; Martinez-Duro *et al.*, 2009, 2012; Olano *et al.*, 2012) and hence is relevant for plant community stability through time (Mall and Singh, 2014). In arid and semi-arid environments, where plants are subjected to high environmental stochasticity due to unpredictable water inputs (Noy-Meir, 1973), the formation of a robust soil seed bank is crucial for plant community endurance (Luzuriaga *et al.*, 2005; Olano *et al.*, 2012). Specifically, semi-arid gypsum environments harbor rare and specialized plant communities whose dynamics often rely on the formation of a large seed bank (Aragón *et al.*, 2007; Caballero *et al.*, 2003). Therefore, a proper conservation and restoration management of gypsum ecosystems requires a better understanding of the processes shaping the soil seed bank in these plant communities (Martinez-Duro *et al.*, 2012; Olano *et al.*, 2012).

In arid and semi-arid plant communities, the typical island-like spatial distribution of shrubs (Maestre and Cortina, 2005) influences the spatial distribution of the soil seed bank (Pugnaire and Lázaro 2000; Caballero *et al.* 2008a; López-Peralta *et al.* 2016). Shrubs can act as seed sinks by physically obstructing and accumulating seeds transported horizontally either by wind or water due to a decrease in flowing speed compared to open areas (Aerts *et al.*, 2006; Bullock and Moy, 2004; Nathan *et al.*, 2002; Thiede and Augspurger, 1996). The physiognomic attributes of shrubs may influence the seed trapping and accumulation by, for example, intercepting more wind-dispersal seeds as the taller the plant is and retaining more seeds as the denser and more crawling the branches are (Aerts *et al.*, 2006; Bullock and Moy, 2004; Thiede and Augspurger, 1996). Moreover, the cumulative effect of shrubs can be reinforced by the physical protection of seeds from predators (Smit *et al.*, 2008) and also because shrubs may be used as perches by birds that deposit seeds in the surroundings (Pausas *et al.*, 2006). On the other hand, shrubs often harbor a high plant diversity under canopies

(Soliveres *et al.*, 2011) by creating favorable microhabitats for plant establishment and protecting seedlings from grazing and trampling (Callaway, 2007a). These diversity patches can act as seed sources (Caballero *et al.* 2008a), accumulating a vast amount of seeds in the vicinity due to short-range seed dispersal typical in these communities (Martinez-Duro *et al.*, 2012; Olano *et al.*, 2005). Therefore, the sink and source effects of shrubs favor the formation of a particularly dense seed bank in their vicinity, showing a gradual decline from the shrub center to the peripheral areas (Bullock and Moy 2004; Caballero *et al.* 2008a). However, our understanding of the sink or source role that shrub species with different size and architecture have on the spatial patterning of the soil seed bank in gypsum plant communities is still limited.

Several studies have contributed to the understanding of the dynamics of the soil seed bank and the spatial relationships with the above-ground vegetation in gypsum plant communities (Caballero *et al.* 2003, 2005; Olano *et al.* 2005; Caballero *et al.* 2008b; López-Peralta *et al.* 2016). However, many of these studies are mainly focused on annuals-rich plant communities, whose persistence depends entirely on seed production (García and Zamora, 2003), shaping robust but transient soil seed banks that generally germinate within a year of initial dispersal (Thompson, 2000). Less is known about the spatial patterning of soil seed banks composed predominantly of perennials, whose population dynamics do not rely so strongly on seed production (García and Zamora, 2003). Seeds of perennials can remain in a dormant state for more than one year thus shaping persistent soil seed banks (Leck, 2012; Thompson, 2000), likely spatially structured by secondary dispersion to a greater extent than transient seed banks (Nathan and Muller-Landau, 2000).

This study aimed to unravel the potential role of different shrub species with diverse physiognomy in determining the spatial structure of the soil seed bank in a semi-arid gypsum plant community of NE Spain, by acting as either seed sinks or seed sources. To do so, we sampled the complete and the persistent soil seed bank from the center to nearby open areas of four dominant shrub species in the community, with contrasting size-type (tall *versus* short) and architecture (cushion-like *versus* erect). We hypothesized that richness and abundance of the soil seed bank would be higher under shrub canopies than in open areas because of their role as seed sinks and seed sources. Specifically, we expected to find the highest richness and abundance of seeds associated with tall shrubs with cushion-like architecture because they may be better able to intercept wind-dispersal seeds and retain them through crawling branches. To discern whether shrubs acted as seed sinks or seed sources, we assessed similarities in species composition between the local soil seed bank and the above-ground vegetation harbored under shrub canopies. We considered that shrubs acted as seed sources when species composition between the soil seed bank and the above-ground vegetation was similar. We investigated the soil seed bank of annuals and perennials separately to detect possible differences in their spatial structure.

Methods

Study area

We conducted the study in the Middle Ebro Valley (NE Spain), which encompasses one of the most massive gypsum outcrops in Europe (Machín and Navas, 1998). The lithology in this area is mainly gypsum alternating with marls, limestones, and clays (Quirantes, 1978). This area has a semi-arid Mediterranean climate with high continental influence (Creus and Ferraz, 1995). The landscape is characterized by low hills and flat-bottomed areas with traditional agro-pastoral use, consisting mainly of cereal crops and extensive sheep livestock (Pueyo and Alados, 2007).

Specifically, we performed the study in “La Lomaza de Belchite” Wildlife Reserve (41º23’33” N 0º42’18” W, 410 m a.s.l.), which consists of a low gypsum hill protected from agro-pastoral activities. The average annual temperature in the study site is 14.7 ºC, and average annual precipitation is 302 mm·yr^{-1}, with the main rainfall events occurring in May and November (“Z02 Belchite” meteorological station; 2004-2017 period; SIAR-Ministerio de Agricultura, Pesca, Alimentación y Medio Ambiente; http://www.siar.es). Plant community in the study site consists of a patchy scrubland-grassland with a predominance of shrubs, subshrubs, and perennial grasses, together with annual forbs and annual grasses [Table A1.1 and Table A8.1]. Many of the species are substrate-specialists (*i.e.*, gypsophytes) as *Gypsophila struthium* Loefl. subsp. *hispanica* (Willk.) G. López, *Ononis tridentata* L., *Helianthemum squamatum* (L.) Pers. and *Herniaria fruticosa* L. (Mota *et al.*, 2011).

Target species

We selected as target species two of the most abundant tall shrubs and two of the most abundant short shrubs, which accounted for a relative abundance of 45 % among shrubs in the study site [Table A3.1, Site 2]. We selected one species with cushion-like architecture and one species with erect architecture per size-type (Figure 29). Target species were a) *Gypsophila struthium* subsp. *hispanica* (*Caryophyllaceae*), a 47 ± 3 cm tall gypsophilous cushion-like nanophanerophyte; b) *Ononis tridentata* (*Fabaceae*), a 53 ± 2 cm tall gypsophilous erect nanophanerophyte; c) *Helianthemum squamatum* (*Cistaceae*), a 21 ± 1 cm tall gypsophilous cushion-like chamaephyte and d) *Thymus vulgaris* L. (*Labiatae*), a 25 ± 1 cm tall non-gypsophilous erect chamaephyte [Table A8.2].

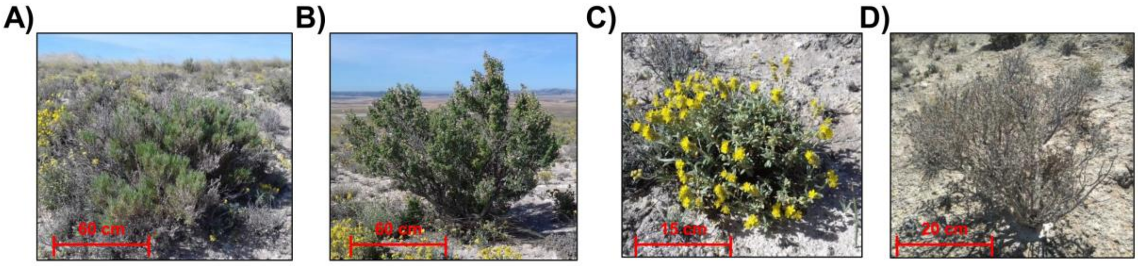


Figure 29 Target species: **A)** *Gypsophila struthium* Loefl. subsp. *hispanica* (Willk.) G. López; **B)** *Ononis tridentata* L.; **C)** *Helianthemum squamatum* (L.) Pers.; and **D)** *Thymus vulgaris* L.

Soil seed bank survey

We collected soil cores in three microsites from the center to peripheral areas of 25 random individuals per target species. Microsites were a) under the shrub canopy, almost in the center of the shrub (UC); b) at the edge of the shrub, whose width was considered the 10% of the canopy radius (ES); and c) open areas not covered by perennial plants, 50 cm away from the edge of the target species (OA). We vertically collected 10 cm deep soil cores (3.5 cm diameter), considered a sufficient depth for sampling the entire seed bank in drylands (Guo *et al.*, 1998). We collected soil samples in winter (February 2015), after the autumn germination peak typical in gypsum communities (Escudero *et al.*, 1997), to quantify the persistent seed bank, and in late summer (September 2015), after seeds shedding, to quantify the complete soil seed bank (Caballero *et al.* 2005). Samples were collected in the prevailing windward direction (W-NW) to account for wind-dispersal seeds (Bullock and Moy, 2004). We measured the height (m) of the target species in each of the 25 individuals in both sampling periods.

To quantify seeds in the soil samples (n = 600 samples = 4 target species x 25 individuals x 3 microsites x 2 sampling periods), we used the seedling emergence method (Heerdt *et al.*, 1996), which enables the identification of only viable seeds (active seed bank; Csontos 2007). Soil samples were kept in airtight plastic bags and stored in a cold chamber at 4°C until we set them in a greenhouse for seeds germination (March 2015 and March 2016 for winter and summer samples respectively). We first soaked the soil samples for ten minutes in a $NaHCO_3$ solution (70 $g \cdot l^{-1}$) for clay disaggregation, and then washed and sieved them over a 4 mm mesh to remove the coarse fraction of the soil and pieces of roots, branches or leaves. To obtain seed-rich samples, we re-sieved samples over a 0.25 mm mesh, which was small enough to retain seeds of all species living in the community [Table A8.1]. Sieving may produce seeds scarification, favoring the germination of hard-coated seeds (Albert *et al.*, 2002; Pérez-García and González-Benito, 2006). We arranged the resulting samples in 23 × 9 × 7 cm trays, using one tray per soil core but differentiating between D1 and D2 depths. We filled the trays with a commercial substrate (70% white peat and 30% pine forest soil) to provide support for the emerged seedlings. To prevent the emergence of potential germinated seeds from the substrate, we laid the samples on a 0.25 mesh nylon cloth placed on top of the substrate. Then, we set the trays in the greenhouse (Estación Experimental Aula Dei-CSIC, Zaragoza: 41º43’31”N 0º48’43”W) under controlled temperature regimes (25°C during the day and 15°C during the night) and natural lightning (12-15 daylight hours).

We monitored seedling emergence once a week for 20 weeks (from March to July) to quantify species richness (number of species) and seed abundance (number of seedlings) at each sample. As soon as we identified an emerged seedling to the species level, it was removed from the tray. When identification at species level was not feasible after two weeks of being emerged, seedlings were transplanted into individual pots and allowed to grow. We watered the trays three times a week with fresh water, simulating a soft rain with a showerhead to avoid seedling damage. After 12 weeks, we irrigated the trays once a week for four weeks with a gibberellic acid solution (1 $g \cdot l^{-1}$

GA_3, GIBERLUQ-L) to induce germination of physiologically dormant seeds (Albert *et al.*, 2002). We then monitored seedling emergence until the end of the assay, but germination hardly occurred (< 5 % of the total emerged seedlings were recorded after 12 weeks; [Figure A9.1]).

Vegetation survey

A vegetation survey performed in spring 2014 allowed evaluating the similarity in species composition between the soil seed bank and above-ground vegetation under shrub canopies. We recorded annuals and perennials occurring under the canopy of 25 random individuals per target species ('canopy' microsite = UC + ES microsites). Sampled areas were defined by rings matching the canopy area of each individual (Cavieres *et al.*, 2014). We also surveyed the vegetation in the surrounding open areas ('open' microsite) in paired rings placed in a random direction 50 cm away from each sampled individual. The total number of rings was 200 (4 target species x 25 individuals x 2 microsites).

Data analyses

For the complete and the persistent seed bank separately, we tested significant effects of the microsite (UC, ES, and OA) and the target species (*G. struthium*, *O. tridentata*, *H. squamatum* and *T. vulgaris*) on richness and abundance of both annuals and perennials by fitting Generalized Linear Models (GLMs). Plant height (m) was included as a covariate in the GLMs. We also fitted GLMs to test significant effects of the shrub architecture (erect and cushion-like) per size-type separately (tall and short) on richness and abundance of annuals and perennials. GLMs were fitted with Poisson error distribution and log link function because count data did not meet the assumptions of normality. When we found significant effects, we applied Tukey's post-hoc multiple comparisons. Seeds from the target species recorded in microsites linked to conspecific shrubs were excluded from the analyses because the donation of seeds by parental plants was not an objective of this study.

For each target species, we assessed similarities in species composition between the soil seed bank and the above-ground vegetation. We considered 'canopy' (UC + ES microsites) and 'open' microsites (OA microsite) separately. We assessed similarities in species composition with the Sorensen's index of similarity (hereafter SSI) for presence and absence data (Hopfensperger, 2007). This index was calculated as

$$SSI = \frac{2\,w}{a + b}$$

where w is the total number of shared species at both the soil seed bank and the above-ground vegetation, a is the total number of species in the soil seed bank and b is the total number of species in the above-ground vegetation. A value of SSI equal to 1 means that soil seed bank and above-ground vegetation share all species, while a value of SSI equal to 0 means that both community components do not share any species (Sørensen, 1948). We analyzed differences in SSI among target species with Kruskal-Wallis non-parametric tests because data did not meet the assumptions of normality. When significant differences among target species were found, we performed post-hoc one-tailed multiple comparisons after Kruskal-Wallis (Siegel and Castellan, 1988).

We performed all statistical analyses in R software, using the packages 'stats' (GLMs for differences in richness and abundance; R Core Team, 2017), 'multcomp' (multiple comparisons after GLMs; Hothorn *et al.*, 2008), 'vegan' (Chao-Jaccard and Sorensen's index; Oksanen *et al.*, 2017) and 'pgirmess' (Kruskal-Wallis non-parametric tests; Giraudoux and Giraudoux 2017).

Results

A total of 685 seedlings belonging to 13 taxa emerged from the persistent soil seed bank, and a total of 1,784 seedlings belonging to 28 taxa emerged from the complete soil seed bank (Table 6). Soil seed bank was mainly formed by perennials, with 69 % of the richness and 94% of the abundance in the persistent seed bank and 61 % of the richness and 86% of the abundance in the complete seed bank (Table 6). The gypsophyte *H. fruticosa* was the most representative perennial species in both the persistent and the complete seed bank, followed by *H. squamatum* (Table 6). Since the species found in the persistent soil seed bank were a subset of the species found in the complete soil seed bank (Table 6), only results of the complete soil seed bank are presented in detail. Results of the persistent soil seed bank are in supplementary material [Table A10.1, Figure A10.1 to Figure A10.6].

Table 6 Total density (individuals/m^2) of annual and perennial species recorded in the persistent and the complete soil seed banks and the above-ground vegetation survey.

	Persistent	Complete	Vegetation
Annuals			
Aegilops geniculata Roth.	-	-	0.05
Alyssum alyssoides (L.) L.	-	-	0.02
Anagallis arvensis L.	-	-	0.02
Asterolinon linum-stellatum (L.) Duby	-	20.79	18.75
Brachypodium distachyon (L.) P. Beauv.	-	-	0.99
Bromus rubens L.	-	3.46	1.93
Bupleurum semicompositum L.	-	-	0.11
Campanula fastigiata Dufour ex A. DC	-	3.46	-
Cerastium pumilum Curtis	-	3.46	0.11
Chaenorrhinum rubrifolium (Robill. & Castagne ex DC.) Fourr.	110.87	675.60	8.27
Clypeola jonthlaspi L.	-	3.46	-
Desmazeria rigida (L.) Tutin	-	-	1.80
Diplotaxis ilorcitana (Sennen) Aedo, Mart.-Laborde & Muñoz Garm.	-	-	0.05
Filago pyramidata L.	20.79	83.15	6.64
Galium verrucosum Huds.	-	13.86	0.05
Helianthemum salicifolium (L.) Mill.	-	-	0.05
Hippocrepis ciliata Willd.	-	-	0.16
Linaria arvensis (L.) Desf.	-	-	0.94
Linum strictum L.	6.93	20.79	0.51
Narduroides salzmannii (Boiss.) Rouy	-	-	1.20
Neatostema apulum (L.) I.M.Johnst.	-	-	0.21
Reseda stricta Pers.	-	10.39	-
Trigonella monspeliaca L.	-	-	0.04
Trisetum loeflinngianum (L.) C. Presl.	-	-	0.95
Unknown annual	6.93	3.46	-
Perennials			
Brachypodium retusum (Pers.) P. Beauv.	-	24.25	0.02
Carduus sp.	-	-	0.02
Ephedra fragilis Desf.	-	-	0.34
Gypsophila struthium Loefl. subsp. *hispanica* (Willk.) G.López	3.46	41.58	0.02
Hedysarum boveanum Bunge ex Basiner	-	-	0.12
Helianthemum squamatum (L.) Pers.	169.77	388.03	7.63
Helianthemum syriacum (Jacq.) Dum. Cours	-	72.76	2.00
Helianthemum violaceum (Cav.) Pers.	-	-	0.18
Helichrysum stoechas (L.) Moench	62.36	377.64	2.60
Herniaria fruticosa L.	1863.95	3835.31	1.59
Koeleria vallesiana (Honck.) Gaudin	-	17.32	2.00
Launaea lanifera Pau	-	24.25	0.30
Linum suffruticosum L.	-	27.72	0.23
Lygeum spartum L	-	-	0.11
Moricandia arvensis (L.) DC.	-	3.46	-
Ononis tridentata L.	-	-	0.02
Plantago albicans L.	27.72	69.29	26.38
Polygala rupestris Pourr.	-	-	0.42
Sedum sediforme (Jacq.) Pau	-	10.39	0.12
Sideritis hirsuta L.	-	6.93	0.12
Sonchus tenerrimus L.	6.93	34.65	-
Stipa sp.	20.79	79.69	3.55
Teucrium capitatum L.	24.25	17.32	0.18
Thymus sp.	48.50	308.35	0.97

Stipa sp. can be either *Stipa lagascae* Roem. & Schult. or *Stipa parviflora* Desf.; and *Thymus sp.* can be either *Thymus vulgaris* L. or *Thymus zygis* L. (difficult to identify at the seedling stage).

We found significant effects of the microsite in the richness of annuals and perennials in the complete soil seed bank (Table 7), being both larger in UC microsite than in the other microsites, and being also larger at ES microsite than in OA microsite (Figure 30A). We also found significant effects of the target species in the richness of annuals (Table 7), being larger in *G. struthium* than in *O. tridentata* and *T. vulgaris*, with intermediate values in *H. squamatum* (Figure 30B). Plant height was positively correlated to the richness of both annuals and perennials (Table 7; Figure 31).

We observed significant effects of the microsite and the target species in the abundance of annuals and perennials, with significant interactions between both independent variables (Table 7). The abundance of annuals was the largest in ES and UC microsites linked to *G. struthium* and in UC microsite linked to *H. squamatum* (Figure 32), and the abundance of perennials was the largest in UC microsite linked to *O. tridentata* (Figure 32). Concerning OA microsite, the abundance of both annuals and perennials were significantly larger in *G. struthium* than in the other target species (Figure 32). We only detected significant correlations between the plant height and the abundance of annuals in *T. vulgaris* (Figure 33). The abundance of perennials was significantly larger when plants were taller in UC and OA microsites, but not in ES microsite (Figure 34). For tall shrubs, we observed significantly more richness and abundance of annuals and less abundance of perennials in the cushion-like shrubs than in the erect shrubs (Figure 35). For short shrubs, we found more abundance of perennials in the cushion-like shrubs (Figure 35).

The similarity in species composition between the complete soil seed bank and the above-ground vegetation was greater in the canopy microsite (10 % < SSI < 30 %) than in the open microsite (SSI < 10%) for all target species. In the canopy microsite, SSI was significantly greater in *G. struthium* than in *H. squamatum*, with intermediate values in the other target species, while no significant differences were found among target species in the open microsite (Figure 36).

Table 7 Results of GLMs to test the effect of the microsite, the target species and the plant height, and the interaction among variables on soil seed bank richness and abundance for annuals and perennials emerged in the complete soil seed bank. Significant effects (p≤0.05) are highlighted in bold.

Dependent variables	Explanatory variables	DF	Deviance	p-value
Richness of annuals	Microsite	2	54.89	**<0.001**
	Target species	3	9.14	**<0.05**
	Plant height	1	5.60	**<0.05**
	Microsite : Target species	6	2.62	0.854
	Microsite : Plant height	2	0.65	0.722
	Target species : Plant height	3	7.09	0.069
	Microsite : Target species : Plant height	6	1.67	0.948
Richness of perennials	Microsite	2	187.05	**<0.001**
	Target species	3	0.52	0.916
	Plant height	1	8.75	**<0.01**
	Microsite : Target species	6	8.86	0.181
	Microsite : Plant height	2	0.60	0.742
	Target species : Plant height	3	0.28	0.964
	Microsite : Target species : Plant height	6	2.61	0.856
Abundance of annuals	Microsite	2	113.06	**<0.001**
	Target species	3	40.01	**<0.001**
	Plant height	1	14.11	**<0.001**
	Microsite : Target species	6	25.94	**<0.001**
	Microsite : Plant height	2	4.993	0.082
	Target species : Plant height	3	42.74	**<0.001**
	Microsite : Target species : Plant height	6	8.62	0.196
Abundance of perennials	Microsite	2	1221.44	**<0.001**
	Target species	3	46.28	**<0.001**
	Plant height	1	37.42	**<0.001**
	Microsite : Target species	6	53.01	**<0.001**
	Microsite : Plant height	2	6.37	**<0.05**
	Target species : Plant height	3	5.20	0.158
	Microsite : Target species : Plant height	6	6.33	0.388

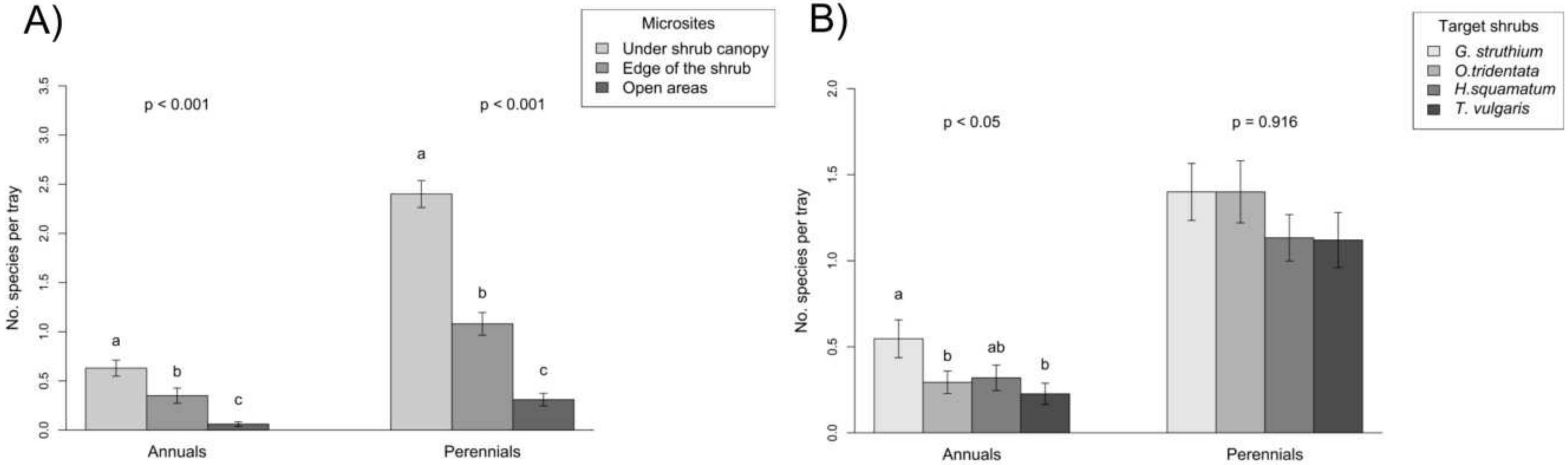


Figure 30 Mean richness per tray (number of species) of annuals and perennials found **A)** at each microsite and **B)** at each target species in the complete soil seed bank. Different letters indicate significant differences after Tukey's multiple comparisons when significant effects of the microsite and the target species were found in GLMs ($p \leq 0.05$).

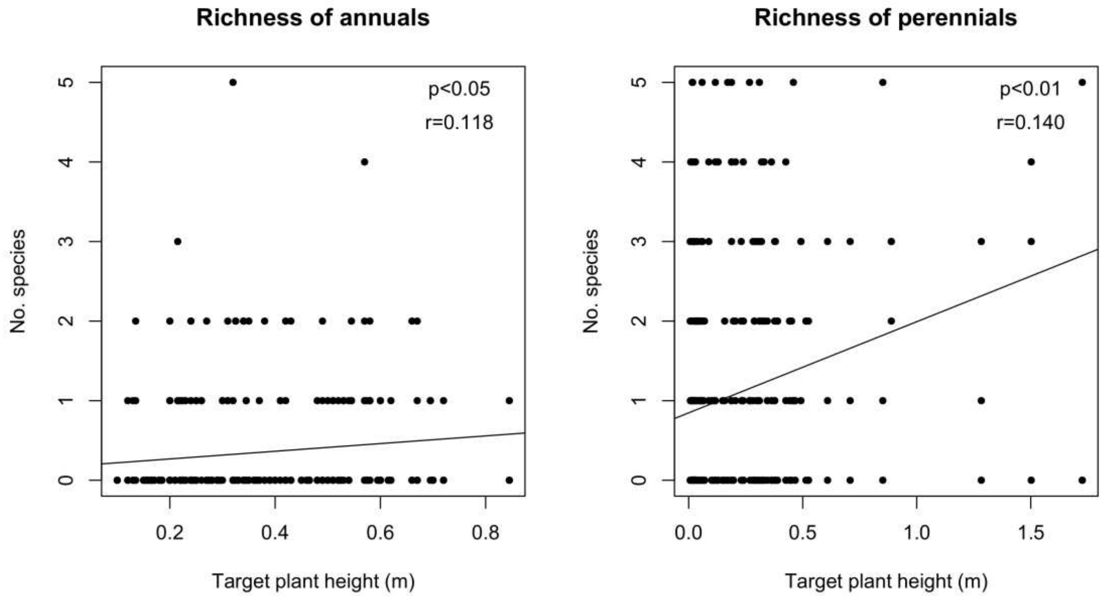


Figure 31 Correlations between richness of annuals and perennials and the plant height (m) in all microsites and target species altogether in the complete soil seed bank. $p \leq 0.05$ indicates significant effects of the plant height (r = Pearson's correlations).

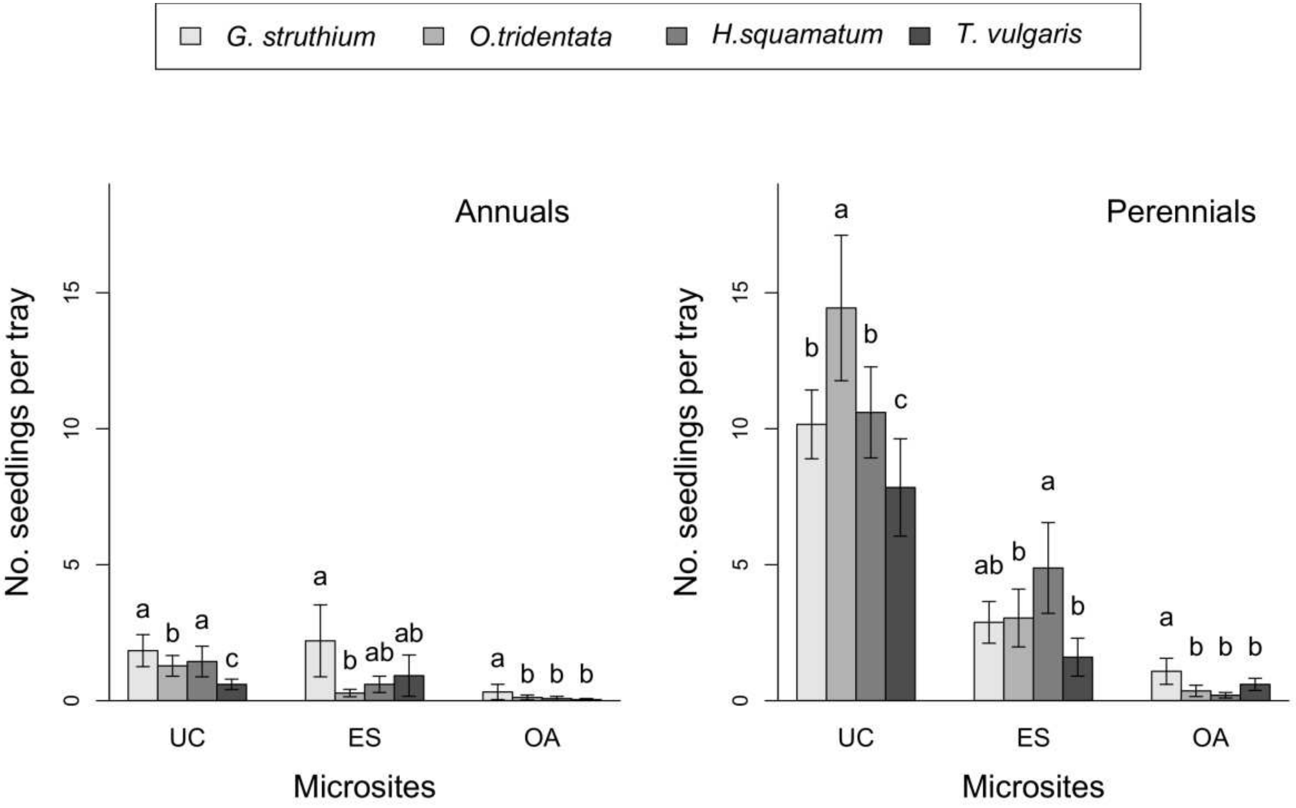


Figure 32 Mean abundance per tray (number of emerged seedlings) of annuals and perennials in different target species (*G. struthium*, *O. tridentata*, *H. squamatum* and *T. vulgaris*) per microsite (UC = under the shrub canopy, ES = at the edge of the shrub, and OA = open areas) in the complete soil seed bank. Different letters indicate significant differences among target species after Tukey's multiple comparisons for each microsite separately.

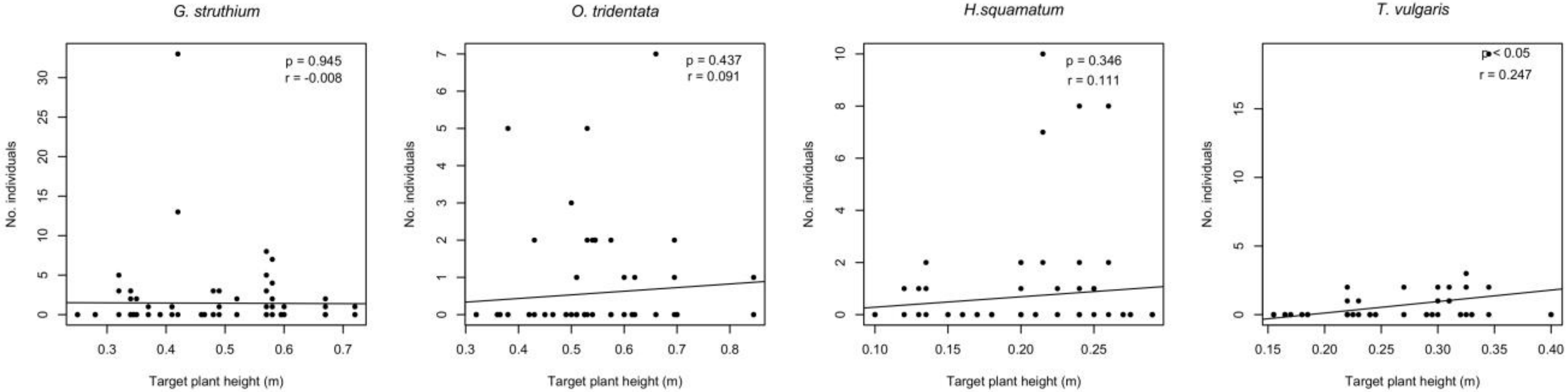


Figure 33 Correlations between abundance of annuals and the plant height (m) per target species (*G. struthium*, *O. tridentata*, *H. squamatum* and *T. vulgaris*) in the complete seed bank. p≤0.05 indicates significant effects of the plant height (r = Pearson's correlations).

Figure 34 Correlations between abundance of perennials and the plant height (m) per microsite in the complete seed bank. p≤0.05 indicates significant effects of the plant height (r = Pearson's correlations).

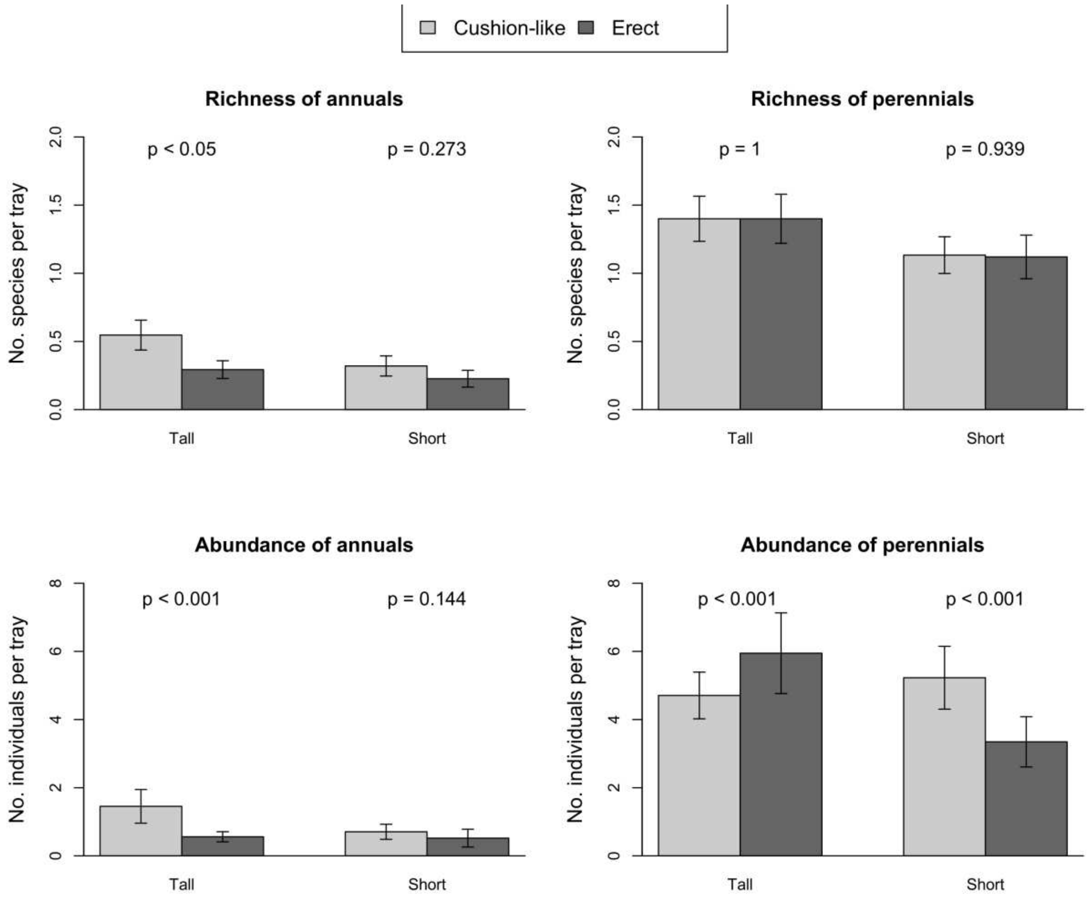


Figure 35 Mean richness and abundance per tray of annuals and perennials found in the complete seed bank in shrubs with different architecture (cushion-like and erect) per size-type (tall and short). $p \leq 0.05$ indicates significant effects of the shrub architecture.

Discussion

In line with previous studies carried out in semi-arid gypsum plant communities, our results highlight the role of shrubs as important elements in the community by spatially structuring the soil seed bank (Caballero *et al.* 2008a; López-Peralta *et al.* 2016). As we expected, soil seed bank richness and abundance were larger in microsites within the shrub canopy than in open areas. Moreover, we found a gradient in seed accumulation from the edge to the center of shrubs, likely because of wind or water-mediated secondary transportation of seeds from the peripheral to the inner parts of shrubs (Aerts *et al*., 2006). Internal branches would act as substantial barriers to wind and water flows (Aerts *et al*., 2006; Bullock and Moy, 2004), thus stopping and accumulating seeds in the center of the shrubs. Redistribution of seeds towards the center of shrubs seemed to be more remarkable for perennials than for annuals, and also it was notably observed in the persistent soil seed bank [Figure A10.1]. This can be owed to the force of time because the longer the seeds are exposed, the more subjected to secondary dispersion they are (Nathan and Muller-Landau, 2000). This could explain the different findings in studies focused on annuals-rich communities, which did not observe significant differences between edge and central locations of vegetation patches (Caballero *et al.* 2008a).

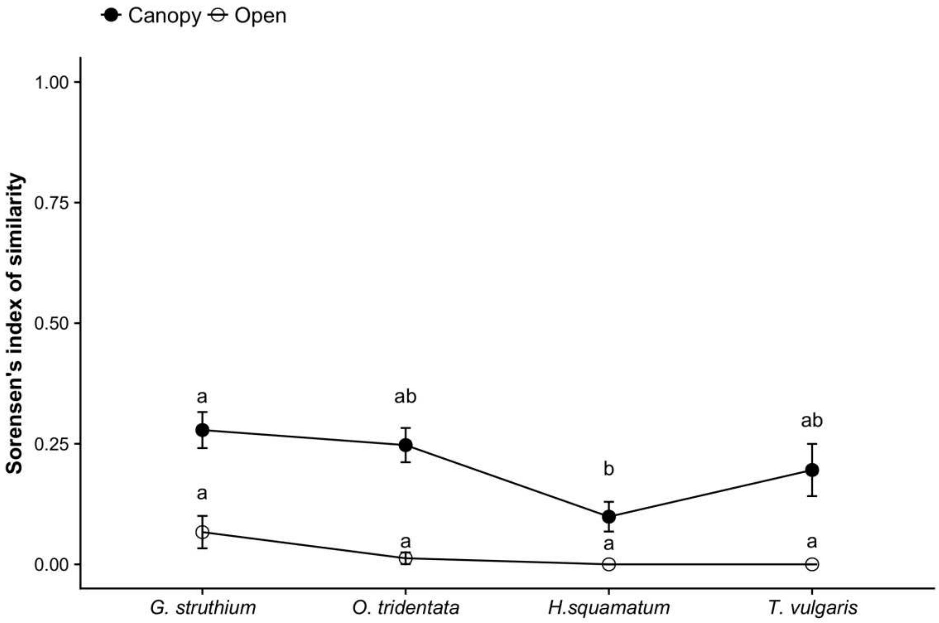


Figure 36 Mean Sorensen's index of similarity (SSI) in species composition between the complete soil seed bank and the above-ground vegetation recorded under target species canopies (*G. struthium*, *O. tridentata*, *H. squamatum* and *T. vulgaris*) and in the paired open areas. Different letters indicate significant differences among target species after Kruskal-Wallis multiple comparisons.

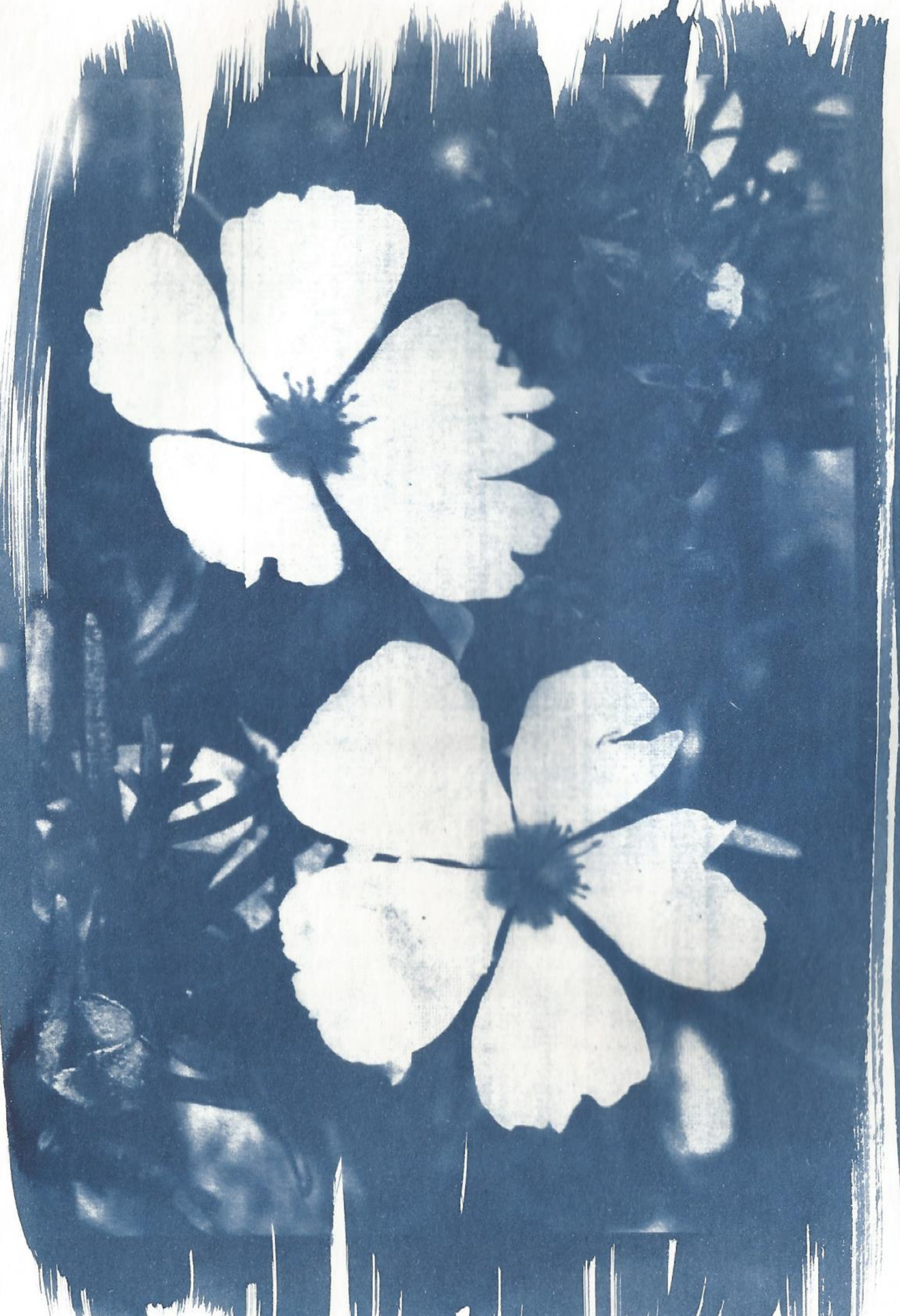
Our main finding was the differential roles of different shrub species with different physiognomy on spatially structuring the soil seed bank. While some of the studied shrub species accumulated a vast and rich seed bank under their canopies (*i.e., G. struthium* and *O. tridentata*), other shrub species did not have such a substantial effect on the spatial structure of the soil seed bank (*e.g., T. vulgaris*). Consistent with other studies, we found that shrub height may influence the accumulation of seeds (Caballero *et al.* 2008a), as the higher was the shrub, the richer was the seed bank it formed under the canopy. Nonetheless, our results suggested a species-specific influence on the accumulation of seeds that may be driven by the architecture of the shrub as well. Within shrub species of the same size-type (*i.e.*, the tall *G. struthium* and *O. tridentata*, and the short *H. squamatum* and *T. vulgaris*), we observed different abilities in accumulating seeds, being the cushion-like shrubs (*i.e.*, *G. struthium* and *H. squamatum*) more able to aggregate seeds than the erect ones (*i.e.*, *O. tridentata* and *T. vulgaris*). The better ability of cushion-like shrubs may be due to the placement of branches that drag in the soil surface acting as physical barriers to seed distribution by runoff (Aerts *et al.*, 2006).

Shrubs can accumulate seeds coming from other vegetation patches and seeds produced by plants co-occurring in their same patch, acting as both seed sinks and seed sources (Soriano *et al.*, 1994). The observed low similarity in species composition between the soil seed bank and the above-ground vegetation hints that seeds accumulated under shrub canopies could come to a greater extent from plants external to the vegetation patch. Then, the studied shrubs may have a predominant role in the community as seed sinks, especially *H. squamatum* that formed a seed bank very dissimilar in composition to the plants it harbored. Otherwise, despite showing low values of the Sorensen's index, the similarity in species composition between the seed bank and the above-ground vegetation underneath *G. struthium* was the highest among the target shrubs. This may imply that besides acting as seed sink, *G. struthium* acted as seed source through the plants harbored underneath, especially through annual plants [Figure A11.1]. Plants in semi-arid gypsum areas typically show short-range dispersal (Martinez-Duro *et al.*, 2012; Olano *et al.*, 2005), thus accumulating their seeds in the local vicinity of parent plants. In our study site, the cumulative effect of *G. struthium* extended to the surrounding open areas, being those richer and more abundant than open areas associated to the other shrubs, likely corroborating the mentioned its seed source effect (Caballero *et al.* 2008a).

Then, this study revealed that size-type and architecture of shrubs have a role in the creation of species-rich islands, being in particular tall shrubs with cushion-like architecture, such as *G. struthium*, the ones that contribute the most acting as both seed sinks and seed sources. Nevertheless, despite the seed accumulation driven by shrubs, a successful seedling establishment from the seed bank is not ensured. Instead, seedling establishment depends on the proper role of the shrub as a nurse plant because the rich seed bank would encounter suitable conditions for seeds germination (Callaway, 2007a). Therefore, identifying shrubs acting not only as seed sinks and seed sources but also as nurse plants would be valuable for plant community conservation and restoration efforts. Previous studies proved the facilitative role of *G. struthium* on a wide array of plant species leading to diverse and abundant understory vegetation (Navarro-Cano *et al.* 2016; Foronda *et al.*, 2019).

In Mediterranean gypsum plant communities, the soil seed bank in late summer (*i.e.*, complete seed bank) is supposed to parallel the above-ground vegetation in the growing season (Olano *et al.* 2005; Caballero *et al.* 2008b). However, in our study, less than one-half of the species were shared by both community compartments. In any case, the complete soil seed bank was more similar to the above-ground vegetation than the persistent soil seed bank [Figure A10.6], indicating that the seeds forming the transient seed bank are those that most resemble the above-ground vegetation in the growing season. Transient soil seed banks are primarily composed of annuals (Leck, 2012) because their persistence in the community often relies on seed production (García and Zamora, 2003). Differently to the assertion that soil seed banks in semi-arid gypsum environments are primarily composed of annuals and short-lived perennial species (Leck, 2012; Olano *et al.*, 2012), we predominantly recorded seeds of perennial species in both the complete and the persistent soil seed bank. This fact may be explained by the dominance of perennial plants in the community [Figure A11.2]. Seeds of perennials would shape persistent soil seed banks, remaining in a dormant state for more than one year (Leck, 2012; Thompson, 2000). Indeed, the persistent soil seed bank in our study site was mainly composed of perennials of which the gypsophytes *H. fruticosa* and *H. squamatum* were dominant (the latter is known to be a short-lived perennial; de la Cruz *et al.*, 2008). This fact supports the studies that argue that gypsum plants maintain a persistent soil seed bank (Caballero *et al.*, 2003). Nevertheless, detailed spatiotemporal studies would be necessary to fully understand this finding.

In conclusion, this study contributed to the understanding of the role that dominant shrub species in the community have in spatially structuring the soil seed bank in semi-arid gypsum plant communities. The shrub species that most contributed to the formation of a rich and abundant soil seed bank in gypsum plant communities of the Middle Ebro Valley was *G. struthium*, likely due to the conjoint sink and source effects. Thus, conservation and restoration efforts on this species are recommended, as they would enhance the stability and regeneration of these rare and specialized plant communities.



Detalle de las flores de *Cistus clusii*

Cianotipia elaborada por Ángeles Muñoz
Fotografía original: Jan Thomas Johansson

Chapter 3

Species-specific interference exerted by the shrub *Cistus clusii* Dunal in a semi-arid Mediterranean gypsum plant community

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BMC Ecology (2018), 18:49

https://doi.org/10.1186/s12898-018-0204-x

Abstract

The gypsovag shrub *Cistus clusii* is locally dominant in semi-arid gypsum plant communities of North-Eastern Spain. This species commonly grows in species-poor patches even though it has nurse potential, suggesting interference on neighboring species. Other *Cistus* species exert a chemically mediated interference on plant communities, suggesting that it might be a common phenomenon in this genus. This study aimed to investigate whether *C. clusii* exerts chemically mediated interference on neighboring species in gypsum plant communities. We tested in a greenhouse whether aqueous extracts from *C. clusii* leaves (L), roots (R) and a mixture of both (RL) affected germination, seedling survival, and growth of nine native species of gypsum communities, including *C. clusii* itself. We further assessed in the field richness and abundance of plants under the canopy of *C. clusii* compared to *Gypsophila struthium* (shrub with a similar architecture having a nurse role) and in open areas. Finally, we specifically assessed in the field the influence of *C. clusii* on the presence of the species tested in the greenhouse experiment. Aqueous extracts from *C. clusii* (R and RL) negatively affected either germination or survival in four of nine species. In the field, richness and abundance of plants were lower under the canopy of *C. clusii* than under *G. struthium*, but higher than in open areas. Specifically, five of nine species were less frequent than expected under the canopy of *C. clusii*. The shrub *C. clusii* shows species-specific interference with neighboring species in the community, which may be at least partially attributable to its phytotoxic activity. To our knowledge, this is the first report of species-specific interference by *C. clusii*.

Keywords: aqueous extracts; germination; interference; nurse plant; survival; richness.

Introduction

Both interference and facilitation influence the composition and structure of plant communities (Cáceres *et al.*, 2015; Gross *et al.*, 2009). The interplay between interference and facilitation may be particularly important in arid and semi-arid environments, where abiotic conditions make facilitation an important process that affects local species composition (Soliveres and Maestre, 2014). Shrubs can act as nurse plants by providing under their canopy favorable microhabitats for plants and thus can harbor highly diverse microcosms (Callaway, 2007a; Cavieres and Badano, 2009). In early developmental stages, plants are highly vulnerable to abiotic stress and often need facilitation by nurse plants to establish (Escudero *et al.*, 2005). Once the facilitated seedlings become adults, they may exert an adverse effect on nurse plants by competitive exclusion for water, nutrients, light or space (Armas and Pugnaire, 2009; Miriti, 2006; Schöb *et al.*, 2014; Valiente-Banuet and Verdú, 2008).

Some plant species produce chemical compounds that are released to the local environment through volatilization, leaf leachates, root exudates or leaf litter decomposition (Bertin *et al.*, 2003; Inderjit and Duke, 2003; Zhang and Fu, 2010). Although these compounds can have positive effects by promoting plant growth (Tsubo *et al.*, 2012) or increasing species richness (Ehlers *et al.*, 2014), they are usually phytotoxic and act as selective agents that affect the performance of other species negatively (Callaway and Ridenour, 2004). The most common phytotoxic compounds found in plants are terpenes and phenolic compounds (Inderjit, 1996; Langenheim, 1994) that may inhibit or reduce germination capacity, may cause a delay in germination time and may hamper root elongation or nutrient absorption thereby reducing plant survival and growth (Escudero *et al.*, 2000; Nielsen *et al.*, 2015; Rice, 1984; Ridenour and Callaway, 2001). The release of phytotoxic compounds may have ecological implications because non-competitive species can take advantage of the affected plants since the latter become less competitive (Verdú and Traveset, 2005). Therefore, chemically-mediated interference might be a way for nurse plants to gain a competitive advantage over neighboring plants that may be potentially competitive (Callaway and Ridenour, 2004).

In arid and semi-arid areas of the Mediterranean region, chemical interference is likely to be a common phenomenon, given the abundance of aromatic plants that produce potential phytotoxic compounds (De Almeida *et al*., 2010; Dhima *et al*., 2010; Linhart *et al*., 2015). This production may be promoted by stressful conditions as lack of water or nutrients, salinization and high solar radiation (Pedrol *et al.*, 2006). Furthermore, in arid and semi-arid environments, the effect of those compounds may increase because of their relative accumulation in the soil (Sosa *et al*., 2010) and an intensification of plant sensitivity (Pedrol *et al*., 2006). The accumulation of phytotoxic compounds in the soil may have incremental effects along plant life-span (Sosa *et al*., 2010), being the longer-lived plants more affected by phytotoxic effects due to more prolonged exposure.

Mediterranean gypsum plant communities are mainly composed of well-adapted gypsophytes (*i.e*., gypsum soil specialists). However, in those communities the gypsovag (*i.e.*, non-specialist) rosemary-leaved rockrose *Cistus clusii* Dunal often forms locally dominant populations that are associated with species-poor plant communities. *Cistus clusii*, a multi-branched perennial shrub (0.5-1.0 m tall), is distributed throughout the western Mediterranean region on alkaline soils, including gypsum, marls, and limestones (Demoly and Montserrat, 1990), and is very tolerant to dry environments (Munné-Bosch *et al*., 2003). This shrub may have nurse potential because it provides shade under its canopy due to its multi-branched architecture, creating favorable microenvironments in which other species can establish (Armas and Pugnaire, 2005). Nevertheless, *C. clusii* commonly grows in patches isolated from other species (personal observation) which suggests that it may exert interference on other plant species in the community.

Several studies have shown that other *Cistus* species have phytotoxic effects, either inhibiting the germination and growth of hetero-specific seedlings via foliar exudates (*e.g*., *Cistus ladanifer*; Alías *et al*., 2006; Chaves *et al*., 2003; Chaves and Escudero, 1997) or immobilising nutrients in the soil via root exudates (*e.g*., *Cistus albidus*; Castells *et al*., 2004). Based on the species-poor patches of *C. clusii* observed in gypsum plant communities and the phytotoxicity of other *Cistus* species, we postulated that the gypsovag *C. clusii* could exert phytotoxic effects on other plants beneath its canopy. To date, no study has demonstrated phytotoxicity in *C. clusii*, even though it is known to produce phenolic compounds and terpenes (Barrajón-Catalán *et al.*, 2011; Hernández *et al.*, 2004; Morales-Soto *et al.*, 2015).

This study aimed to test whether or not *C. clusii* interferes with neighboring species in gypsum plant communities through chemical mechanisms of interference. A controlled seeding experiment was performed in a greenhouse to identify potential phytotoxic effects of aqueous extracts from *C. clusii* leaves, roots and a mixture of both plant tissues on the development of neighboring species in the community. We hypothesized that 1) aqueous extracts from *C. clusii* would affect those species negatively and more specifically, we expected that 2) the effect of leaf extracts would be more intense than the effect of root extracts, as previously shown for other species (Dorning and Cipollini, 2006). Moreover, we expected the effect of the mixture of leaf and root extracts to be the most significant through a combined activity of both extracts. We predicted that 3) affections by aqueous extracts from *C. clusii* would be exhibited on seed germination inhibition or delay, early survival decline and plant growth reduction (Escudero *et al.*, 2000; Nielsen *et al.*, 2015; Rice, 1984; Ridenour and Callaway, 2001). Complementary to the greenhouse experiment, plant spatial associations were evaluated in the field in the local vicinity of *C. clusii*, compared to a shrub species of similar architecture in the community. Plant spatial associations were assessed as an indicator for biotic interactions to disentangle the potential interference exerted by this shrub on neighboring plants in gypsum plant communities. We hypothesized that 4) interference exerted by *C. clusii* would imply less plant-plant associations, resulting in an impoverishment of species in its local vicinity compared to the other shrub. Since the effects of phytotoxic compounds can increase along the plant life-span (Sosa *et al.*, 2010), we expected 5) a more evident depletion of perennial plants (especially at the adult stage) than of annual plants in the local vicinity of *C. clusii*.

Methods

Study area

The study was conducted in Sierra de Alcubierre (41º41’N 0º32’W, municipality of Leciñena), in the Middle Ebro Valley, Zaragoza (NE Spain), one of the largest gypsum outcrops in Europe (Machín and Navas, 1998). This area has a semi-arid Mediterranean climate with high continental influence. Average precipitation is 382 mm·year^{-1}, and average annual temperature is 13.5 ºC (“HU05 Lanaja” meteorological station, 2004-2017 period; source: Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente, http://www.siar.es). The landscape is characterised by low hills (480 m a.s.l. average) with mainly gypsiferous lithology, and flat valleys, most of which have been cultivated. In the gypsiferous hills, plant communities are composed predominantly of highly specialised flora (the gypsophytes *Helianthemum squamatum* (L.) Pers., *Gypsophila struthium* Loefl. ssp. *hispanica* (Willk.) G. López, *Ononis tridentata* L. and *Lepidium subulatum* L.) and some widespread Mediterranean shrub species, *e.g., Rosmarinus officinalis* L., *Thymus vulgaris* L. and *C. clusii* (Braun-Blanquet and Bolòs, 1958). The vegetation structure is a scattered scrubland comprising large open areas interspersed with patches of vegetation. This unique habitat (*Gypsophiletalia*) has a high ecological value and is listed as a conservation priority in international directives (European Commission, 1992).

Greenhouse experiment

A greenhouse seeding experiment was performed under controlled conditions to identify potential phytotoxic effects of *C. clusii* leaf and root aqueous extracts on the germination, early survival, and growth of nine species. The choice of species included the most abundant perennials co-occurring with *C. clusii* in the study area (Pueyo *et al.*, 2008). Selected species were *G. struthium* ssp. *hispanica* (hereafter *G. struthium*), *H. squamatum*, *Helianthemum syriacum* (Jacq.) Dum. Cours., *R. officinalis*, *T. vulgaris*, *Helichrysum stoechas* (L.) Moench, *Linum suffruticosum* Orteg. ex Planch. and S*tipa lagascae* Roem. & Schult. *Cistus clusii* was also included in the experiment to test autotoxicity. Ripe fruits were collected at the study site from ten similar-sized individuals per test species. Most species seeds were collected in June 2015, except for *G. struthium* seeds, which were collected in September 2014 and *R. officinalis* seeds, which were collected in February 2015, matching the fructification peak respectively. Seeds were separated from the fruits, discarding any malformed seeds.

Aqueous solutions were prepared from leaves and roots of *C. clusii* and used as watering treatments in the experiment. Water was used as a solvent to simulate the leaching of phytotoxic compounds by rainfall. Solutions were prepared by "cool extraction", soaking fresh plant material in distilled water for 24 h at room temperature in darkness (Herranz *et al.*, 2005). For fresh material, we used leaves recently collected from natural communities and roots from plants grown for three months in a nursery. It was unfeasible to collect a sufficient amount of roots from natural communities given the difficulties encountered due to the deep taproots of *C. clusii* (Guerrero-Campo, 1999). The seeds used to grow *C. clusii* in the nursery were collected from the same population as the collected leaves. The treatments were aqueous leaf extracts 1.5 $g{\cdot}l^{-1}$ concentration (L), aqueous root extracts 0.025 $g{\cdot}l^{-1}$ concentration (R), a mixture of both extracts 1.525 $g{\cdot}l^{-1}$ concentration (RL) and water as the control (C). The water: leaf and water: root volumetric ratios were equivalent, and were within the range that occurs in natural conditions [Appendix 12].

The seeding experiment was performed in July 2015 in a greenhouse maintained at 25 ºC during the day and 15 ºC during the night. Trays (60 x 40 x 20 cm) were filled with a peat-based substrate in which a known seed mixture was sown (9 species x 15 seeds per species in each tray). Seeding density was 0.06 $seeds{\cdot}cm^{-2}$. Each of the four extract treatments (L, R, RL, and C) had five replicates (trays). To assure germination, hard seeds were pretreated to break coat-imposed dormancy (Thanos *et al.*, 1992); specifically, *H. squamatum*, *H. syriacum* and *T. vulgaris* seeds were mechanically scarified using sandpaper (Pérez-García and González-Benito, 2006) and *C. clusii* seeds received a dry-heat shock at 100 °C for 5 minutes (Castro and Romero-García, 1999). Before sowing, all seeds were soaked in distilled water for 20 h to stimulate germination. Extract treatments (L, R, RL and C) were applied twice a week by watering trays with one liter of the specific aqueous solution. To record the potential effects of the extract treatments on the delay of germination and possible cumulative effects on seedling survival, the experiment was monitored once per week. Germinated seedlings were labeled with a toothpick indicating the date of emergence, and seedling survival was recorded throughout the experiment. To avoid any position effects, trays were randomized once a week. After ten weeks, living seedlings were harvested and washed, and the below-ground and above-ground parts of each plant were separated and kept in individual paper bags. Plants were dried in an oven at 70 °C for 48 hours and weighed using a 0.01 mg precision balance. Total dry biomass and the ratio of below-ground/above-ground biomass were used as growth estimators.

Vegetation survey

To test the potential interference exerted by *C. clusii* on neighboring plants under natural conditions, a vegetation survey was conducted in May 2014, at the peak of vegetation growth. We surveyed the plants growing under the canopy of *C. clusii*, and also under the canopy of *G. struthium* for comparison purpose. The latter is a gypsophyte shrub which has a proved nurse role (Navarro-Cano *et al.*, 2016) and, to our knowledge, without any phytotoxic effects. Both shrubs have similar architecture, providing similar soil temperature and surface compaction under their canopies, which were improved, compared to open areas [Figure A13.1 and Figure A13.2]. To obtain comparable samples from the surrounding open areas, we surveyed the vegetation in paired areas placed in a random direction ≥50 cm away from each sampled target plant. Sampled areas were defined by circles matching the size of the area under the canopy of the paired target plant (Cavieres *et al.*, 2006). For both focal species, 25 sets of paired plant-open areas were sampled (n=100 circles). All plants growing within the circles were recorded and identified to the species level. To assess the potentially differential phytotoxic effects along plant life-span, plants were categorized as either annual (short-lived) or perennial (long-lived), and within perennial, as either seedling or adult. For each category, we estimated richness (number of species present) and abundance (number of individuals present of all plant species) at each microsite: in open areas, under the canopy of *C. clusii*, and under the canopy of *G. struthium*.

Data analyses

In the greenhouse experiment, the effects of the extract treatments either on total germination, as well as germination delay or on seedling survival gradual decline were evaluated considering germination and survival rates through time. Differences among extract treatments in seed germination rate and seedling survival rate of the nine test species were evaluated using Cox proportional hazard models and, for data visualization, Kaplan-Meier curves (Bewick *et al.*, 2004). Pairwise comparisons among extract treatments were performed with Tukey's post-hoc tests. For each test species, differences in total biomass and the ratio of below-ground/above-ground biomass among extract treatments were tested using Linear Mixed Models (LMMs) with the tray as a random factor. Time since germination and size of seedlings were strongly correlated; therefore, the number of weeks from germination to the end of the experiment was included as a covariate. To attain the assumption of normality, the dependent variables were log-transformed.

Differences in richness and abundance among microsites were analyzed by fitting Generalized Linear Models (GLMs) with the assumption of a Poisson error distribution and log link function. The size of the sampled area (circle area) was included as a continuous covariate because it might have influenced the number of plants recorded. When a significant effect of the microsite was found, Tukey's post-hoc tests were applied for pairwise comparisons. In addition, for each of the nine test species used in the greenhouse experiment, G-tests (log likelihood ratio tests) were implemented to compare the observed frequencies with the haphazardly expected frequencies at each microsite (in open areas, under the canopy of *C. clusii*, and under the canopy of *G. struthium*). The expected frequencies were estimated as the total observed frequency of each species multiplied by the proportion of the area occupied by each microsite.

All statistical analyses were performed using R software (R Development Core Team, 2014). To fit Cox models and construct Kaplan-Meier curves for germination and survival, the "survival" package was used (Therneau, 2014). To fit LMMs for growth, the "nlme" package was used (Pinheiro *et al.*, 2007). To fit GLMs for richness and abundance, the "stats" package was used (R Development Core Team, 2014). All pairwise comparisons were performed using the "multcomp" package (Hothorn *et al.*, 2008).

Results

Greenhouse experiment

C. clusii aqueous extracts had a negative effect on seed germination rates of three of the nine test species (*H. squamatum*, *H. stoechas* and *C. clusii*; Figure 37). Seed germination of *H. squamatum* was lower in the trays subjected to extracts than it was in the control trays and did not differ significantly among extract treatments. Seed germination of *H. stoechas* was lower in all extract treatments compared to the control treatment, being the lowest in the RL treatment. Seed germination of *C. clusii* was

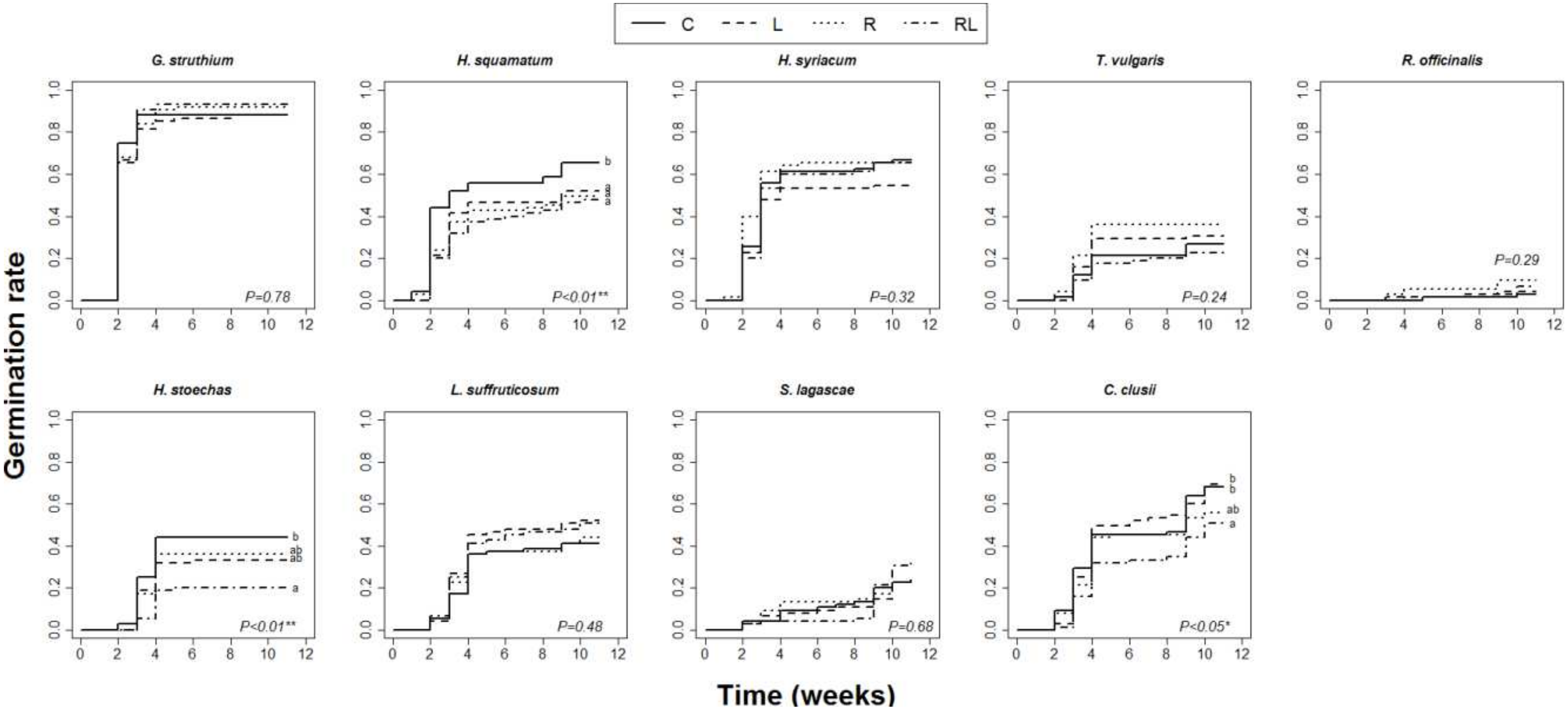

Figure 37 Kaplan-Meier curves representing seed germination rate over time (weeks from the experiment start) under each extract treatment: C (control), L (leaf extracts), R (root extracts) and RL (root and leaf extracts mixture) for each test species separately. Different letters represent statistically significant differences between extract treatments after Tukey's post-hoc tests ($p \leq 0.05$).

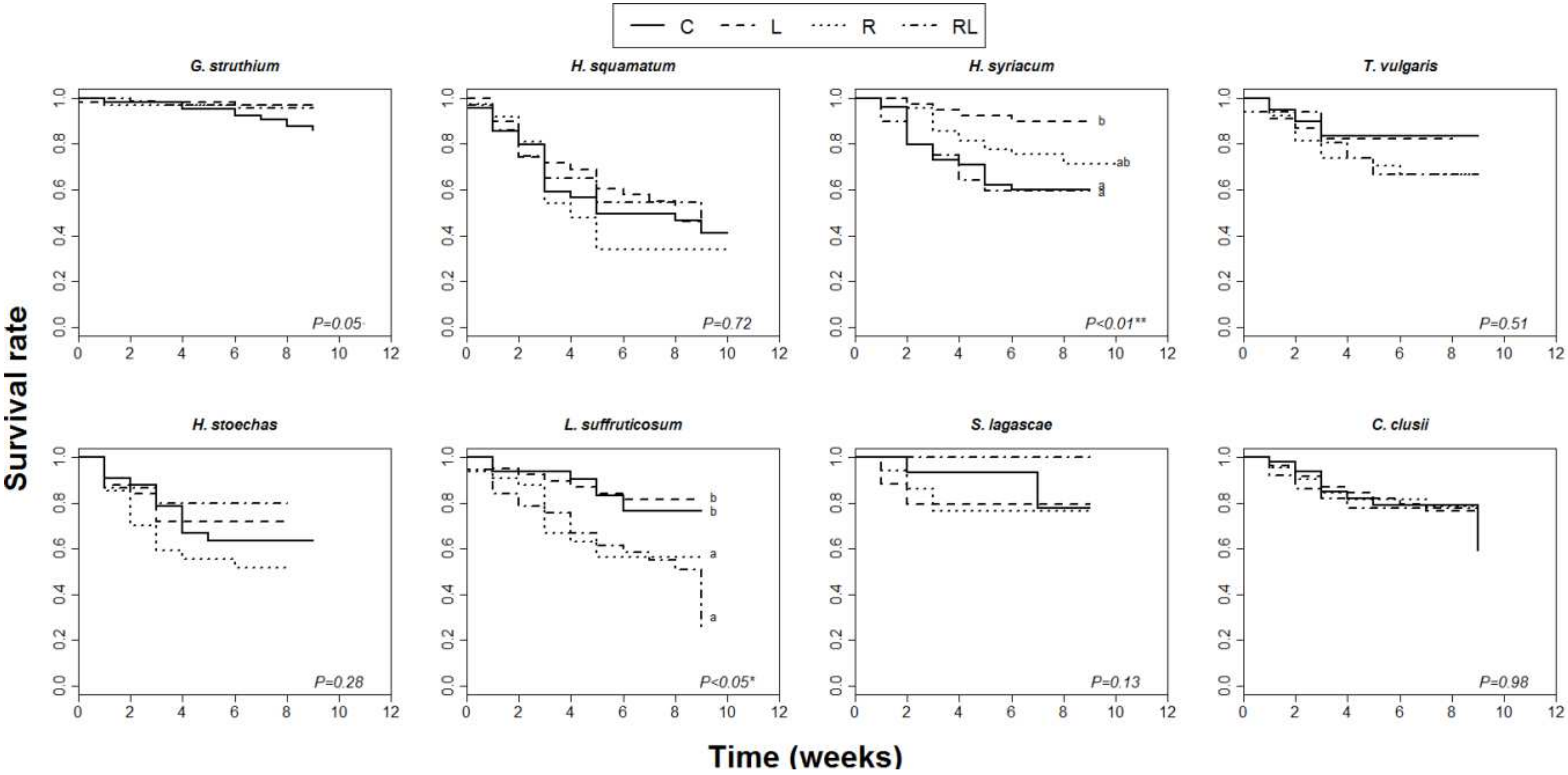

Figure 38 Kaplan-Meier curves representing seedling survival rate over time (weeks since germination) under each extract treatment: C (control), L (leaf extracts), R (root extracts) and RL (root and leaf extracts mixture) for each test species separately. Different letters represent statistically significant differences between extract treatments after Tukey's post-hoc tests ($p \leq 0.05$).

significantly lower in the R and the RL treatments than it was in the control treatment. However, germination rates did not differ significantly between the trays watered with leaf extracts (L) and the control trays. At the end of the experiment, <10% of *R. officinalis* seeds had germinated (Figure 37); therefore, this species was excluded from the survival and growth analyses. Survival rates differed significantly among extract treatments in two of the test species (*H. syriacum* and *L. suffruticosum*). Seedling survival of *L. suffruticosum* was lower in the R and the RL treatments compared to both the L treatment and the control treatment. Seedling survival of *H. syriacum* was the lowest in the control treatment and the RL treatment and the highest in the L treatment (Figure 38). Neither total biomass nor the ratio of below-ground/above-ground biomass differed significantly among extract treatments for any of the test species [Table A14.1 and Table A14.2].

Vegetation survey

Microsite had a significant effect on richness and abundance of annual and perennial plants (Figure 39). In all cases, richness and abundance were lower in open areas than under the canopies of both shrubs. Significantly fewer species of perennial adults were found under the canopy of *C. clusii* than under the canopy of *G. struthium*; however, richness under the shrubs did not differ significantly for seedlings of perennial species and annual plants. The abundances of annual plants and perennial adult plants were lower under the canopy of *C. clusii* than under the canopy of *G. struthium*; however, the abundance of perennial seedlings did not differ significantly between the two shrubs (Figure 39).

Except for *G. struthium*, *H. stoechas* and *S. lagascae*, the seedlings of the test species were significantly less frequent in open areas than they were under the canopy of *C. clusii*. Among adults, *H. syriacum* and *H. squamatum* were less frequent in open areas than they were under the canopy of *C. clusii*. The other test species showed no difference in frequency between these two microsites (Table 8). Most test species were significantly less frequent in open areas than they were under the canopy of *G. struthium,* except seedlings of *S. lagascae* and adults of *H. squamatum* and *R. officinalis* (Table 8). Five of the test species were significantly less frequent under the canopy of *C. clusii* than they were under the canopy of *G. struthium*; specifically, seedlings of *H.*

syriacum, *H. stoechas* and *C. clusii*, and adults of *T. vulgaris*, *H. stoechas*, *S. lagascae* and *C. clusii*. The observed frequency of *C. clusii* seedlings under the canopy of *C. clusii* was significantly higher than expected (Table 8).

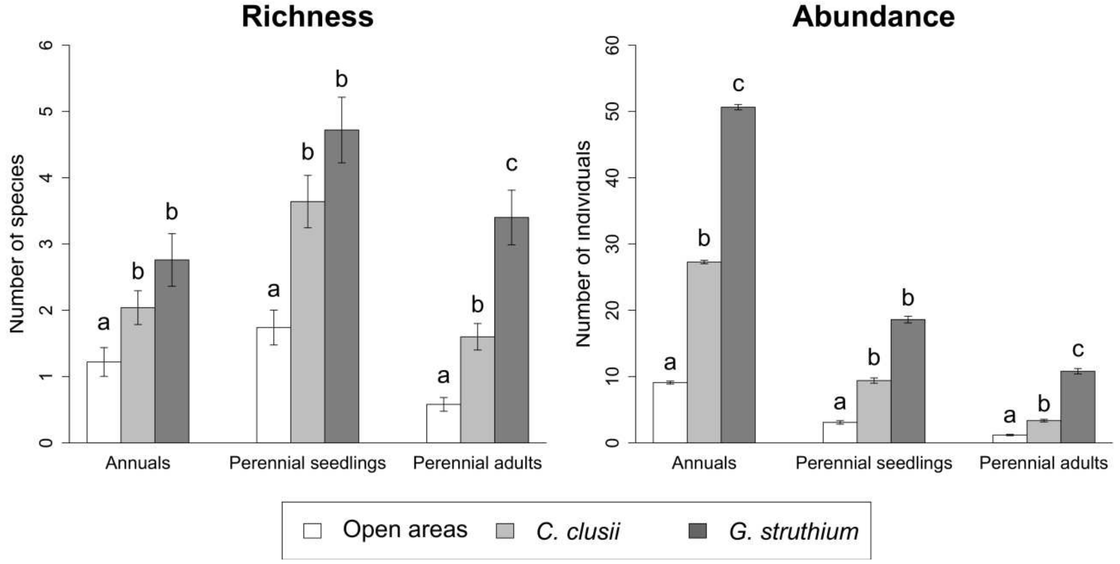

Figure 39 Mean richness and abundance of annuals, perennial seedlings and perennial adults among microsites: in open areas, under the canopy of *C. clusii* and under the canopy of *G. struthium*. Microsite effect was significant in GLMs in all cases ($p \leq 0.001$). Different letters indicate statistically significant differences between microsites after Tukey's post-hoc tests ($p \leq 0.05$).

Discussion

We combined a controlled experiment with a field survey aiming to disentangle the potential chemical mechanisms of interference exerted by *C. clusii* on neighboring plant species in gypsum plant communities of the Middle Ebro Valley. While the controlled experiment allowed us to isolate the phytotoxic effect of *C. clusii* root and leaf aqueous extracts on the early establishment of the test species, the field survey showed a more complex picture. In the field, chemical interference influences the net plant-plant interactions outcome together with facilitation and competition for resources (Inderjit and Del Moral, 1997). Thus, a complementary assessment considering experimental and field effects of *C. clusii* on our test species can help unravel the relative relevance of its potential chemical interference compared to other types of interference (*i.e.*, competition for resources; Table 9).

Table 8 Summary of the pairwise comparisons of the G-test, indicating significant differences ($p<0.05$) between observed frequencies (f) and expected frequencies (f_e) of the nine test species at each microsite.

Seedlings	**$f\ (f_e)_{Op}$**	**$f\ (f_e)_{Cc}$**	**G**	***P***	**$f\ (f_e)_{Op}$**	**$f\ (f_e)_{Gs}$**	**G**	***P***	**$f\ (f_e)_{Cc}$**	**$f\ (f_e)_{Gs}$**	**G**	***P***
G. struthium	8 (11)	6 (3)	2.51	0.057	8 (14)	14 (8)	5.83	**<0.01**	6 (7)	14 (13)	0.11	0.372
H. squamatum	2 (21)	26 (7)	61.71	**<0.001**	2 (29)	45 (18)	72.14	**<0.001**	26 (24)	45 (47)	0.33	0.284
H. syriacum	6 (10)	7 (3)	74.98	**<0.001**	6 (25)	35 (16)	202.94	**<0.001**	7 (14)	35 (28)	147.02	**<0.001**
T. vulgaris	43 (76)	56 (23)	49.27	**<0.001**	43 (90)	103 (56)	62.88	**<0.001**	56 (53)	103 (106)	0.23	0.314
R. officinalis	18 (24)	14 (8)	6.25	**<0.01**	18 (27)	25 (16)	7.02	**<0.01**	14 (13)	25 (26)	0.11	0.371
H. stoechas	6 (7)	3 (2)	0.43	0.255	6 (33)	48 (21)	60.63	**<0.001**	3 (17)	48 (34)	22.79	**<0.001**
L. suffruticosum	21 (34)	23 (10)	16.79	**<0.001**	21 (32)	30 (19)	8.90	**<0.01**	23 (18)	30 (35)	2.28	0.065
S. lagascae	5 (6)	3 (2)	0.77	0.190	5 (6)	5 (4)	0.58	0.223	3 (3)	5 (5)	0.06	0.404
C. clusii	21 (50)	44 (15)	56.52	**<0.001**	21 (46)	54 (29)	35.34	**<0.001**	44 (33)	54 (65)	5.56	**<0.01**
Adults	**$f\ (f_e)_{Op}$**	**$f\ (f_e)_{Cc}$**	**G**	***P***	**$f\ (f_e)_{Op}$**	**$f\ (f_e)_{Gs}$**	**G**	***P***	**$f\ (f_e)_{Cc}$**	**$f\ (f_e)_{Gs}$**	**G**	***P***
G. struthium	0 (-)	0 (-)	-	-	0 (-)	0 (-)	-	-	0 (-)	0 (-)	-	-
H. squamatum	5 (8)	5 (2)	3.26	**<0.05**	5 (7)	6 (4)	1.21	0.135	5 (4)	6 (7)	0.69	0.204
H. syriacum	3 (6)	5 (2)	5.46	**<0.05**	3 (15)	21 (9)	25.28	**<0.001**	5(9)	21 (17)	2.58	0.054
T. vulgaris	12 (12)	4 (4)	0.02	0.449	12 (38)	49 (23)	45.50	**<0.001**	4 (18)	49 (35)	20.25	**<0.001**
R. officinalis	3 (3)	1 (1)	0.00	0.474	3 (2)	1 (2)	0.31	0.289	1 (1)	1 (1)	0.23	0.314
H. stoechas	2 (2)	0 (0)	1.08	0.150	2 (6)	8 (4)	7.34	**<0.01**	0 (3)	8 (5)	6.50	**<0.01**
L. suffruticosum	0 (-)	0 (-)	-	-	0 (2)	3 (1)	5.78	**<0.01**	0 (1)	3 (2)	2.44	0.059
S. lagascae	0 (-)	0 (-)	-	-	0 (6)	10 (4)	19.28	**<0.001**	0 (3)	10 (7)	8.13	**<0.01**
C. clusii	7 (6)	1 (2)	0.63	0.214	7 (11)	11 (7)	3.87	**<0.05**	1 (4)	11 (8)	4.25	**<0.05**

Op: open patches; Cc: under the canopy of *C. clusii*; Gs: under the canopy of *G. struthium*.

Table 9 Comparison of the potential effects exerted by *C. clusii* under experimental and field conditions.

	Experimental conditions		
	Negative effect	**No effect**	**Positive effect**
Field conditions			
Negative effect	Chemical interference: *H. stoechas, C. clusii* (adults)	Other sources of interference (i.e., competition for resources), and phytochemicals accumulation and/or transformation in soils: *T. vulgaris*, *S. lagascae*	Other sources of interference (i.e., competition for resources), and phytochemicals accumulation and/or transformation in soils: *H. syriacum*
No effect	Neutral interaction outcome (facilitation + chemical interference): *H. squamatum*, *L. suffruticosum*	*C. clusii*-tolerant species: *G. struthium*, *R. officinalis*	-
Positive effect	Limited seed dispersal: *C. clusii* (seedlings)	-	-

The greenhouse experiment confirmed our hypothesis that aqueous extracts of *C. clusii* affect the development of some species from gypsum plant communities. Supplementary chemical analyses of *C. clusii* tissues confirmed the presence of water-soluble terpenes and phenolic compounds with potential phytotoxic activity [Appendix 15]. We hypothesized that aqueous extracts from *C. clusii* would affect germination rates, survival rates and growth of the test species negatively. Even though it was not visible for all test species, our hypothesis was supported by experimental results for germination and survival rates. However, it was not evident for seedling biomass, manifesting that *C. clusii* aqueous extracts do not affect seedling growth, at least at the short-term. Chemically inhibition or retardation of germination and seedling survival decline may have ecological implications in the community. This may result in an advantage of low-competitive species over the affected species at early life stages (Escudero *et al.*, 2000; Verdú and Traveset, 2005), likely causing a species shift in the community. Germination inhibition by phytotoxic compounds is a phenomenon widely reported by other studies in semi-arid communities (Arroyo *et al.*, 2018; Escudero *et al.*, 2000).

Diverse effects of aqueous extracts were found on germination and survival rates depending on the plant tissue tested in the experiment. Other studies found that leaves of allelopathic plants contained more water-soluble phytotoxic compounds than roots (Whitehead *et al.*, 1983), likely resulting in stronger phytotoxic effects, as observed by Dorning and Cipollini (2006) in an invasive shrub. Our additional chemical analyses confirmed that leaves from *C. clusii* contain more water-soluble potential phytotoxic compounds than its roots. We expected the leaf extracts to exert a stronger negative effect on the test species than root extracts. On the contrary, despite containing fewer compounds than did its leaves, solutions containing root extracts more often had negative effects on germination and seedling survival compared to pure leaf extracts. As predicted, roots combined with leaves was the most inhibiting treatment likely due to a synergic effect of the compounds contained in both plant tissues (Kruse *et al.*, 2000).

The outcomes of the field survey and the experiment denoted that chemical interference could explain why some species are less frequent in the local vicinity of *C. clusii*. This fact was especially evident for *H. stoechas* because the low number of individuals found under the canopy of *C. clusii* compared to those under the canopy of

G. struthium paralleled the low germination of seeds treated with *C. clusii* extracts. These results confirm our hypothesis of the lessening of plant-plant associations due to the chemical interference exerted by *C. clusii* on neighboring plants, deriving to an impoverishment of species around this shrub. It has already been evidenced in semi-arid plant communities that phytotoxic effects on neighboring plants result in species-poor islands around the allelopathic plant (Arroyo *et al.*, 2015). Other negative interactions between *C. clusii* and neighboring species in the field could not be corroborated in our seeding experiment because other factors beyond the releasing of phytotoxic compounds may influence plant establishment under natural conditions (Inderjit and Callaway, 2003). For example, in the field, fewer than the expected number of individuals of *H. syriacum* were found under the canopy of *C. clusii*; however, in the experiment, *C. clusii* aqueous extracts had a positive effect on *H. syriacum* seedling survival. Similarly, *T. vulgaris* and *S. lagascae* were found in low abundance under the canopy of *C. clusii*; however, the extract treatments did not significantly affect their performance. Negative interactions between those species and *C. clusii* are thus more likely caused by competition for space and resources rather than chemical interference (Inderjit and Del Moral, 1997).

The limitations of the greenhouse experiment could have led to an underestimation of the phytotoxic effects of *C. clusii* that may occur under natural conditions. For example, the effects of hydrophobic compounds present in *C. clusii* (*e.g.,* β-pinene; Morales-Soto et al., 2015) were not tested in the experiment because they were not extracted in the aqueous solutions [Appendix 15]. Those compounds can be released to the environment by volatilization and are potentially phytotoxic (Arroyo *et al.*, 2018; Chowhan *et al.*, 2011). Also, soil microorganisms are known to transform chemical compounds (Jilani *et al.*, 2008), which can increase phytotoxicity under natural conditions (Gagliardo and Chilton, 1992), dissimilar to the controlled conditions of the experiment. On the other hand, it should be noted that phytotoxic compounds can have a cumulative effect over a plant life-span (Sosa *et al.*, 2010) and our seeding experiment did not last long enough to detect potential long-term negative effects of *C. clusii*. Nevertheless, accordingly to our expectations, long-term negative effects were found in the field survey since *C. clusii* consistently harbored fewer species than *G. struthium*, mainly perennials at the adult stage.

Both in the greenhouse experiment and the field *C. clusii* did not show a negative effect on *G. struthium*, suggesting that this species tolerates *C. clusii*. In the field, *R. officinalis* also appeared to exhibit tolerance to *C. clusii*; however, this could not be confirmed experimentally because *R. officinalis* exhibited an overall very low germination rate. Those species may have adapted to the potential phytotoxic compounds of *C. clusii* because they frequently co-occur with this species in gypsum plant communities of the Middle Ebro Valley. Tolerance to “chemical neighbors” is a well-studied co-evolutionary phenomenon that allows a species to coexist with phytotoxic plant species (Grøndahl and Ehlers, 2008; Jensen and Ehlers, 2010). Moreover, although *C. clusii* extracts reduced seedling survival of *H. squamatum* and *L. suffruticosum* in the greenhouse experiment, this was not evident in natural conditions. Possibly, in nature, the net interaction outcome between *C. clusii* and those species tends to be neutral because of positive interactions. *Cistus clusii* has the potential of behaving as a nurse plant since the area under its canopy provides microsites similar to known nurse plants in this habitat (*e.g., G. struthium;* Navarro-Cano *et al.*, 2016) and may suit the establishment of *C. clusii*-tolerant species (Callaway, 2007a). Indeed, there was a positive net effect of *C. clusii* when richness and plant abundance were compared to open areas. Positive effects of *C. clusii* on the establishment of other species compared to open areas had been documented in semi-arid plant communities before (Armas and Pugnaire, 2005).

Few of the studies that investigated phytotoxicity in plants also evaluated autotoxicity (Alías *et al.*, 2006). In the greenhouse experiment, *C. clusii* aqueous extracts inhibited the germination of its own seeds, indicating a phytotoxic potential against itself. In natural conditions, more seedlings of *C. clusii* than expected were found under its canopy, probably because of high seed accumulation (Callaway, 2007b); however, fewer than the expected numbers of adult *C. clusii* were found under its canopy, in agreement with an auto-inhibiting effect. The low establishment of adult *C. clusii* in the vicinity of *C. clusii* shrubs could have important implications for *C. clusii* population dynamics as auto-inhibition could lead to a strong reliance on other nurse species to establish under the highly restrictive conditions that occur in gypsum environments.

Despite ameliorating micro-environmental conditions under its canopy, and having a nurse role compared to open areas (Armas and Pugnaire, 2005), *C. clusii* did not present such a positive role as the other shrub with similar architecture, suggesting interference with neighboring plants in the community. We found potential phytotoxic compounds in *C. clusii* leaves and roots, and the associated vegetation showed a species-specific sensitivity to *C. clusii*. Among test species, there were possible *C. clusii*-vulnerable species (*H. stoechas*, *H. syriacum*, *S. lagascae*, and *T. vulgaris*), but also potential *C. clusii*-tolerant species (*G. struthium*, *H. squamatum*, *L. suffruticosum*, and *R. officinalis*). Species-specific phytotoxicity has been previously described (Linhart *et al.*, 2015) and might have important ecological implications for the dynamics of plant communities, by affecting the recruitment of some species and thereby their abundance in the community (Schenk, 2006). Besides *C. clusii*, other gypsovags that are very common in gypsum environments (*e.g., T. vulgaris* and *R. officinalis*) leach chemical compounds with known phytotoxic activity to their local environments (Thompson *et al.*, 2003; Vokou *et al.*, 1993). Phytotoxicity might be a mechanism that allows gypsovags to succeed in competition for resources with neighboring plant species that may be better adapted to the harsh conditions in gypsum soils (Escudero *et al.*, 2015). For example, phosphorous is scarce in gypsum hills (Guerrero-Campo *et al.*, 1999; Pueyo *et al.*, 2007), and excluding other plants from the local vicinity might be a means of minimizing local phosphorous depletion.

This study provides novel results of species-specific interference of *C. clusii* on other plant species. Phytotoxicity of *C. clusii* at least partly affects species richness in its local vicinity in gypsum plant communities in the Middle Ebro Valley. The importance of the role of the phytotoxicity of *C. clusii* in plant-plant interaction outcomes at the community level should be investigated in other Mediterranean plant communities.

Vista general de la zona experimental en el vertedero de yeso de la Mina de carbón "Barrabasa" (Andorra, Teruel, España)

Cianotipia elaborada por Ángeles Muñoz
Fotografía original: Ana Foronda

Chapter 4

Substrate specialist plants enhance vegetation recovery effectiveness in post-mining gypsum substrates

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Catena (in rev.)

Abstract

Spontaneous vegetation recovery in degraded gypsum areas is challenging due to physical-chemical limitations of gypsum soils. Facilitation mediated by the stress-adapted gypsophytes may be a key process in restoration because it potentially favors the establishment of lower stress-adapted species underneath, promoting plant succession. This study aimed to assess the suitability of the gypsophyte *Gypsophila struthium* Loefl. for vegetation recovery in gypsum mines, focusing on its role as a pioneer species that may act as an ecosystem engineer and hence as a nurse plant. We conducted a vegetation survey in a gypsum mine in NE Spain, in plots where the original topsoil was reinserted in different years after the mineral extraction to study plant community succession and reorganization through time. Complementary, we set a sowing and planting experiment in a gypsum spoil dump to assess how *G. struthium* may positively affect soil and micro-environmental conditions and, consequently, may act as a nurse species facilitating plant establishment and development. To test the nurse role of *G. struthium*, we sowed seeds and planted seedlings of *Helianthemum squamatum* (L.) Pers. and *Stipa lagascae* Roem. & Schultz under the canopy of adult individuals of *G. struthium,* and in the bare soil. We found that *G. struthium* appeared as a pioneer shrub in the restored gypsum mine and accumulated plant diversity in the local vicinity in late stages of plant succession. This shrub had a positive effect on seed germination and seedling growth of the test species in the experiment and improved soil and micro-environmental conditions under its canopy. Therefore, we recommend two non-exclusive actions to enhance restoration in post-mining gypsum substrates: (1) to promote the establishment of the pioneer plant *G. struthium* by improving the physical-chemical and biological soil properties through the re-insertion of the original topsoil that contains propagules of the former community; (2) to apply active restoration actions, using *G. struthium* as a nurse plant through sowing seeds or planting seedlings of other species of interest under its canopy.

Keywords: gypsophytes; gypsum spoil; nurse plants; pioneer species; plant succession; restoration.

Introduction

Gypsum outcrops shelter rare and specialized plant communities listed in European directives as a priority for conservation (92/43/CEE; European Commission, 1992). Gypsum mineral extraction activities usually endanger these communities by resulting in the thorough removal of the vegetation and the degradation of the substrate to low-quality materials that often form cemented layers (Bradshaw, 1997; Espigares *et al.*, 2011; Mota *et al.*, 2004; Sharma *et al.*, 2001). Vegetation recovery is generally a challenging process in post-mining substrates (*e.g.*, Wiegleb and Felinks, 2001) and more specifically in gypsum spoils owed to physical and chemical limitations that hinder plant development (Escudero *et al.*, 2015). Among the mentioned limitations, gypsum substrates have surface crusts, poor water availability, low concentrations of nutrients and high content of sulfate and calcium (Palacio *et al.*, 2007; Pueyo *et al.*, 2007; Romão and Escudero, 2005). Moreover, gypsum outcrops and hence gypsum mines are mostly located in arid and semi-arid environments (Boyadgiev and Verheye, 1996), where primary plant succession is a long-lasting process (Dana and Mota, 2006; Mota *et al.*, 2003). These constraining conditions hamper spontaneous vegetation recovery, and thus restoration actions are required to recover the former species-rich gypsum ecosystems.

Active restoration practices such as planting and sowing have exhibited successful results in the recovery of gypsum plant communities in the short-term (Ballesteros *et al.*, 2014, 2012). However, socio-economic constraints might compromise the application of active restoration practices due to their occasionally high economic costs (Miller and Hobbs, 2007). By comparison, natural plant succession, which assumes lower economic costs, often shows a notable recovery of the former gypsum plant community but in the long-term (Dana and Mota, 2006; Martínez-Duro *et al.*, 2010; Mota *et al.*, 2003). Natural plant succession in bare post-mining substrates depends on the likelihood of seeds arrival by dispersal from well-conserved habitat fragments remaining in the vicinity of the mine (Kirmer *et al.*, 2008). Moreover, plant establishment is a critical stage for natural vegetation recovery, especially in harsh environments because it depends on the availability of resources such as water and nutrients (Maestre *et al.*, 2001; Noy-Meir, 1973). In general, arrived seeds in mined substrates encounter limiting factors such as resource-scarce conditions and unsuitable physical soil conditions that prevent plant development (Bradshaw, 1997).

Plant succession in low-productivity substrates (*e.g.*, gypsum spoils) can be accelerated by enhancing the physical-chemical and biological soil properties with technical actions (Prach and Hobbs, 2008). These technical actions often consist of organic amendments (Castillejo and Castelló, 2010) or the addition of the original topsoil that contains the soil seed bank together with nutrients and microbiota (Abdul-Kareem and McRae, 1984; Hall *et al.*, 2010). In addition, plant succession can be enhanced by promoting biotic interactions such as facilitation, which is being recently considered a key process in restoration practices (Gómez-Aparicio, 2009; Navarro-Cano *et al.*, 2016; Padilla and Pugnaire, 2006; Pueyo *et al.*, 2009). Facilitation can be regulated through handling facilitative shrubs, also known as nurse shrubs, that act as ecosystem engineers by modifying the local environment (Jones *et al.*, 1997). Shrubs, especially large shrubs (*i.e.*, nanophanerophytes), provide shade, thus reducing environmental stress under their canopy by diminishing solar radiation, increasing soil water availability, decreasing soil compaction, and softening extreme temperatures (Callaway, 2007a). Shrubs also provide leaf litter, whose decomposition provide nutrients and organic matter to the soil and ameliorate soil physical properties (Boyle *et al.*, 1989; Callaway, 2007a; Delgado-Baquerizo *et al.*, 2013). The amelioration of the soil conditions would increase the niche of lower stress-adapted species thereby facilitating their establishment and, consequently, stimulating plant succession and creating species-rich patches in the local vicinities (Gómez-Aparicio *et al.*, 2005; Soliveres *et al.*, 2011). Identifying ecosystem engineers and including them in restoration plans might lead to restoration success and the reduction of time and economic costs (Byers *et al.*, 2006).

Gypsum substrate-specialist plants (*i.e.*, gypsophytes) can establish as pioneer species in the community by overcoming gypsum harsh environmental conditions (Escudero *et al.*, 2015; Martínez-Duro *et al.*, 2010). Particularly, previous studies have shown that the gypsophyte shrub *Gypsophila struthium* Loefl. commonly emerges as a pioneer species in gypsum mine spoils (Dana and Mota, 2006). This is a large shrub with crawling branches (a cushion *sensu* Cornelissen *et al.*, 2003) with proven nurse role in natural plant communities (Foronda *et al.*, 2019; Navarro-Cano *et al.*, 2016) that can be essential for promoting an efficient re-structuration of the diversity in degraded gypsum plant communities. The potential role of pioneer shrub species like *G. struthium* as nurse plants in the restoration of gypsum mines is promising but needs further evidence.

This study aimed to assess the suitability of pioneer shrubs with a nurse role for enhancing vegetation recovery in gypsum areas degraded by mining. We specifically proposed a) to investigate how plant communities reorganized at different stages of plant succession in a gypsum mine, focusing on the role of the potential pioneer *G. struthium*, and b) to experimentally test *G. struthium* effectiveness as ecosystem engineer and hence as a nurse shrub for the recovery of other species of interest in gypsum plant communities.

To study vegetation reorganization through time in a restored gypsum mine, we conducted a vegetation survey in plots where the original topsoil had been reinserted in different years. We hypothesized that the gypsophyte *G. struthium* might be able to regenerate from the seed bank, establishing in the bare soil and thus becoming a pioneer species in the community. Facilitation driven by *G. struthium* might affect community structure and composition by creating species-rich areas in its vicinity. Complementary, we conducted an experimental study in a gypsum spoil dump to assess how planted individuals of this shrub might act as ecosystem engineers and thus may affect the establishment and development of other plants. We sowed and planted two test species (*Helianthemum squamatum* (L.) Pers. and *Stipa lagascae* Roem. & Schultz) under *G. struthium* and in the bare soil, and measured soil and micro-environmental conditions at both microsites. We expected that *G. struthium* would increase soil water storage and nutrient content, diminish surface compaction and soften extreme temperatures under canopy. According to this soil and micro-environmental amelioration, the germination, survival, and growth of the test species are expected to increase.

Methods

Observational study

We performed an observational study in a restored gypsum mine to evaluate the reorganization of the vegetation at different stages of plant succession, focusing on the role that pioneer shrub species may have on community structure. The gypsum mine is in Gelsa municipality (N 41º 25’ 40.24” W 0º 22’ 47.26”), located in the Middle Ebro Valley in North-Eastern Spain, one of the largest gypsum outcrops in Europe (Machín and Navas, 1998). Average annual temperature in the study site is 15.8 ºC and average annual precipitation is 306 $mm \cdot yr^{-1}$ (“Quinto” meteorological station, 1988-2012 period; source: Gobierno de Aragón, http://opendata.aragon.es). Plant communities in the surrounding areas of the mine consist of patchy scrublands composed predominantly of gypsophyte shrubs, Mediterranean widespread gypsovag shrubs and perennial grasses [Table A16.1].

Restoration actions in the gypsum mine were carried out in different phases after mineral extraction, corresponding each one to a different year from 2008 to 2012 [Figure A16.1]. These actions consisted of the replenishment of the exploitation pits with the mine waste, which is a by-product obtained after gypsum processing in the mine, composed of the materials adjacent to gypsum strata and traces of non-exploitable gypsum. Technical actions to improve the physical-chemical and biological properties of the restored area included the addition of an organic amendment and the re-insertion of the original topsoil containing the soil seed bank of the former plant community. We selected one of the youngest plots restored in 2010-2011 (R1; 2.68 ha), one of the oldest plots restored in 2008-2009 (R2; 3.40 ha), and a control plot in the surrounding undisturbed gypsum plant community (C). We discarded the very youngest plots restored in 2011-2012 because they were too small [Figure A16.1]. We arranged six paralleled 225-m linear transects at each plot in May 2015. We recorded all species in contact with the transect line at 20 cm intervals (n = 1225 points per transect x 6 transects per plot = 6750 points per plot) with the line-point intercept sampling method (Goodall, 1952). Recorded plants were categorized in shrubs, perennial grasses, perennial herbs, and annuals.

To assess changes in vegetation among plots (R1, R2 and C) we considered species richness per transect (*i.e.*, the total number of species recorded) and the relative abundance of shrubs, perennial grasses, perennial herbs and annuals (*i.e.*, the percentage of species of each category over the total species recorded). We tested for differences among plots in species richness and relative abundance of the different plant categories using one-way ANOVAs. When significant differences among plots were found, we performed Tukey's post-hoc pairwise comparisons. To determine which perennial species (among dwarf shrubs and large shrubs) appeared as pioneers in the community, we tested for differences among species in the abundance of individuals per transect at each plot by computing zero-inflated Poisson regressions. When significant differences in abundance of individuals were found among species, we computed Tukey's post-hoc pairwise comparisons based on least-squares means.

To evaluate the role of *G. struthium* in the reorganization of the diversity in the community, we assessed its spatial associations with other plant species. For comparison purposes, we assessed plant spatial associations with the rest of large shrubs recorded (the nanophanerophytes *Cistus clusii* Dunal, *Ephedra fagilis* Desf., *Genista scorpius* (L.) DC., *Rosmarinus officinalis* L., *Salsola genistoides* Juss. ex Poir., and *Salsola vermiculata* L.). At each plot, we analyzed plant diversity in the vicinity of the target species *t* (*i.e.*, either *G. struthium* or the other shrub species altogether) with Individual Species-Area Relationships (hereafter ISAR) proposed by Wiegand *et al.* (2007). The $\mathrm{ISAR}_t(d)$ of a target species *t* is defined as the number of different species *j* present within a distance *d* from all of the individuals of the target species *t* along the transect (Arroyo *et al.*, 2015; Wiegand *et al.*, 2007):

$$\mathrm{ISAR}_t(d) = \sum_{j=1}^{N} [1 - P_{tj}(0, d)]$$

where $1 - P_{tj}(0, d)$ is the probability that species *j* was present within a distance *d* of individuals of target species *t*. The $\mathrm{ISAR}_t(d)$ value will be the sum of the probabilities of all the species *N* present (Wiegand *et al.*, 2007). To detect whether the ISAR was significantly different than expected, a confidence envelope was calculated using Monte Carlo simulations of 199 Poisson null models (Wiegand *et al.*, 2007). Then, when ISAR value for a given distance *d* was greater than the maximum expected (*i.e.*, over the 97.5 percentile of the confidence interval), the shrub was considered a diversity accumulator. Conversely, when the ISAR value for a given distance *d* was lower than the minimum expected (*i.e.*, 2.5 percentile of the confidence interval), the shrub was considered a diversity repeller. When the ISAR value was within the confidence envelope at a given distance *d*, it was considered neutral (Wiegand *et al.*, 2007). We set a maximum distance *d* of 4 m, considered an adequate detection range of plant-plant interactions in

Mediterranean semi-arid ecosystems (Arroyo *et al*., 2015; Chacón-Labella *et al*., 2016; Foronda *et al*., 2019).

To detect species-specific interactions, we evaluated pairwise spatial associations between species at each plot, considering the same target species *t*. To do so, we compared the observed number of co-occurrences and the expected number of co-occurrences of the pair of species *tj* (Saiz and Alados, 2012). The observed co-occurrence was the number of times that *t* and *j* were at the same location in all the surveyed transects per plot, and the expected number of co-occurrences was the probability of finding *t* and *j* at the same location in the transects multiplied by the total number of points surveyed per plot. We calculated the probability of finding the pair of species at the same location by multiplying the proportion of each species in the plot (*i.e*., the total recorded abundance of each species divided by the total points surveyed in the plot). To compare the observed and the expected frequencies for each pair of species, we computed the lower and the upper limit of the 95% confidence interval of the Poisson distribution fitted with the expected frequency (Saiz and Alados, 2012). The spatial association between target species and each of the other species, *tj*, was significantly positive (+1) when the observed frequency was above the upper limit of the confidence interval, significantly negative (-1) when the observed frequency was under the lower limit of the confidence interval, and neutral or random spatial association (0) when the observed frequency was within the confidence interval (Saiz and Alados, 2012).

Statistical analyses were performed with R (R Core Team, 2017). ANOVAs were performed using the “stats” package (R Core Team, 2017), and zero-inflated Poisson regressions and Tukey’s pairwise comparisons after least-square means were performed using the “pscl” package (Jackman *et al*., 2007). ISAR analyses and comparisons with Poisson null models were performed using MATLAB R2013a.

Experimental study

We performed an experimental study in a gypsum spoil dump inside an old opencast coal mine to test the potential effects of *G. struthium* as a nurse plant for seedling establishment and as an ecosystem engineer by improving the soil and micro-environmental conditions under its canopy. Gypsum spoil was formed in a coal-fired power station during the process of desulfurization of lignite by limestone forced oxidation (Srivastava and Jozewicz, 2001). The mine is in Andorra municipality (N 40º 59’ 38.45” W 0º 28’ 42.76”) in Teruel coalfield, Central-Eastern Spain. Average annual temperature in the study site is 14.03 ºC, and average annual precipitation is 378 mm·yr^{-1}(“Andorra-Central térmica” meteorological station, 1983-2011 period; source: Gobierno de Aragón, http://opendata.aragon.es). Since the lithology in the area is sand and silt intercalated with marls, shales and coal strata, the experimental site consists of a “gypsum island”.

We set a planting and sowing experiment in the gypsum spoil dump in November 2014 to test the effects of *G. struthium* on the germination, survival and growth of other species of interest for vegetation recovery in gypsum spoils. As nurse plants, we employed 80 well-established adults of *G. struthium* planted in 2012 in the experimental area [Figure A16.2]. As test species, we selected the gypsophyte shrub *Helianthemum squamatum* and the gypsovag perennial grass *Stipa lagascae*. Both plant species are abundant in gypsum plant communities in NE Spain and contribute to organize mature gypsum plant communities (Foronda *et al.*, 2019). We sowed one seed per test species under the canopy of each of 80 nurse plants and in paired points in the surrounding bare soil (n=160 seeds per test species). The location of each sowed seed was marked with a nail to ensure the detection of germinated seedlings. In a second experiment, we planted one seedling per test species under the canopy of 40 nurse plants and in paired points in the surrounding bare soil (n=80 seedlings per test species). We used one-year-old seedlings grown in a nursery managed by the regional government (“Viveros Forestales DGA”, Ejea de los Caballeros municipality, Zaragoza). Sowing and planting under nurse plants were performed at northern canopy exposure. We monitored seed germination and emerged seedling survival three times a year during two years (*i.e.*, in early spring, early summer, and early autumn), and planted seedling growth (height, cm) and survival twice a year during two years (*i.e.*, in early summer and early autumn).

In the sowing experiment, we tested differences between microsites (under *G. struthium* canopy and in the bare soil) in seed germination and seedling survival rates through time for the two test species separately using Cox proportional hazard models. We constructed Kaplan-Meier curves for germination and survival rates visualization

(Bewick *et al.*, 2004). In the plantation experiment, we tested differences in seedling growth between microsites with one-way ANOVAs for each test species separately. Height data were square root transformed to reach normality assumptions. Survival of the planted seedlings was 100% in both test species, and hence it was excluded from the statistical analyses.

Additionally, we evaluated soil and micro-environmental conditions under *G. struthium* canopy compared to the bare soil to quantify the potential role of this shrub as an ecosystem engineer. To this end, we collected soil samples under the canopy of five random individuals of *G. struthium* and in five random points in the bare soil (n=10 samples) in October 2016. We randomly collected and combined five soil cores (4 x 4 cm surface and 7.5 cm deep) per point. Soil samples were dried and sieved over a 2 mm mesh sieve and analyzed in the laboratory for available phosphorus, total organic carbon, and total nitrogen. We estimated available phosphorus extracted with Bray nº1 reagent (Bray and Kurtz, 1945) using a spectrometer (UNICAM 8625 UV/Vis Spectrometer) with the absorbance at 430 nm. We estimated total organic carbon in samples subjected to chromatic acid digestion (Heanes, 1984), using the spectrometer with the absorbance at 590 nm. Finally, we measured the total nitrogen in samples ground to fine particle size, using a Vario MAX CN analyzer (Elementar Vario MAX CN). Also, from January 2015 to October 2016 we seasonally measured surface mechanical resistance ($kg \cdot cm^{-2}$) and six cm-deep soil temperature (ºC) under the canopy of each of 80 *G. struthium* individuals and in paired points in the bare soil (n=160 points). We measured surface mechanical resistance using a force gauge equipped with a compression plate with a diameter of 2 cm (MECMESIN Basic Force Gauge 500N) and soil temperature using a T-bar digital stem thermometer (ATM Ltd ST-9265A). Finally, we investigated the spatiotemporal patterns of soil water availability for the seeds and seedlings in the experiment. We installed five soil moisture EC-5 probes (Decagon Devices Inc., Pullman, USA) under *G. struthium* shrubs employed as nurse plants and five in the bare soil (n=10 points) in November 2014. Volumetric water content (VWC, m^3/m^3) was continuously gathered every one hour from November 24, 2014 to July 29, 2016.

We tested significant differences between microsites in available phosphorus, total organic carbon, total nitrogen, surface mechanical resistance and soil temperature with one-way ANOVAs. Surface mechanical resistance and soil temperature were analyzed for each season separately. To assess soil water content at each microsite during the experiment period, we constructed probability density functions (PDFs) per season with the hourly VWC measures.

Since nurse size can affect soil and micro-environmental conditions under the canopy though the amount of leaf litter and the shade provided (Callaway, 2007a; Tewksbury and Lloyd, 2001), modulating the effect on nursed test species, we seasonally measured the volume (m^3) of each nurse plant considering an approximated semispherical shape

$$V = \frac{2}{3} \times \pi \times H \times D \times d$$

where H is the maximum height, D is the maximum diameter and d is the diameter perpendicular to the maximum diameter. We computed the Pearson's correlation coefficient r to assess the effect of the average shrub size (*i.e.*, the average volume of the seasonal measurements per individual) on seedling growth, nutrient content, surface mechanical resistance and soil temperature under *G. struthium* canopy.

All statistical analyses were performed using R software (R Core Team, 2017). To fit Cox models and construct Kaplan-Meier curves for germination and survival, the "survival" package was used (Therneau, 2014). To fit ANOVAs for seedling growth and for soil and micro-environmental conditions, the "stats" package was used (R Core Team, 2017).

Results

Community structure and composition reorganization after mining disturbances: is G. struthium a pioneer species? Which is its role in the community?

The observational study carried out to evaluate the reorganization of the vegetation along time after the disturbance showed a progression in the structure and composition of plant communities among the surveyed plots (R1, R2 and C). Species richness was the largest in the youngest restored plot R1, with no significant differences between R2 and the undisturbed plot C (Figure 40A). We also found significant differences among plots in the relative abundance of shrubs, perennial grasses, perennial herbs and annuals (Figure 40B). The relative abundance of shrub species increased with time after exploitation being the highest in the undisturbed plot C and the lowest in the youngest restored plot R1. The relative abundance of perennial grasses was also the lowest in the youngest restored plot R1, with no difference between the restored plot R2 and the undisturbed community. The relative abundance of perennial herbs and annual species decreased with time after exploitation, being the highest in the youngest restored plot R1 than in the other plots (Figure 40B).

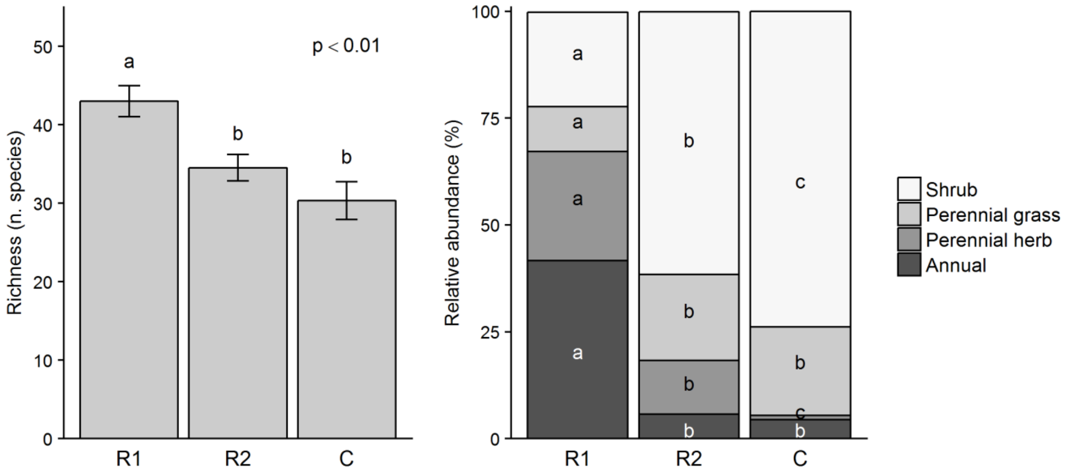

Figure 40 Changes in vegetation among plots in the gypsum mine: (A) Mean species richness and (B) Relative abundances of different plant categories (shrubs, perennial grasses, perennial herbs and annuals) at each plot in the gypsum mine. Different letters indicate significant differences in the relative abundance of each habitat category among plots after Tukey's post-hoc pairwise comparisons.

Particularly for shrubs, we found significant differences among species in the mean abundance per transect at each plot (Figure 41). In both restored plots R1 and R2, *G. struthium* was the shrub with the largest number of individuals, followed by the other gypsophyte shrubs (*Lepidium subulatum* L., *Herniaria fruticosa* L. and *Helianthemum squamatum*). Gypsovag shrubs showed a low abundance of individuals in the restored plots R1 and R2 (Figure 41). In the undisturbed plot, the shrub species that showed the highest abundance was the gypsophyte *H. squamatum*, followed by *H. fruticosa* and the gypsovags *Helianthemum syriacum* (Jacq.) Dum. Cours., *Helichrysum stoechas* (L.) Moench and *Thymus vulgaris* L. We found a more considerable abundance of gypsovag shrubs in this plot compared to the restored ones (Figure 41).

The ISAR computed to evaluate the role of *G. struthium* in the reorganization of the diversity in its local vicinity showed that this shrub acted as a diversity repeller in the restored plots R1 and R2, indicating that plant diversity under the canopy of the shrub was smaller than expected by chance and acted as diversity accumulator in the undisturbed plot C, indicating that plant species richness under its canopy was larger than expected (Figure 42A). The other large shrubs altogether (*C. clusii*, *E. fragilis*, *G. scorpius*, *R. officinalis*, *S. genistoides* and *S. vermiculata*) acted as neutrals in the restored plots R1 and R2 and as diversity accumulators in undisturbed communities (Figure 42B).

The pairwise species-specific analyses of the spatial association at close distances (coexisting in the same location = 0 cm distant) showed that *G. struthium* was positively associated with two, six and seven plant species at R1, R2 and C respectively. These numbers were similar to the number of positive associations found between the other large shrubs and the rest of plant species (two, six and six at R1, R2 and C respectively; Table 10). Mainly herbs and grasses were positively associated with *G. struthium* in the restored plots R1 and R2 (Table 10), while four shrubs appeared positively associated with *G. struthium* in the undisturbed plot C (Table 10).

Testing the role of G. struthium *as a nurse plant*

In the sowing experiment, we found significant differences between microsites in the germination rate through time (Figure 43). Germination of *S. lagascae* was significantly higher under the canopy of *G. struthium* than in the bare soil. Contrarily, germination of *H. squamatum* was significantly higher in the bare soil than under the canopy of *G. struthium*. The survival rate through time did not differ significantly between microsites for any of the test species (Figure 43). In the planting experiment, we found a significant effect of the microsite on seedling growth (Figure 44 A-B). Seedling growth of *H. squamatum* and *S. lagascae* planted under *G. struthium* canopy was higher than in the bare soil. For the individuals planted under *G. struthium* canopy, we found a significant effect of the shrub size (*i.e.*, nurse volume) on seedling growth for *S. lagascae* but not for *H. squamatum* (Figure 44C). All the planted seedlings were alive at the end of the experiment and thus seedling survival was not analyzed.

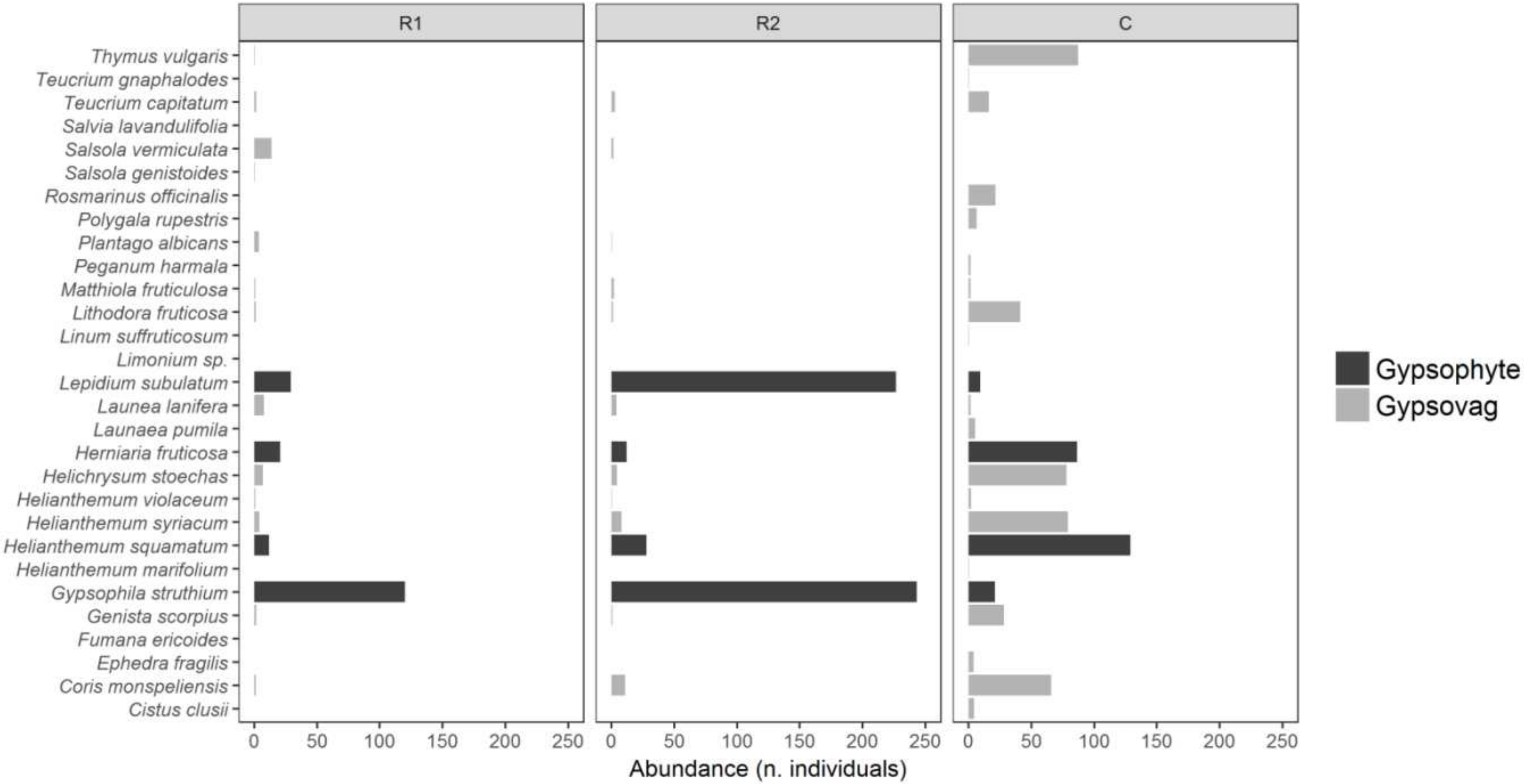

Figure 41 Mean abundance per transect of shrub species at each plot in the gypsum mine.

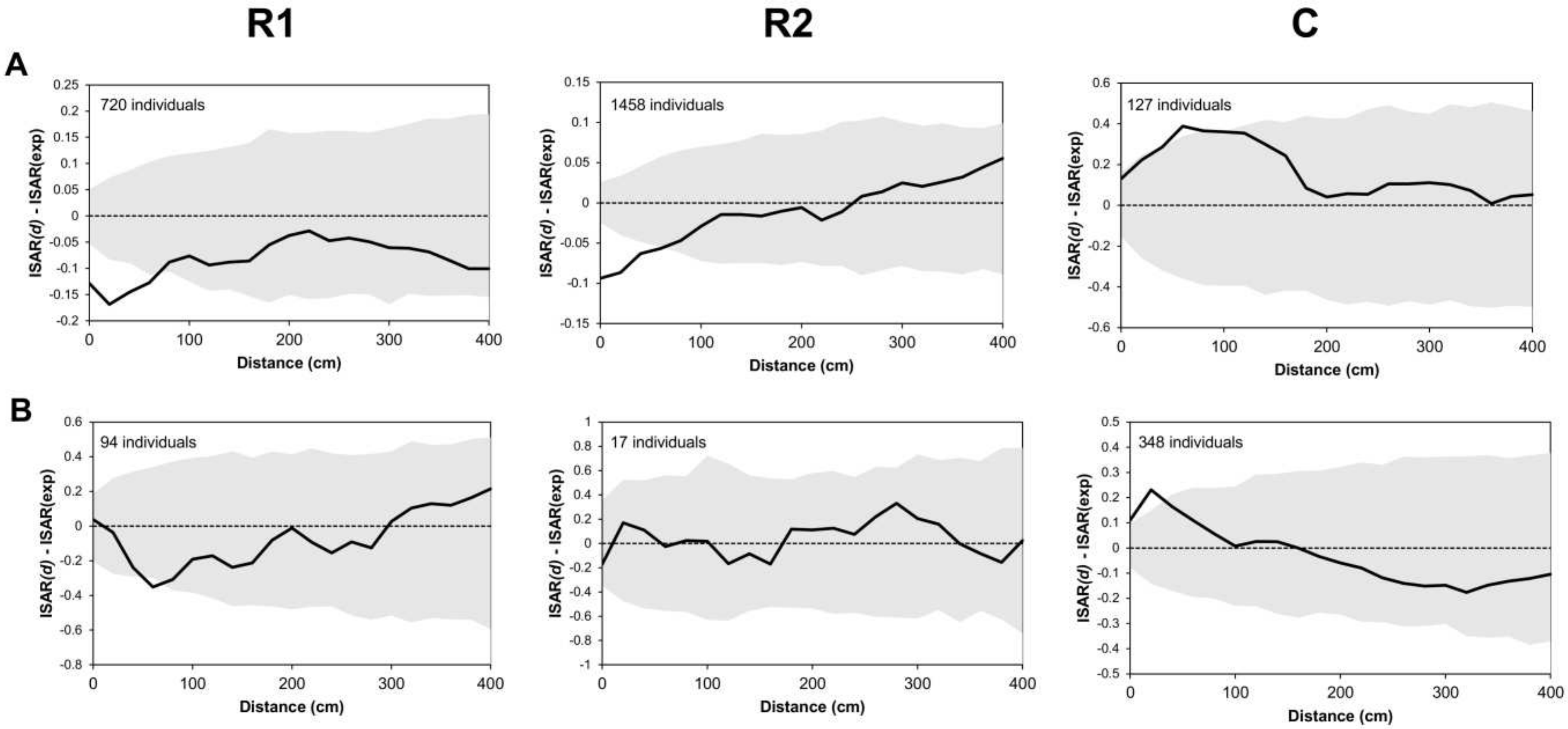

Figure 42 ISAR curves of **A)** *G. struthium* and **B)** the other large shrubs per plot in the gypsum mine (R1, R2 and C). When ISAR curve is represented above the confidence envelope (grey shaded), the shrub act as diversity accumulator, when it is represented below the confidence envelope, the shrub act as diversity repeller and when it is represented within the confidence envelope, the shrub act as neutral.

Table 10 Positive (+1), negative (-1) and neutral (0) spatial associations between each recorded species and *G. struthium* compared to the spatial associations between each species and the other shrubs.

		G. struthium			**Other shrubs**[1]		
Species[2]	**Category**	**R1**	**R2**	**C**	**R1**	**R2**	**C**
Perennial species							
Agropyron cristatum (L.) Gaertn.	Grass	0	0	0	0	0	-1
Coris monspeliensis L.	Dwarf shrub	0	0	0	0	0	-1
Convolvulus lineatus L.	Ruderal herb	0	-1	0	0	0	0
Diplotaxis erucoides DC.	Ruderal herb	0	-1	0	0	0	0
Dittrichia viscosa (L.) Greuter	Ruderal herb	0	0	0	-1	0	0
Echium vulgare L.	Ruderal herb	0	0	0	0	+1	0
Eruca vesicaria (L.) Cav.	Ruderal herb	0	-1	0	0	0	0
Fumana ericoides (Cav.) Gand.	Dwarf shrub	0	0	0	0	0	+1
Genista scorpius (L.) DC.	Shrub	0	0	-1	0	0	-1
Helianthemum syriacum (Jacq.) Dum. Cours.	Dwarf shrub	0	0	+1	0	0	0
Helianthemum squamatum (L.) Pers.	Dwarf shrub	0	-1	0	0	0	+1
Helichrysum stoechas (L.) Moench	Dwarf shrub	0	0	0	0	0	0
Herniaria fruticosa L.	Dwarf shrub	0	-1	-1	0	0	0
Lepidium subulatum L.	Dwarf shrub	-1	-1	0	+1	0	+1
Lolium perenne L.	Ruderal grass	-1	+1	0	0	+1	0
Lygeum spartum L.	Grass	-1	-1	0	0	0	-1
Onopordum nervosum Boiss.	Ruderal herb	0	+1	0	0	0	0
Piptatherum miliaceum (L.) Coss.	Ruderal grass	0	+1	0	0	0	0
Polygala rupestris Pourr.	Dwarf shrub	0	0	+1	0	0	+1
Rosmarinus officinalis L.	Shrub	0	0	0	0	0	-1
Salsola vermiculata L.	Shrub	-1	0	0	0	0	0
Scorzonera laciniata L.	Ruderal herb	-1	0	0	0	0	+1
Sonchus tenerrimus L.	Ruderal herb	+1	+1	0	+1	0	0
Stipa parviflora Desf.	Grass	-1	+1	+1	0	0	+1
Teucrium gnaphalodes L'Hér.	Dwarf shrub	0	0	+1	0	0	0
Thymus vulgaris L.	Dwarf shrub	0	0	+1	0	0	0
Annual species							
Avenula bromoides (Gouan) H. Scholz	Ruderal grass	0	0	0	+1	0	0
Bromus diandrus Roth.	Ruderal grass	+1	0	0	+1	0	0
Bromus rubens L.	Ruderal grass	-1	0	0	0	0	0
Chaenorrhinum rubrifolium (Robill. & Castagne ex DC.) Fourr.	Herb	0	0	0	0	0	-1
Filago pyramidata L.	Herb	0	0	0	+1	0	0
Galium verrucosum Huds.	Herb	0	+1	+1	0	0	0
Hordeum murinum L.	Ruderal grass	-1	0	0	+1	0	0
Hippocrepis ciliata Willd.	Ruderal herb	-1	-1	0	0	0	0
Linum strictum L.	Herb	0	0	+1	0	0	0
Neatostema apulum (L.) I.M. Johnst.	Ruderal herb	0	-1	0	0	0	0
Unknown species							
Unknown 1	Unknown	0	0	0	+1	0	0

[1] Nanophanerophytes *(Cistus clusii, Ephedra fragilis, Genista scorpius, Rosmarinus officinalis, Salsola genistoides* and *S. vermiculata).*

[2] The species showing neutral associations with both *G. struthium* and the other shrubs in all the plots are not displayed (but see Table A17.1).

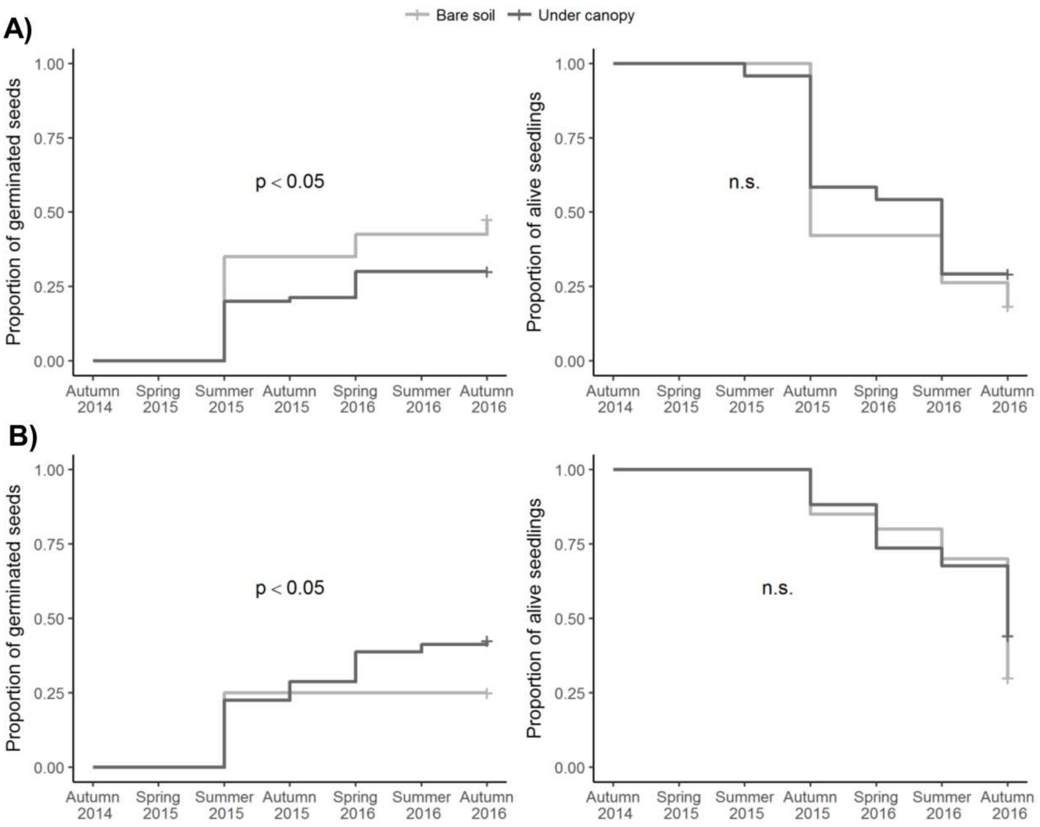

Figure 43 Germination and survival rate through time per microsite (in the bare soil and under *G. struthium* canopy) of seeds planted of the test species A) *Helianthemum squamatum* and B) *Stipa lagascae*. Significant differences between microsites when $p \leq 0.05$.

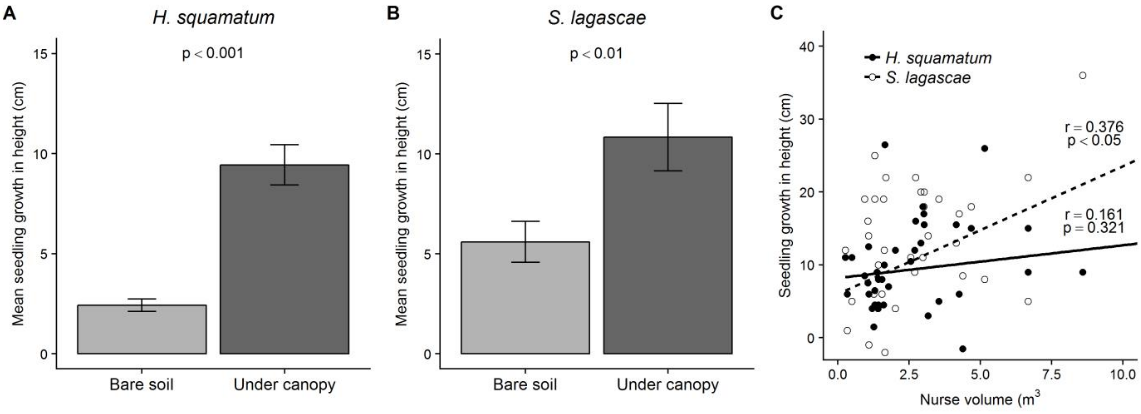

Figure 44 Mean seedling growth in height per microsite (in the bare soil and under *G. struthium* canopy) of planted seedlings of the test species **A)** *Helianthemum squamatum* and **B)** *Stipa lagascae*; and **C)** Pearson's correlation *r* between the nurse volume and the seedling growth for both test species. Significant differences between microsites when $p \leq 0.05$.

Testing the role of G. struthium as an ecosystem engineer

Total organic carbon was significantly higher under the shrub canopy than in the bare soil (Figure 45). There were no significant differences in available phosphorous and total nitrogen between both microsites. Surface mechanical resistance and soil temperature were significantly lower under the shrub canopy than in the bare soil in all seasons, being this trend more marked in summer (Figure 46). While nutrients content was not affected by the shrub size [Figure A18.1], the surface mechanical resistance and the soil temperature were lower under larger shrubs, but only in spring (Figure 47, [Figure A18.2]).

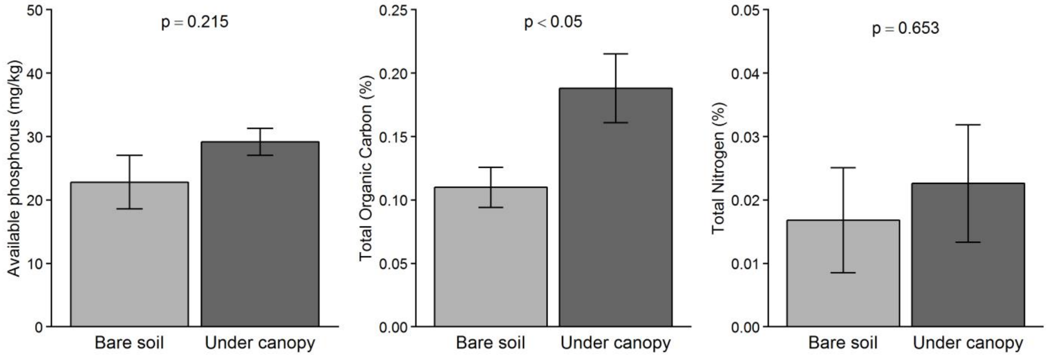

Figure 45 Mean values of the nutrient content in the soil (available phosphorus, total organic carbon and total nitrogen) per microsite surveyed in the gypsum spoil dump (in the bare soil and under *G. struthium* canopy). Significant differences between microsites and significant effect of the nurse volume when $p \leq 0.05$.

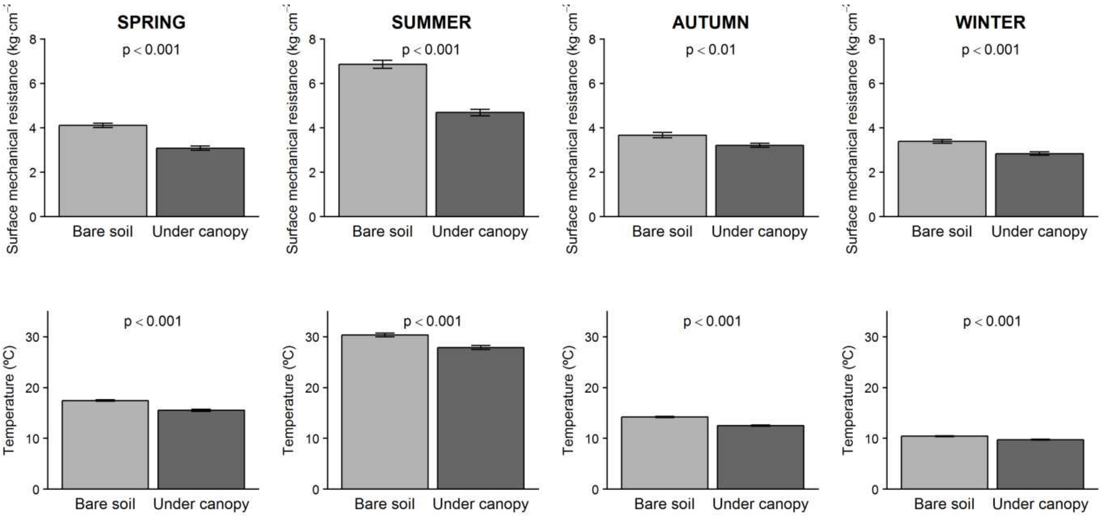

Figure 46 Mean values per season of surface mechanical resistance and soil temperature at each microsite surveyed in the gypsum spoil dump (in the bare soil and under *G. struthium* canopy). Significant differences between microsites when $p \leq 0.05$.

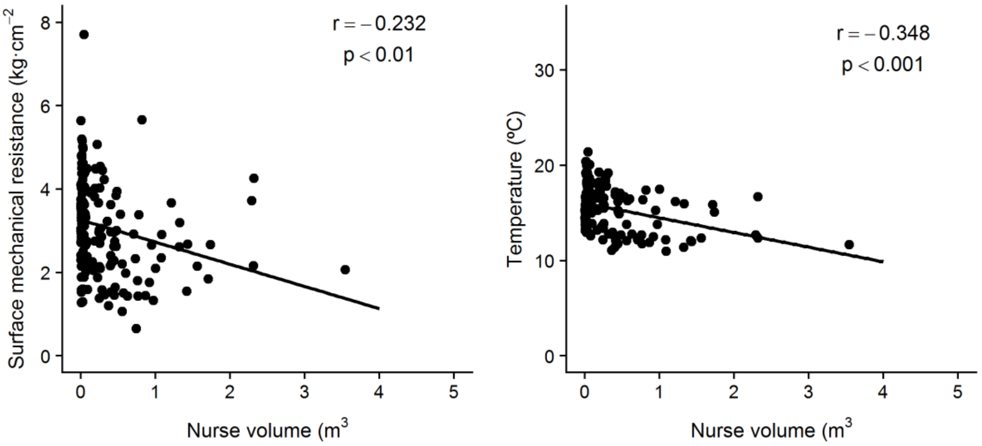

Figure 47 Pearson's correlations between the nurse volume and surface mechanical resistance and soil temperature of the soil under canopy in spring. Significant effect of the nurse volume when $p \leq 0.05$.

The probability density functions (PDFs) of the volumetric water content (VWC) per season showed higher peak PDFs in the bare soil than under canopy at intermediate VWC values (*c.* 0.1 m^3/m^3), being this trend more marked in spring and autumn. At the lowest and highest VWC values registered, PDFs were higher under *G. struthium* canopy than in the bare soil in all the seasons (Figure 48).

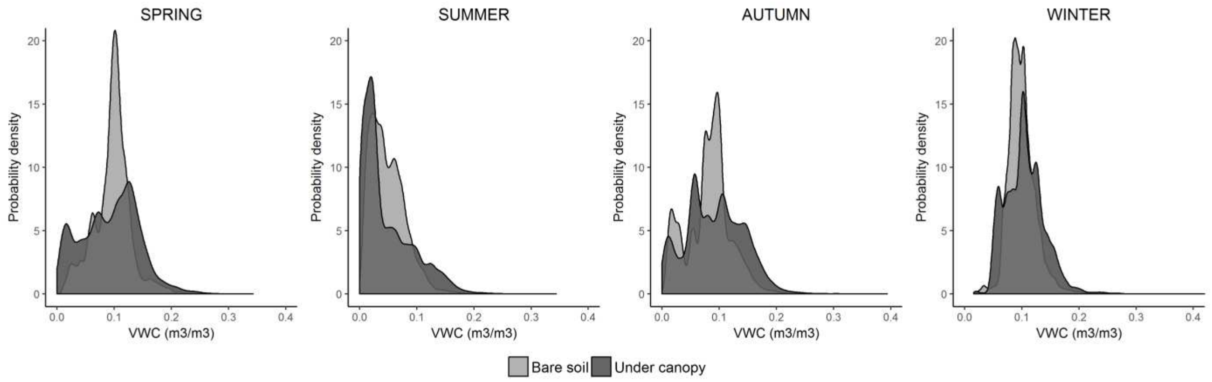

Figure 48 Probability density functions per season for the VWC data hourly gathered with EC-5 humidity probes at each microsite in the gypsum dump: in the bare soil and under *G. struthium* canopy.

Discussion

Facilitation is an important structuring mechanism in natural plant communities (Brooker *et al.*, 2008) and is increasingly being considered in the restoration of disturbed areas, particularly in harsh environments (*e.g.*, Maestre *et al.*, 2001). In this study, we combined observational and experimental data to assess the suitability of a shrub emerging as a pioneer in post-mining gypsum substrates for enhancing vegetation recovery in these degraded areas. Our study unveiled that the gypsophyte shrub *G. struthium* appeared spontaneously as a pioneer species in the first stages of plant succession in gypsum spoils and that it promoted the germination and growth of other plants by improving soil and micro-environmental conditions under its canopy.

In the observational study performed in a gypsum mine, we found that vegetation was spontaneously regenerated in the reinserted topsoil. Propagules came either from the original seed bank contained in the topsoil or by seed dispersal from the surrounding undisturbed communities (Kirmer *et al.*, 2008; Martínez-Duro *et al.*, 2010). As observed in other mines, younger plant communities are richer in species in the younger, but it mainly consisted of transitory annual species and more specifically of ruderals, matching with the results in other studies (*e.g.*, Prach, 2003). Nutrients limitation for plant development inherent in gypsum substrates (Pueyo *et al.*, 2007) were corrected through the addition of the topsoil and organic amendments to the gypsum spoil, thus favoring the establishment of non-specialist and ruderal gypsum plants in the first stages after restoration actions. Nevertheless, approximately the 25 % of the species in the youngest community were shrubs, being gypsophytes the most abundant likely because they can overcome gypsum harsh environmental conditions with no need for facilitation (Escudero *et al.*, 2015; Martínez-Duro *et al.*, 2010). Among pioneer gypsophyte shrubs, *G. struthium* was the most abundant, outperforming the others (*H. fruticosa*, *H. squamatum,* and *L. subulatum*) by more than three times their abundance. Therefore, in line with previous findings in another gypsum mine in SE Spain, and satisfying the postulated hypothesis in this study, *G. struthium* appeared as a pioneer species in the community (*e.g.*, Dana and Mota, 2006).

In the observational study, we considered the spatial association of *G. struthium* with other species as a proxy to infer the potential nurse role of this shrub in different stages of plant succession (Saiz and Alados, 2012). We found that *G. struthium* had a positive role in structuring diversity in gypsum plant communities, but only in late successional stages. Specifically, we found positive spatial associations between *G. struthium* and mainly perennial species in the undisturbed community. Although *G. struthium* also showed positive spatial associations with perennial species in plot R2, in general, we found diversity repulsion in both restored plots. As well as for *G. struthium*, the other large shrubs did not act as nurses in the youngest communities, likely due to the broad niche suitability of the annuals that first colonized the substrate (Grime, 2006). Thus, in general, all the large shrubs including *G. struthium* showed a similar trend, from repelling diversity in the youngest communities towards accumulating diversity in the undisturbed communities, where large shrubs play an important role accumulating plant diversity underneath (Tewksbury and Lloyd, 2001). Despite having a similar role to the other shrubs, the occurrence of *G. struthium* as a pioneer species in the community and the ecological relevance that gypsophytes have would make it more suitable for the restoration of gypsum spoils.

Complementary, the sowing and planting experiment showed beneficial effects of *G. struthium* on the development of the gypsophyte dwarf shrub *H. squamatum* and the gypsovag perennial grass *S. lagascae*. These are perennial species commonly growing in gypsum plant communities of NE Spain (Braun-Blanquet and Bolòs, 1958) and thus are target species for the restoration of the former community. Specifically, the positive effects of *G. struthium* on the germination and growth of *S. lagascae* in the experiment paralleled its positive spatial association with *S. parviflora*, of similar morphological traits. Conversely, although the growth of *H. squamatum* was positively affected by *G. struthium* in the experiment, these species were not spatially associated at any plant community surveyed in this study. Besides, more seeds of *H. squamatum* germinated in the bare soil than under *G. struthium* canopy. Both findings support the idea that this gypsophyte does not need to be facilitated to establish in the community (Escudero *et al.*, 1999). In any case, positive interactions of *G. struthium* with perennial gypsovag species may have a key role by inducing plant succession towards a community that endures over time (Connell and Slatyer, 1977). However, given the temporal variability in plant interactions (Miriti, 2006), longer-term studies including other potential nurse plants would be desirable to assess the net beneficial effect on plant succession.

The confirmed nurse effect of the gypsophyte shrub *G. struthium* is related to its role as ecosystem engineer by changing the physical environment under the canopy through providing shade and nutrients and thus reducing environmental stress for plant establishment (Callaway, 2007a; Jones *et al.*, 1997). The detailed comparisons of soil and micro-environmental conditions under *G. struthium versus* bare soil helped to understand what are the specific factors involved in the role of *G. struthium* as a nurse plant. As expected, adult individuals of this shrub improved these conditions under canopy compared to the bare soil. We found significantly more total organic carbon under the shrub canopy than in the bare soil likely related to a higher amount and decomposition of leaf litter, which in turn is related to higher soil biological activity and nutrient cycling (Delgado-Baquerizo *et al.*, 2013). Higher availability of nutrients would facilitate the development of plants, especially of those that are poorly tolerant to nutrient-scarcity conditions (Callaway, 2007a). The hypothesized effect of *G. struthium* canopy on the diminishing of the surface mechanical resistance was also observed, especially in the dry season. The soil physical amelioration mediated by *G. struthium* could have positive effects on seed establishment because physical crusts act as a barrier for root elongation (Romão and Escudero, 2005). Our study also confirmed that soil temperature was softened under *G. struthium* compared to the bare soil. This effect was primarily observed in summer when the surface temperature in the bare soil can be lethal for plants (Valladares and Pearcy, 2002). On the other hand, we found complex patterns of water availability under *G. struthium* compared to the bare soil. In general, for all seasons, high VWC values were more frequent under the plants than in the bare soil, which can be interpreted as larger infiltration rates during rainfall events under the plants (Pueyo *et al.*, 2016). Plant roots open channels and microporous and organic matter enhances soil structure, facilitating water infiltration under the plants compared to the bare soil (Boyle *et al.*, 1989; Burgess *et al.*, 2001). However, low VWC values were also more frequent under the canopy of plants than in the bare soil in all seasons, which can be interpreted as evidence of the depletion of soil water storage through plants water uptake. In summary, the detected nurse effect of the gypsophyte shrub *G. struthium* could be explained by its role as ecosystem engineer by improving nutrient content, ameliorating physical soil properties and reducing extreme temperatures and to a lesser extent, soil water scarcity.

There is an increasing demand for low-cost but fast and efficient restoration techniques for maintaining ecosystem functions (Hobbs *et al.*, 2007). While active restoration actions often derive in a rapid vegetation recovery in post-mining bare soils (*e.g.*, Ballesteros *et al.*, 2012), natural plant succession is a slow process, especially in harsh environments such as gypsum areas (Mota *et al.*, 2003). Nonetheless, natural plant succession is often preferred by managers because it assumes lower economic costs than active restoration actions (Miller and Hobbs, 2007). As a medium-cost alternative and in order to accelerate the recovery of gypsum plant communities, we propose applying technical actions to direct spontaneous succession instead of relying entirely on the latter (Prach, 2003; Prach and Hobbs, 2008). As in other investigations, this study revealed that shrubs with a nurse role could be essential for promoting plant succession and the efficient re-structuration of the diversity in degraded plant communities (*e.g.*, Gómez-Aparicio, 2009). Using nurse plants naturally growing in degraded areas might be a useful measure to reduce economic cost, accelerate plant succession, and restore a self-sustainable community (Byers *et al.*, 2006; Padilla and Pugnaire, 2006). However, in ecosystems degraded by mining, vegetation is thoroughly removed, and thus the re-establishment of nurse plants is crucial as the first step in restoration.

Particularly, favoring the establishment of *G. struthium* after gypsum mining can imply essential improvements to the effectiveness of revegetation in gypsum spoils by facilitating the colonization of non-specialist but desirable plants, and hence leading to species enrichment. Specifically, we recommend two non-exclusive but complementary actions to enhance restoration in post-mining gypsum substrates. One is to promote the establishment of *G. struthium* by the re-insertion of the original topsoil that contains propagules of the former community together with nutrients and microbiota (Abdul-Kareem and McRae, 1984; Hall *et al.*, 2010). This action offered good results in our study site because the nurse shrub *G. struthium* regenerated spontaneously as a pioneer from the bare soil. Vegetation recovery can then rely on natural succession because seeds of other species contained in the soil seed bank or arrived from the surrounding undisturbed communities (Kirmer *et al.*, 2008; Martínez-Duro *et al.*, 2010) will encounter suitable conditions for their establishment under the canopy of well-established individuals of *G. struthium*. The other proposed restoration action is to subsequently use those well-established individuals of *G. struthium* as nurse plants through actively sowing seeds or planting seedlings of different species of interest for restoration under its canopy. These plants will successfully develop and then contribute to the organization of mature gypsum plant communities.

Individuo de *Chaenorrhinum rubrifolium* germinado en las muestras del banco de semillas recogido en La Lomaza de Belchite

Cianotipia elaborada por Ángeles Muñoz
Fotografía original: Ana Foronda

General Discussion

This thesis contributed to understanding the role of plant-plant interactions at the community level in semi-arid gypsum plant communities of the Middle Ebro Valley (NE Spain) and tests the application of this knowledge to the restoration of habitats degraded by mining. This community-level study provided insights into the relevance of plant-plant interactions in maintaining and structuring plant diversity. Plant-plant interactions at the community level have been previously studied in arid and semi-arid ecosystems (Soliveres and Maestre, 2014), but these are poorly studied specifically in gypsum outcrops. Our findings corroborated the importance of positive interactions for plant establishment in gypsum environments, where plants are subjected to severe environmental and soil conditions that limit their development (Escudero et al., 2015). The main purpose for studying biotic interactions in these communities was to understand the understudied biotic mechanisms that modulate gypsum ecosystems, and subsequently to apply the acquired knowledge to the conservation and restoration of these rare and specialized communities. The study had a particular focus on the identification of key nurse species that maintain local species richness, which would be useful for including them as examples in restoration plans, favoring the restoration success and the reduction of time and economic costs of vegetation recovery (Byers et al., 2006). In fact, we successfully found the suitability of using the species identified as nurse plants in the restoration of gypsum areas.

We investigated biotic interactions in the community in a broad sense, including all the plant species present, covering different life/ontogenetic stages going from seeds to standing plants, and considering different methodologies that include observational and experimental proxies. This multi-specific and multi-proxy study allowed the identification of key nurse species in Iberian gypsum plant communities. Specifically, the gypsophyte shrub *Gypsophila struthium* was the one that showed the most facilitative effect in the community. Unlike other studies that previously demonstrated facilitation driven by *G. struthium* only regarding the phylogenetic diversity it harbored (Navarro-Cano et al., 2016), we determined its positive role at the community level from different perspectives and methodologies. The observational data revealed that this shrub appears as a pioneer species in the community, and that accumulates other plants under its canopy and in the local vicinity, shaping species-rich vegetation patches. The experimental data revealed that this shrub accumulates plants underneath due to a twofold positive effect. This positive effect seems to be the combined result of acting as a seed accumulator and ameliorating the germination and growth of other species by its ecosystem engineer abilities.

Biotic interactions at the community level in gypsum plant communities

Biotic interactions between plants in arid and semi-arid areas have been widely studied, but they have usually been evaluated considering the effect of a single species or the interplay between a pair of species (*e.g.*, Armas and Pugnaire, 2005). Given that biotic interactions work in complex networks of individual plants interacting with other plants (Verdú and Valiente-Banuet, 2008), studies considering all the species constituting the community are required to understand the net effect of biotic interactions on ecosystems fully. Although the assessment of biotic interactions at the community level is gaining more attention (*e.g.*, Maestre et al. 2008), studies are still scarce, especially in gypsum ecosystems, where they have been poorly studied (*e.g.*, Saiz et al., 2014). This thesis provides new evidence on the importance of biotic interactions in gypsum plant communities, considering all the species (both perennials and annuals) constituting gypsum vegetation in the study area. Studies such as this one, considering biotic interactions at the community level, provide broader knowledge on plant community structuring, which would be useful for ecosystems conservation and restoration purposes. Moreover, considering all the species constituting the community allow the identification of species-specific effects (Callaway, 1998), difficult to detect when focused only in a few species or in the effect of a single species. Indeed, we were able to identify species-specific interactions with this multi-specific study (*e.g.*, species-specific interference exerted by *Cistus clusii* found in Chapter 3).

Conducting field experiments to explore biotic interactions at the community level is logistically challenging (Schöb et al., 2012); therefore, biotic interactions in the community were evaluated mainly through observational studies. Given that plants are sessile organisms and biotic interactions occur between individuals closely located in space, plant spatial patterns are considered good proxies to infer the interaction outcome in the community (Gross et al., 2013; Saiz and Alados, 2012; Tirado and Pugnaire, 2003). As in other studies, biotic interactions were ascertained from the spatial associations of plants (Arroyo et al., 2015; Cavieres et al., 2006; Saiz and Alados, 2012). Specifically, the aim was to approximate the potential plant-plant interactions by assessing whether or not species aggregated in space. We predominantly found species aggregation in the studied plant communities in the Middle Ebro Valley (Chapter 1). Spatial aggregation of plants was found from the standpoint of the facilitated plants, which were found associated with adult shrubs more often than expected, but also from the standpoint of the facilitator plants (*i.e.*, shrubs), which accumulated highly diverse microcosms underneath. Plant spatial aggregation may be driven by direct facilitation on plant establishment through the amelioration of micro-environmental conditions under the canopy of shrubs, but also by indirect facilitation through the protection from herbivores or the seed trapping effect of shrubs (Bullock and Moy, 2004; Callaway, 2007a, 2007b; Smit et al., 2006).

Importantly, other factors such as the spatial distribution of favorable resources, the micro-topography or seed dispersal can act simultaneously generating aggregated vegetation patterns (Aguiar and Sala, 1997; Ramón et al., 2018; Rubio and Escudero, 2000). Thus, inferring biotic interactions based only on observational data should be taken with caution. For this reason, we combined observational data with experimental data under field and greenhouse conditions. The combination of both the observational and the experimental data allowed confirming the spatially inferred interactions, and the identification of the potential mechanisms that resulted in species spatial aggregation or segregation. More specifically, the greenhouse experiment using aqueous extracts allowed the identification of the chemical interference exerted by *C. clusii* (Chapter 3). The observational study showed a species-impoverishment under its canopy (Chapter 1), but the underlying chemically mediated mechanism would not have been discerned without an experimental approach. In addition, sowing and planting experiments were set to test the effect that key nurse species may have on the establishment and development of other species (Chapter 4), which in the long term will lead to species accumulation under nurse canopies. Finally, the combination of the quantification of the soil seed bank and the above-ground vegetation surveys demonstrated that species aggregation around nurse shrubs is due to both direct and indirect facilitation (Callaway, 2007a, 2007b).

Positive interactions (*i.e.*, facilitation), but also negative interactions (*i.e.*, competition or allelopathy) operate simultaneously and bidirectionally between pairs of neighboring species (Holzapfel and Mahall, 1999), and both influence the spatial structure of the community in arid and semi-arid environments (Brooker, 2006). The dominance of the aggregated spatial pattern found in the observational study (Chapter 1) highlighted a predominance of positive interactions under stressful gypsum environments. Nevertheless, the net balance of interactions at the community level depends on the species involved and the abiotic conditions (Bertness and Callaway, 1994; Liancourt et al., 2005). Therefore, in order to obtain general conclusions about the role of biotic interactions in gypsum environments, it is necessary to test the relevance of the identified interaction outcome in other gypsum plant communities. In fact, we found more positive plant associations under lower aridity and greater diversity repulsion under higher aridity conditions. Therefore, the observational study carried out in two natural plant communities under different aridity conditions suggested a minimization of facilitation towards an increment of interference along with stress increment. These results match with other studies in the same study area (Saiz et al., 2014) but also in other semi-arid areas (Maestre et al., 2009; Soliveres and Maestre, 2014). These findings are contrary to what was expected following other studies that postulate that facilitation would have a higher relevance under more stressful environments (SGH; Bertness and Callaway, 1994). One possible explanation is a collapse of facilitation at the extreme of stress gradients (Michalet et al., 2014). Nevertheless, this result must be taken with caution, as only two levels along the aridity gradient were considered.

Plants need for facilitation in semi-arid gypsum plants communities

The presence of gypsum in the soil determines a stressful and extreme ecological environment for plant life (Escudero et al., 2015). Gypsum soils have physical and chemical limitations such as low water availability, nutrients scarcity, potentially toxic concentrations of sulfate and calcium and hard surface crusts that prevent root penetration (Moret-Fernández and Herrero, 2015; Palacio et al., 2014; Pueyo et al., 2007; Romão and Escudero, 2005). Such stressful conditions make gypsum areas considerably unsuitable for plants, especially at the establishment stage, being germination the first bottleneck that plants must overcome for a successful establishment (Martínez-Duro et al., 2009). The observational study of Chapter 1 highlighted the relevance of facilitation for plant establishment in semi-arid gypsum plant communities. Plants, especially in early life stages, were found more frequently than expected spatially associated with adult shrubs that provide shade and ameliorate micro-environmental conditions (Callaway, 2007a). Conversely, seedlings were recorded less frequently than expected in open areas, where plants are subjected to potentially lethal conditions such as high solar radiation, elevated surface temperature, and intense dryness (Valladares and Pearcy, 2002). At the adult stage, the spatial association of plants with adult shrubs was not so evident, thus revealing that facilitation is especially important at the establishment phase. Although it is widely recognized that facilitative interactions are crucial for seedling establishment in stressful environments (Soliveres and Maestre, 2014), studies based on plant establishment at the community level are not frequent. This study contributes to the understanding of the recruitment processes in gypsum plant communities focusing on the spatial patterning of plant establishment.

In general, gypsophytes have a range of adaptations to gypsum, such as the ability to exploit the crystallization water contained in gypsum molecules, or the ability to surpass the hard surface crust (Palacio et al., 2014; Romão and Escudero, 2005). Adaptations to gypsum may allow gypsophytes to overcome the stressful conditions of gypsum soils and establish with no need for a nurse plant (Escudero et al., 2000; Romão and Escudero, 2005). This ability may lead to the occurrence of gypsophytes as pioneer species in gypsum plant communities, which was observed in the gypsum mine surveyed in this study, as well as in other gypsum mines (Dana and Mota, 2006). On the other hand, gypsovags in early life stages have been found to be more vulnerable to gypsum stressful conditions, and often benefit from the presence of nurse plants to establish and survive in gypsum plant communities (de la Cruz et al., 2008). However, in this thesis, and contrary to what we expected, in general seedlings of both gypsophytes and gypsovags were found close to adult shrubs at frequencies higher than expected (Chapter 1). This finding contrast with other studies, which found that gypsophytes establish in open areas with no need for a nurse plant (*i.e.*, *Helianthemum squamatum*; de la Cruz et al., 2008). The ability to establish under the harsh conditions of gypsum bare soils may be species-specific. Since our study takes in consideration all the species in the community, these potential species-specific abilities to establish in open areas have been observed. For example, despite *G. struthium* is able to establish in open areas, it showed a preference to establish under the canopy of other shrubs. This goes in line with results from Chapter 4, where *G. struthium* appeared as a pioneer in gypsum plant communities, suggesting that it does not need to be facilitated to establish in gypsum bare soils. Nevertheless, despite having the ability to establish without assistance, gypsophyte plants could also benefit from the presence of nurse plants in stressful environments. These observations further reinforce the importance of facilitation for plant establishment in stressful environments, such as gypsum ecosystems.

Although proximity to nurse plants may enhance seedling establishment, the positive effects may vary in intensity depending on the relative distance to the nurse plant (Reisman-Berman, 2007). As the facilitated plants become adults, they may be harmed by nurse shrubs due to competition for water or nutrients, or due to the limitation of light availability under the canopy (Coomes and Grubb, 2000; Miriti, 2006; Valladares and Pearcy, 2002). Despite having found more seed accumulation in the center of the shrubs than at the edge of the canopy of shrubs, the most favorable microsite in which facilitated gypsophytes and gypsovags emerged and established was at the edge of the canopy. At the edge of the canopy, the positive effects of shrubs through the micro-environmental amelioration may overcome shrubs competition for water, nutrients, and light, as found in other study (Reisman-Berman, 2007). Thus, accumulated seeds would germinate and establish at the edge of the shrubs to a higher extent than under the canopy of shrubs. This suggest that, although seeds accumulation may be an important mechanism that form species-rich areas (Caballero et al., 2008), biotic interactions related directly to resource use are the most significant modulators of the community structure.

In general, adult gypsophytes appeared more frequently than expected at the edge of the canopy and less frequently than expected under the canopy of shrubs, suggesting competitive exclusion by nurse plants. Adult gypsovags also appeared more frequently at the edge of the canopy, but different to gypsophytes, they appeared as frequently as expected under the canopy of shrubs. This fact supported the idea that the competitive response of a species may be inverse to its ability to tolerate stress (Liancourt et al., 2005; Maestre et al., 2009). To our knowledge, this is the first report on how the positive effect of nurse plants is modulated by the combined effect of nurse plant tolerance to gypsum (gypsophytes *versus* gypsovags) and the position of facilitated plants with reference to the canopy of nurse plants.

Dominant shrubs with a positive role in gypsum plant communities

Generally, shrubs are considered to have a facilitative role in plant communities (Gómez-Aparicio et al., 2005). In our study, to a lesser or a greater extent, most target shrubs harbored more richness and abundance of plants than open areas. Nevertheless, the studied shrubs showed different abilities to accumulate diversity in the local vicinity, being large shrubs (nanophanerophytes) more capable of accumulating diversity than small shrubs (chamaephytes). The target shrubs whose understories harbored the highest richness and abundance of plants were the gypsophytes *G. struthium* and *Ononis tridentata,* hence having the most significant positive effect structuring diversity in their local vicinities. Similar findings were obtained by Saiz et al. (2014) in the same gypsum plant communities following a different methodology. In addition, other studies also found a positive role of *G. struthium* and *O. tridentata* in other gypsum plant communities from the Iberian Peninsula (Navarro-Cano et al., 2016, 2014). These are very abundant gypsophyte shrubs in the gypsum plant communities of the Middle Ebro Valley, which reinforces their positive key role in maintaining species richness and structuring plant diversity in the community. The positive role of these shrubs may be driven by direct facilitation (*e.g.*, micro-environmental amelioration; Callaway, 2007a) or indirect facilitation (*e.g.*, seed trapping; Callaway, 2007b), or by the interplay between both. In this thesis, the inclusion of studies focused on the soil seed bank, and the assessment of micro-environmental conditions under shrub canopies provided a more comprehensive view of the underlying mechanisms that result in species aggregation around shrubs in gypsum plant communities. For example, regarding species aggregation found associated with *G. struthium*, the results of the thesis suggest that the mechanism that drive species aggregation could be the combination of the seed accumulation (Chapter 2) and the micro-environmental amelioration (Chapter 1) under its canopy.

In line with previous studies carried out in semi-arid gypsum plant communities, this study highlights the role of shrubs as important elements in the community by spatially structuring the soil seed bank (Caballero et al., 2008; López-Peralta et al., 2016). One of the main findings of Chapter 2 was the contrasting roles of shrub species with different physiognomy on spatially structuring the soil seed bank. Consistent with Caballero et al. (2008), shrub size influenced the formation of the soil seed bank, being richer below taller shrubs. Within shrub species of the same size-type, cushion-like shrubs were more capable of aggregate seeds than the erect ones. This better ability of cushion-like shrubs may be due to the placement of branches, which drag in the soil surface and act as physical barriers to seed distribution by runoff (Aerts et al., 2006). The target shrub that accumulated the richest seed bank under its canopy was *G. struthium* that is a tall cushion-like shrub. This shrub acts as a seed sink likely by physically obstructing seeds more efficiently due to its height, and accumulating seeds underneath through crawling branches (Aerts et al., 2006; Bullock and Moy, 2004; Caballero et al., 2008). Moreover, this species was found to act simultaneously as a seed source through the plants harbored underneath, accumulating seeds in the local vicinity due to the short-range dispersal typical in species from gypsum areas (Martinez-Duro et al., 2012; Olano et al., 2005). The cumulative effect of *G. struthium* extended to the surrounding open areas, likely corroborating the seed source effect (Caballero et al., 2008). Therefore, results derived from Chapter 2 (*i.e.*, seeds accumulation under shrubs) link to the results derived from Chapter 1 (*i.e.*, species aggregation under shrubs), highlighting the positive role of *G. struthium* in forming species-rich vegetation patches through the formation of species-rich soil seed banks in its vicinity.

However, despite the seed accumulation driven by shrubs, a successful seedling establishment from the seed bank is not ensured. Instead, seedling establishment depends on the potential nurse role of shrubs that allow the rich seed bank to encounter suitable conditions for seed germination (Callaway, 2007a). Shrubs often act as ecosystem engineers by reducing environmental stress under their canopies, hence favoring plant establishment (Callaway, 2007a; Jones et al., 1994). Data on micro-environmental and soil conditions and data on seed germination and seedling growth under a potential nurse shrub complemented the observational study in natural plant communities. In the observational study, the target shrubs harboring the highest richness and abundance of plants (*i.e.*, *G. struthium* and *O. tridentata*) also enhanced the micro-environmental and physical-chemical soil conditions under their canopies compared to open areas. Specifically, these shrubs diminished surface mechanical resistance, softened temperature and increased nutrient content (especially total organic carbon). In addition, the experiment set in the gypsum dump showed that the positive effect of *G. struthium* on the germination and growth of *Stipa lagascae* could be attributed to reduced surface compaction, softening of temperature and increased organic carbon content in the soil. Other measured variables, such as soil water content, did not seem to be as decisive. Studying these different variables allowed unraveling the mechanisms and factors behind facilitation, which appear to be correlated to the physical-chemical soil properties to a greater extent than to the hydrological ones. These findings contrast with other studies demonstrating that the nurse effect of shrubs in semi-arid ecosystems is closely related to the hydrological improvement under shrubs (Berdugo et al., 2014; Callaway, 2007a; Pueyo et al., 2016). Thus, plants in gypsum environments benefit from the amelioration by shrubs of the most limiting factors for plant establishment, which particularly in gypsum soils seem to be the physical surface crust and the nutrient imbalance in the soil to a greater extent than the low water availability.

In summary, the detected positive effect of the gypsophyte shrub *G. struthium* on the formation of species-rich vegetation patches in the community could be explained by the combination of direct and indirect facilitation mechanisms. On the one hand, this shrub accumulates a robust and rich seed bank underneath and in its local vicinity (indirect facilitation; Callaway, 2007b). On the other hand, it has an ecosystem engineer role by reducing environmental stress for plant establishment (direct facilitation; Callaway, 2007a). Both direct and indirect facilitation mechanisms make *G. struthium* a key nurse species in gypsum plant communities of the Middle Ebro Valley. In order to unravel the mechanisms that drive species accumulation under *O. tridentata* (Chapter 1), it would be necessary to experimentally test the effect that this shrub has on the germination and growth of other species as well as it was tested for *G. struthium* in Chapter 4.

Interference in gypsum plant communities

Although in the observational study almost all shrubs showed a nurse role when comparing the plants harbored underneath with those harbored in open areas, there were significant differences between shrubs in their nursing abilities. The gypsovag nanophanerophytes *C. clusii* and *Rosmarinus officinalis* showed less evident positive roles than the gypsophilous nanophanerophytes *G. struthium* and *O. tridentata*. These gypsovag shrubs have an architecture similar to the latter (*i.e.*, potential nurse architecture) and were found to improve micro-environmental conditions under their canopy in the same way as these gypsophytes. Nevertheless, *C. clusii* and *R. officinalis* harbored less richness and abundance of plants than the referred gypsophytes. These findings suggest that, despite having a positive interaction outcome compared to open areas, these shrubs might have adverse effects on neighboring species that preclude stronger facilitation, potentially caused by a higher competitive capacity or by chemical interference. Chemical inhibition of germination is widely reported in semi-arid communities (Arroyo et al., 2018; Escudero et al., 2000), where it results in species impoverishment in neighboring areas (Arroyo et al., 2015). Well is known that the gypsovag *R. officinalis* has phytotoxic activity (Vokou et al., 1993) but, as far as we know, potential chemical interference exerted by *C. clusii* on neighboring plant species had not been assessed yet.

We combined observational field data with a sowing experiment in a greenhouse to study the possible interference mechanism that this shrub could be exerting on neighboring species in the community (Chapter 3). Results under controlled conditions confirmed that aqueous extracts from *C. clusii* exerted an adverse effect on the germination and survival of neighboring plants, as well as other species of the same genus (*e.g*., *C. ladanifer*, Alías *et al*., 2006). The isolation of the possible phytotoxic effect of *C. clusii* root and leaf aqueous extracts in the greenhouse experiment and the supplementary chemical analyses that detected water-soluble compounds with potential phytotoxic activity (*e.g*., bornyl acetate; Verdeguer et al., 2012), confirmed the hypothesis that the interference exerted by this shrub was, at least partially, chemically mediated. This thesis has provided a novel report of chemical interference of *C. clusii* on the establishment of neighboring species in gypsum plant communities. Chemical interference could be a mechanism of gypsovags to take advantage over other species at early life stages (Escudero et al., 2000), and then avoid competition for scarce resources with neighboring plant species that may be better adapted to gypsum (Escudero et al., 2015). This mechanism may cause a species shift in the community and would allow gypsovags succeeding in gypsum plant communities, even becoming locally dominant, but this idea deserves further investigations in other gypsum areas.

Notably, *C. clusii* did not affect equally all neighbor plant species, but the chemical interference was species-specific. Species-specific effects are likely a result of differential sensitivity of neighbor species to the phytotoxic compounds released by this gypsovag (Linhart et al., 2015). It was proved for some species that were not adversely affected by *C. clusii* neither in the field nor in the greenhouse experiment (*e.g.*, *G. struthium*). Those species may have locally adapted to the potential phytotoxic compounds of *C. clusii* because they frequently co-occur with this species in gypsum plant communities of the Middle Ebro Valley. Tolerance to "chemical neighbors" is a well-studied co-evolutionary phenomenon that allows a species to coexist with phytotoxic plant species (Grøndahl and Ehlers, 2008; Jensen and Ehlers, 2010). Species-specific phytotoxicity has been previously described in other species (Linhart et al., 2015) and might have important ecological implications for the dynamics of plant communities by affecting the recruitment and abundance of some species in the community (Schenk, 2006). In this case, species tolerant to *C. clusii* benefit of the micro-environmental amelioration under the shrub canopy and also of the absence of potential competitors in this microsite. In summary, the presence of this species with phytotoxic activity acts as a selective agent in gypsum plant communities of the Middle Ebro Valley, benefiting the establishment of some species and adversely affecting the establishment of other species that could compete with it at the adult stage.

Nurse shrubs in vegetation recovery of gypsum plant communities

Despite the relevance of gypsum habitats as biodiversity hotspots and their priority for conservation planning, they are often degraded by anthropogenic activities such as intensive agriculture, overgrazing or mining, among others (Mota et al., 2011). Therefore, it is necessary to elaborate efficient restoration plans to return the ecosystem to its natural state. Recently, facilitation has been considered as a crucial mechanism for promoting plant succession and an efficient tool for ecosystem restoration, particularly in harsh environments (Gómez-Aparicio, 2009; Maestre et al., 2001). The use of nurse plants would reduce economic costs, accelerate plant succession, and restore a self-sustainable community (Byers et al., 2006; Padilla and Pugnaire, 2006). However, its efficiency in gypsum environments has been much less studied. In this thesis, we tested the efficiency of *G. struthium*, which acts as a key nurse species in well-preserved gypsum plant communities, for vegetation recovery in gypsum mines. The study combined observational and experimental data that showed successful results of using this gypsophyte as a nurse plant in post-mining gypsum substrates.

The observational study performed in a gypsum mine unveiled that several species, and especially the gypsophyte *G. struthium*, spontaneously regenerated either from the original seed bank contained in the topsoil or from propagules arrived by seed dispersal from the surrounding undisturbed communities (Kirmer et al., 2008; Martínez-Duro et al., 2010). In line with previous findings in other gypsum mines, *G. struthium* appeared as a pioneer species (Dana and Mota, 2006) because, as well as other gypsophytes, it can overcome gypsum harsh environmental conditions with no need for facilitation (Escudero et al., 2015; Martínez-Duro et al., 2010). Although the nurse effect of *G. struthium* was not evident in the first stages of plant succession, likely due to the abundance of ruderal annuals that do not need to be facilitated to establish (Grime, 2006), in later stages it had a proven nurse role that would end in a successful natural vegetation recovery. Observational data from the undisturbed communities surveyed in both Chapter 4 and Chapter 1 showed that *G. struthium* facilitates the creation of species-rich areas in its vicinity. The role of *G. struthium* as a nurse plant in the establishment of species-rich microcosms, and consequently in the restoration of the diversity in gypsum areas has been highlighted by other researchers (Navarro-Cano et al., 2016). Complementary, the experimental study in the gypsum spoil dump (Chapter 4) showed beneficial effects of *G. struthium* on the development of the gypsophyte dwarf shrub *Helianthemum squamatum* and the gypsovag perennial grass *Stipa lagascae*. These are particularly interesting plant species for the restoration of gypsum areas of the Middle Ebro Valley because of their presence in the mature gypsum plant communities (Braun-Blanquet and Bolòs, 1958). Therefore, applying *G. struthium* as a nurse plant in restoration actions would facilitate the colonization of desirable plants to recover the former gypsum community. Other studies found that using shrubs as nurse plants after a disturbance would facilitate the establishment of desirable plants that shaped the pre-disturbance communities (*e.g.*, Gómez et al., 2001). Together with the positive effect of *G. struthium* on plant establishment, its occurrence as a pioneer species in the community and the ecological relevance that gypsophytes have in gypsum plant communities would make this nurse the most suitable for the restoration of gypsum spoils. These conclusions combining observational and experimental evidence reinforce the knowledge about the potential role that *G. struthium* has in increasing restoration success of gypsum mines.

Instead of relying entirely on active restoration actions, favoring the establishment of *G. struthium* after gypsum mining can imply important improvements to the effectiveness of revegetation by accelerating the natural succession towards the desired community (Prach, 2003; Prach and Hobbs, 2008). In addition to the ecological reasoning, favoring spontaneous revegetation can be a medium-cost alternative to other restoration techniques (Miller and Hobbs, 2007). Specifically, we recommend favoring the establishment of *G. struthium* in post-mining gypsum substrates by improving the soil properties (*e.g.*, by adding the original topsoil) and by planting or sowing the nurse species *G. struthium*. Then, to rely on the capacity of *G. struthium* to recruit other plants or to use the well-established individuals of *G. struthium* as nurse plants by planting those species of interest under its canopy. The combined use of these actions will diminish economic and time costs of restoration activities.

Limitations of this study and future research directions

This study corroborated the importance of positive interactions for plant establishment in gypsum environments, allowing the identification of key nurse species in gypsum plant communities of the Middle Ebro Valley for conservation and restoration purposes. The study was carried out in gypsum plant communities representative of the Iberian Peninsula (habitat *Gypsophiletalia*), which enables a particular generalization of our findings. Nevertheless, there could be local particularities in such plant communities because environmental conditions or species composition may vary from site to site (Mota et al., 2011). Therefore, in order to generalize our conclusions to other gypsum environments, further research should test the observed patterns of plant establishment and the role of key plant species in different gypsum plant communities, under contrasting environmental conditions and with diverse species involved. Surveying gypsum plant communities along an aridity gradient would clarify the results of the shift in the interaction outcome at the community level depending on stress degree, and would allow testing more properly the Stress Gradient Hypothesis proposed by Bertness and Callaway (1994). Moreover, to generalize our findings of the positive role that gypsophytes have in structuring plant communities compared to gypsovags, it is recommended to survey gypsum plant communities other than those included in the order *Gypsophiletalia*, with other taxa involved (*e.g.*, the Chihuahuan Desert; Meyer et al., 1989).

We have investigated biotic interactions from different perspectives in the community, taking all the plant species into account, going from seeds to standing plants, and considering different methodologies that include observational and experimental proxies that allowed unraveling the mechanisms involved in the observed patterns. However, biotic interactions at the community level were assessed considering plant spatial patterns from an observational point of view. Given that inferring biotic interactions based on observational data may be controversial because, together with biotic interactions, other biotic and abiotic factors influence the spatial pattern of the community, experimental studies at the community are recommended. In this thesis, the effects of *G. struthium* and *C. clusii* on the germination, survival, and growth of other species have been evaluated experimentally. However, field experiments at the community level are needed to corroborate some of the predictions of the observational work. For example, testing the potential nurse effects of the other abundant shrubs would help drawing firm conclusions about the underlying mechanisms that generate the observed spatial patterns. Nevertheless, the experiments associated with *G. struthium* and *C. clussi* suggest that, at least for our study site, the spatial pattern could be a reasonable proxy of the outcome of the biotic interactions in the community.

On the other hand, the combination of observational and experimental data allowed the identification of species-specific chemical interference exerted by *C. clusii* in gypsum plant communities of the Middle Ebro Valley. Chemical interference has been understudied in comparison to other negative interactions in plant communities (*e.g.*, competition; Tilman, 1994), and particularly its role at the community level. Thus, the importance of the phytotoxic role of *C. clusii* in plant-plant interaction outcomes at the community level should be investigated in other Mediterranean plant communities. In addition, further research on the soil seed bank in microsites associated with this species is recommended because it would help to strengthen the conclusions of germination inhibition, but under natural conditions. Finally, it is necessary to check whether allelopathy is a mechanism of the dominance of gypsovags by testing for phytotoxicity in those others that are also dominant in gypsum plant communities.

To conclude, we successfully tested the suitability of using the pioneer species *G. struthium* as a nurse plant in the restoration of gypsum areas. However, the use of other nurse species as potential restoration tools in gypsum ecosystems remains unknown. In order to diversify the restored community and have alternative species to be used in restoration, further experiments similar to the one in Chapter 4 are recommended to prove the suitability of other pioneer nurses in the restoration of gypsum ecosystems. On the other hand, the structuring role of *G. struthium* was not observed in the youngest restored communities, due to the presence of ruderal species in the first stages of plant succession (Grime, 2006). Given the temporal variability in plant interactions, longer-term studies are needed to assess whether this nurse shrub has a net beneficial effect on plant succession. Observational and experimental studies included in this thesis suggest so.

Detalle de las inflorescencias de
Gypsophila struthium **subsp.** ***hispanica***
en un espécimen recolectado en las inmediaciones de
La Lomaza de Belchite

Cianotipia elaborada por Ángeles Muñoz
Espécimen recolectado y prensado por Ana Foronda

Conclusions / Conclusiones

I. Both gypsophytes and gypsovags, especially in early life stages, appear more than expected in microsites associated with perennials already established in the community, and less than expected in open areas (where conditions are more stressful). This fact shows that, regardless of their degree of linkage to gypsum, plants take advantage of the presence of nurse plants to overcome the stressful environmental conditions of gypsum outcrops to establish in the community.

II. In general, the large shrubs (*Gypsophila struthium*, *Ononis tridentata*, *Cistus clusii* and *Rosmarinus officinalis*) harbour more richness and abundance of plants under their canopies than in open areas. Micro-environmental conditions under the canopy of these shrubs are more benign for plants than those in open areas because nutrient and water content increase, temperatures are softened and soil compaction decrease. The combination of both findings shows that large shrubs act as ecosystem engineers by creating favourable conditions for the establishment of plants and thus act as nurses.

III. Among shrubs with a nurse effect, the gypsophytes *G. struthium* and *O. tridentata* shelter more richness and abundance of plants under their canopies than the gypsovags *C. clusii* and *R. officinalis*. Therefore, the studied shrubs have different facilitation capacity depending on their degree of linkage to gypsum (*i.e.*, gypsophytes facilitate more than gypsovags).

IV. The facilitation mediated by gypsophyte shrubs is reflected in the accumulation of species-rich patches the areas adjacent to these shrubs, thus favouring a patchy structure of the plant community.

V. The weaker positive effect of *C. clusi* and *R. officinalis* on plant richness and abundance suggest that, despite the dominant role of facilitation in the net interaction outcome, these gypsovags exert some interference on their neighbor plants, likely to eliminate competitors. In addition to the known chemical interference of *R. officinalis* in other plant communities, *C. clusii* has been shown to interfere with some of the neighboring species through the release of phytotoxic compounds. This is reflected in a species impoverishment under its canopy. This is the first evidence of species-specific chemical interference in C. clusii.

VI. Regardless of their degree of linkage to gypsum, shrubs create seed-rich patches in the soil under canopy by trapping and accumulating seeds in gypsum plant communities. Tall cushion-like shrubs are more capable of trapping and accumulating seeds, acting as seed sinks and thus forming rich and abundant seed banks in their local vicinity.

VII. Shrubs with a nurse effect have the ability to act as seed sources through the plants harboured under their canopy. Provided seeds accumulate in neighbouring areas due to the short-distance seed dispersal of the species that form gypsum plant communities.

VIII. The gypsophyte *G. struthium* forms the richest and most abundant seed bank in gypsum plant communities due to its double role as a seed sink (driven by its tall size and cushion-like shape) and as a seed source (driven by its nurse role). The formation of a rich and abundant seed bank under its canopy influences the formation of species-rich vegetation patches.

IX. Gypsophytes appear as pioneers in gypsum mines, with *G. struthium* being the dominant shrub in early stages of plant succession. This shrub acts as an ecosystem engineer, improving the micro-environmental conditions under its canopy, and influencing the germination and growth of other plant species of interest for the restoration of gypsum plant communities (*Helianthemum squamatum* and *Stipa lagascae*).

X. It is recommended to promote the establishment of G. struthium for using it as a nurse plant in the restoration of degraded gypsum plant communities due to its ability of regenerating as a pioneer species and its ability to facilitate plant establishment and form rich and abundant seed banks. This species would promote natural plant succession and the persistence of the plant community, therefore resulting in reduced economic and time costs of restoration plans.

I. Tanto los gipsófitos como los gipsovagos aparecen más de lo esperado por azar en micrositios asociados a plantas perennes ya establecidas en la comunidad, y menos de lo esperado al azar en áreas abiertas (donde las condiciones son más estresantes), especialmente en las primeras fases de su vida. Este hecho pone de manifiesto que, independientemente de su grado de vinculación al yeso, las plantas se aprovechan de la presencia de plantas nodrizas para superar las condiciones ambientales estresantes de los afloramientos de yeso y establecerse en la comunidad vegetal.

II. En general, los arbustos grandes (*Gypsophila struthium*, *Ononis tridentata*, *Cistus clusii* y *Rosmarinus officinalis*) albergan bajo su dosel más riqueza y abundancia de especies que las áreas abiertas. Las condiciones microambientales bajo el dosel de estos arbustos son más benignas que las de las áreas abiertas, aumentando el contenido en nutrientes y humedad, amortiguando las temperaturas extremas y disminuyendo la compactación del suelo. La combinación de ambos hallazgos pone de manifiesto que los arbustos grandes actúan como ingenieros del ecosistema, creando condiciones favorables al establecimiento de plantas, actuando por tanto como nodrizas.

III. De entre los arbustos con efecto nodriza, los gipsófitos *G. struthium* y *O. tridentata* albergan bajo su dosel más riqueza y abundancia de plantas que los gipsovagos *C. clusii* y *R. officinalis*. Por tanto, los arbustos estudiados tienen diferente capacidad de facilitación en función de su grado de vinculación al yeso (los gipsófitos facilitan más que los gipsovagos).

IV. La facilitación mediada por los arbustos gipsófitos se ve reflejada en la acumulación de manchas de vegetación ricos en especies en las áreas vecinas a estos arbustos, estructurando la vegetación en las comunidades vegetales gipsícolas.

V. El efecto positivo más débil de *C. clusi* y *R. officinalis* sobre la riqueza y abundancia de plantas pone de manifiesto que, a pesar del papel dominante de la facilitación en el balance neto de las interacciones, estos gipsovagos ejercen cierta interferencia sobre sus plantas vecinas, probablemente para eliminar competidores. Además de la conocida interferencia química de *R. officinalis* en otras comunidades vegetales, se ha demostrado que *C. clusii* ejerce interferencia sobre algunas de las especies vecinas, al menos en parte, por medio de la liberación de productos químicos. Esto se ve reflejado en un empobrecimiento de especies bajo su dosel. Esta es la primera evidencia de interferencia química especie-específica en *C. clusii*.

VI. Independientemente de su grado de vinculación al yeso, los arbustos atrapan y acumulan semillas, estructurando el banco de semillas de las comunidades vegetales gipsícolas en manchas alrededor de los arbustos. Los arbustos altos con forma de cojín tienen más capacidad de atrapar y acumular semillas bajo su dosel, actuando como sumideros de semillas y formando así, bancos de semillas ricos y abundantes en su vecindad local.

VII. Los arbustos con un efecto nodriza tienen la capacidad de actuar como fuentes de semillas por medio de las plantas que albergan bajo su dosel. Debido a la dispersión a corta distancia de las plantas que forman las comunidades vegetales gipsícolas, las semillas se acumulan en áreas vecinas.

VIII. El gipsófito *G. struthium* forma el banco de semillas más rico y abundante en las comunidades vegetales gipsícolas debido a su doble papel como sumidero de semillas (por su tamaño y forma) y como fuente de semillas (por su efecto nodriza). La formación de un banco de semillas rico y abundante bajo su dosel influencia la formación de parches de vegetación ricos en especies.

IX. Los gipsófitos aparecen como pioneros en canteras de yeso, siendo *G. struthium* el arbusto que domina en las primeras etapas de la sucesión vegetal. Este arbusto actúa como ingeniero del ecosistema, mejorando las condiciones microambientales bajo su dosel, influyendo en la germinación y crecimiento de otras especies vegetales de interés para la restauración de las comunidades vegetales gipsícolas (*Helianthemum squamatum* y *Stipa lagascae*).

X. Debido a su capacidad de regeneración como especie pionera y a sus habilidades de facilitar el establecimiento de plantas y formar bancos de semillas ricos y abundantes, se recomienda promover el establecimiento de *G. struthium* para su uso como especie nodriza en la restauración de comunidades gipsícolas degradadas. Su uso resultaría en una disminución de los costes económicos y de tiempo en los planes de restauración de canteras, ya que esta planta promovería la sucesión vegetal natural y la persistencia de la comunidad vegetal.

Reserva de Fauna Silvestre
“La Lomaza de Belchite”
(Belchite, Zaragoza, España)

Cianotipia elaborada por Ángeles Muñoz
Fotografía original: Ana Foronda

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Plantación de *Stipa lagascae* para testar el efecto de las nodrizas sobre su crecimiento

Cianotipia elaborada por Ángeles Muñoz
Fotografía original: Ana Foronda

Glossary of abbreviations

ANOVA	Analysis of Variance
Biocrusts	Biological soil crusts
CMIP5	Fifth Coupled Model Intercomparison Project of the World Climate Research Programme
GC-MS	Gass Chromatography-Mass Spectrometer
GLM	Generalized Linear Model
GLMM	Generalized Linear Mixed Model
ISAR	Individual Species-Area Relationships
LMM	Linear Mixed Model
Microsite ES	On the edge of the canopy of adult shrubs
Microsite OA	Open areas
Microsite UC	Under the canopy of adult shrubs
PDFs	Probability density functions
Plot C	Control plot in the undisturbed communities in Gelsa
Plot R1	Restoration 1 plot in the mine in Gelsa (2010)
Plot R2	Restoration 2 plot in the mine in Gelsa (2008)
SGH	Stress Gradient Hypothesis
SSI	Sorensen's index of similarity
Treatment C	Control treatment (water)
Treatment L	Leaf extract (Cistus clusii)
Treatment R	Root extract (Cistus clusii)
Treatment RL	Root and Leaf aqueous extract (Cistus clusii)
VWC	Volumetric Water Content in the soil

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AF, YP, RR and CLA conceived the study and designed the field sampling; AF, MLG and RR collected soil samples; AF, MLG and AIA washed samples, set them in the greenhouse and monitored seed germination; AF and MLG carried out the vegetation surveys; AF and YP designed the manuscript; AF analyzed the data and wrote the draft; YP, AIA, RR and CLA commented on the manuscript. All authors read and approved the manuscript.

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Vista general de las dunas de yeso de White Sands National Monument (New Mexico, Estados Unidos)

Cianotipia elaborada por Ángeles Muñoz
Fotografía original: Ana Foronda

Supplementary material

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Appendices

General Methodology

Appendix 1 Characteristics of gypsum plant communities in the study area

Table A1.1 Species list recorded during numerous field surveys carried out in different sites in the study area (2003-2015 period). Asterisks (*) indicate that the species is occurring in the site.

Species	Life form	Family	Leciñena	Lomaza	Gelsa	Osera	Pina	Farlete
Aegilops geniculata Roth.	therophyte (annual grass)	*Poaceae*			*	*		*
Agropyron cristatum(L.) Gaertn.	perennial grass	*Poaceae*	*		*			
Aizoon hispanicum L.	therophyte (annual herb)	*Aizoaceae*		*				
Alyssum minus (L.) Rothm.	therophyte (annual herb)	*Brassicaceae*	*	*				
Artemisia herba-alba Asso.	chamaephyte (subshrub)	*Asteraceae*		*		*		*
Asphodelus fistulosus L.	hemichryptophyte (perennial herb)	*Liliaceae*	*	*	*			
Asterolinon linum-stellatum (L.) Duby	therophyte (annual herb)	*Primulaceae*	*	*	*	*	*	*
Astragalus alopecuroides L.	hemichryptophyte (perennial herb)	*Fabaceae*		*		*		*
Astragalus incanus L.	chamaephyte (subshrub)	*Fabaceae*		*				
Astragalus stella Gouan	therophyte (annual herb)	*Fabaceae*		*				
Avenula bromoides (Gouan) H. Scholz	perennial grass	*Poaceae*	*					
Avenula gervaisii Holub	perennial grass	*Poaceae*		*				*
Avenula pratensis (L.) Dumort.	perennial grass	*Poaceae*		*				
Beta vulgaris L.	hemichryptophyte (perennial herb)	*Chenopodiaceae*	*	*		*	*	*
Brachypodium distachion (L.) P. Beauv	therophyte (annual grass)	*Poaceae*	*	*				*
Brachypodium retusum (Pers.) P. Beauv.	perennial grass	*Poaceae*	*	*				*
Bromus rubens L.	therophyte (annual grass)	*Poaceae*	*	*	*	*		*
Bupleurum semicompositum L.	therophyte (annual herb)	*Umbelliferae*		*		*		*
Carduus tenuiflorus Curtis	therophyte (annual herb)	*Asteraceae*		*				
Carex halleriana Asso.	perennial grass	*Poaceae*	*	*				*
Carlina corymbosa L.	hemichryptophyte (perennial herb)	*Asteraceae*		*		*		
Centaurea melitensis L.	therophyte (annual herb)	*Asteraceae*		*	*			
Cerastium pumilum Curtis	therophyte (annual herb)	*Caryophyllaceae*	*	*	*			
Chaenorrhinum rubrifolium (Robill. & Castagne ex DC.) Fourr.	therophyte (annual herb)	*Scrophulariaceae*	*	*	*	*	*	
Cistus clusii Dunal	nanophanerophyte (shrub)	*Cistaceae*	*		*		*	
Clypeola jonthlaspi L.	therophyte (annual herb)	*Brassicaceae*		*				
Convolvulus lineatus L.	hemichryptophyte (perennial herb)	*Convolvulaceae*	*					
Coris monspeliensis L.	chamaephyte (subshrub)	*Primulaceae*	*	*	*	*	*	*
Coronilla minima L.	chamaephyte (subshrub)	*Fabaceae*	*					

Appendix 1 Characteristics of gypsum plant communities in the study area

Species	**Life form**	**Family**	**Leciñena**	**Lomaza**	**Gelsa**	**Osera**	**Pina**	**Farlete**
Cuscuta sp.	therophyte (annual herb)	*Cuscutaceae*	*					
Dactylis glomerata L.	perennial grass	*Poaceae*	*	*				
Desmazeria rigida (L.) Tutin	therophyte (annual grass)	*Poaceae*	*	*	*	*	*	*
Dipcadi serotinum (L.) Medik.	geophyte (annual herb)	*Aspagaraceae*	*	*		*		
Diplotaxis harra (Forssk.) Boiss.	chamaephyte (subshrub)	*Cruciferae*	*	*				*
Dorycnium pentaphyllum Scop.	chamaephyte (subshrub)	*Fabaceae*	*					
Echinops ritro L.	hemichryptophyte (perennial herb)	*Asteraceae*					*	
Echium vulgare L.	chamaephyte (subshrub)	*Boraginaceae*			*			
Elymus pungens (Pers.) Melderis	perennial grass	*Poaceae*	*			*		
Ephedra fragilis Desf	nanophanerophyte (shrub)	*Ephedraceae*		*	*		*	
Erodium cicutarium (L.) L'Hér.	therophyte (annual herb)	*Geraniaceae*		*	*	*		
Eruca vesicaria (L.) Cav.	therophyte (annual herb)	*Brassicaceae*		*	*	*		*
Eryngium campestre L.	hemichryptophyte (perennial herb)	*Asteraceae*		*				*
Euphorbia sp.	therophyte (annual herb)	*Euphorbiaceae*		*				
Filago pyramidata L.	therophyte (annual herb)	*Asteraceae*	*	*	*	*	*	*
Fumana ericoides (Cav.) Gand.	chamaephyte (subshrub)	*Cistaceae*	*		*		*	*
Galium verrucosum Huds.	therophyte (annual herb)	*Rubiaceae*	*	*	*	*	*	*
Genista scorpius (L.) DC.	nanophanerophyte (shrub)	*Fabaceae*	*	*	*	*	*	*
Gypsophila struthium Loefl. subsp. *hispanica* (Willk.) G.López	chamaephyte (subshrub)	*Caryophyllaceae*	*	*	*	*	*	*
Hedypnois cretica (L.) Dum. Cours.	therophyte (annual herb)	*Asteraceae*		*		*		*
Hedysarum humile L.	chamaephyte (subshrub)	*Fabaceae*		*		*	*	
Helianthemum marifolium (L.) Mill.	chamaephyte (subshrub)	*Cistaceae*	*	*	*	*	*	*
Helianthemum pilosum (L.) Pers.	chamaephyte (subshrub)	*Cistaceae*	*	*		*	*	*
Helianthemum salicifolium (L.) Mill.	therophyte (annual herb)	*Cistaceae*	*	*		*	*	*
Helianthemum squamatum (L.) Pers.	chamaephyte (subshrub)	*Cistaceae*	*	*	*	*	*	
Helianthemum syriacum (Jacq.) Dum. Cours.	chamaephyte (subshrub)	*Cistaceae*	*	*	*	*	*	*
Helianthemum violaceum (Cav.) Pers.	chamaephyte (subshrub)	*Cistaceae*			*			
Helichrysum stoechas (L.) Moench	chamaephyte (subshrub)	*Asteraceae*	*	*	*	*	*	*
Herniaria fruticosa L.	chamaephyte (subshrub)	*Caryophyllaceae*	*	*	*	*	*	*
Hippocrepis ciliata Willd.	therophyte (annual herb)	*Fabaceae*		*	*	*		
Hordeum murinum L.	therophyte (annual grass)	*Poaceae*		*	*			
Juniperus phoenicea L.	phanerophyte (tree)	*Cupressaceae*	*				*	

Appendix 1 Characteristics of gypsum plant communities in the study area

Species	Life form	Family	Leciñena	Lomaza	Gelsa	Osera	Pina	Farlete
Juniperus sabina L.	nanophanerophyte (shrub)	*Cupressaceae*				*		
Juniperus thurifera L.	phanerophyte (tree)	*Cupressaceae*	*				*	
Koeleria vallesiana (Honck.) Gaudin	perennial grass	*Poaceae*	*	*	*	*	*	*
Launaea lanifera Pau	chamaephyte (subshrub)	*Asteraceae*	*	*	*			
Launaea pumila (Cav.) Kuntze	chamaephyte (subshrub)	*Asteraceae*			*			
Leontopodium sp.	therophyte (annual herb)	*Asteraceae*		*				
Lepidium subulatum L.	chamaephyte (subshrub)	*Brassicaceae*	*		*			*
Linaria arvensis (L.) Desf.	therophyte (annual herb)	*Scrophulariaceae*	*	*				
Linum strictum L.	therophyte (annual herb)	*Linaceae*		*	*	*	*	*
Linum suffruticosum L.	chamaephyte (subshrub)	*Linaceae*	*	*	*	*	*	*
Lithodora fruticosa (L.) Griseb.	chamaephyte (subshrub)	*Boraginaceae*	*		*	*		
Lithospermum arvense L.	therophyte (annual herb)	*Boraginaceae*		*				
Lolium perenne L.	perennial grass	*Poaceae*	*					*
Lygeum spartum L.	perennial grass	*Poaceae*		*	*	*	*	*
Matthiola fruticulosa (L.) Maire	chamaephyte (subshrub)	*Brassicaceae*			*	*		
Medicago sativa L.	hemichryptophyte (perennial herb)	*Fabaceae*	*	*				
Narduroides salzmanii (Boiss.) Rouy	therophyte (annual grass)	*Poaceae*	*	*		*		
Neatostema apulum (L.) I.M. Johnst.	therophyte (annual herb)	*Boraginaceae*	*	*		*		*
Odontites sp.	therophyte (annual herb)	*Orobanchaceae*				*		
Ononis reclinata L.	therophyte (annual herb)	*Fabaceae*		*				
Ononis tridentata L. subsp. *tridentata*	nanophanerophyte (shrub)	*Fabaceae*	*	*		*	*	*
Onopordum sp.	hemichryptophyte (perennial herb)	*Asteraceae*	*					
Orobanche arenaria Borkh.	geophyte (annual herb)	*Orobanchaceae*		*				
Peganum harmala L.	chamaephyte (subshrub)	*Zygophyllaceae*	*	*	*	*		
Pinus halepensis Mill.	phanerophyte (tree)	*Pinaceae*	*				*	
Plantago afra L.	therophyte (annual herb)	*Plantaginaceae*		*				
Plantago albicans L.	chamaephyte (subshrub)	*Plantaginaceae*		*		*		*
Polygala monspeliaca L.	therophyte (annual herb)	*Polygalaceae*	*	*				
Polygala rupestris Pourr.	chamaephyte (subshrub)	*Polygalaceae*	*	*	*	*	*	*
Quercus coccifera L.	nanophanerophyte (shrub)	*Fagaceae*	*				*	
Quercus ilex L. subsp. *rotundifolia* (Lam.) Tab. Morais	phanerophyte (tree)	*Fagaceae*	*					
Reseda stricta Pers.	hemichryptophyte (perennial herb)	*Resedaceae*	*	*	*	*		

Appendix 1 Characteristics of gypsum plant communities in the study area

Species	Life form	Family	Leciñena	Lomaza	Gelsa	Osera	Pina	Farlete
Retama sphaerocarpa (L.) Boiss.	nanophanerophyte (shrub)	*Fabaceae*	*					
Rhamnus lycioides L.	nanophanerophyte (shrub)	*Rhamnaceae*	*					
Rosmarinus officinalis L.	nanophanerophyte (shrub)	*Lamiaceae*	*	*	*	*	*	*
Ruta angustifolia Pers.	chamaephyte (subshrub)	*Rutaceae*	*					
Salsola genistoides Juss. ex Poir.	nanophanerophyte (shrub)	*Chenopodiaceae*	*			*	*	
Salsola vermiculata L.	nanophanerophyte (shrub)	*Chenopodiaceae*		*		*		
Salvia lavandulifolia Vahl	chamaephyte (subshrub)	*Lamiaceae*	*					
Scorzonera laciniata L.	hemichryptophyte (perennial herb)	*Asteraceae*	*	*	*	*	*	*
Sedum sediforme (Jacq. Pau, non Raym.-Hamet	chamaephyte (subshrub)	*Crassulaceae*	*	*		*		
Senecio auricula Bourg. ex Coss.	hemichryptophyte (perennial herb)	*Asteraceae*			*			
Senecio gallicus Chaix	therophyte (annual herb)	*Asteraceae*		*				*
Sideritis hirsuta L.	chamaephyte (subshrub)	*Lamiaceae*	*	*				
Sonchus tenerrimus L.	hemichryptophyte (perennial herb)	*Asteraceae*	*	*	*			*
Spergularia diandra (Guss.) Boiss.	therophyte (annual herb)	*Caryophyllaceae*	*	*				*
Staehelina dubia L.	chamaephyte (subshrub)	*Asteraceae*	*					
Stipa lagascae Roem. & Schult.	perennial grass	*Poaceae*	*	*		*	*	*
Stipa parviflora Desf.	perennial grass	*Poaceae*	*	*	*	*		*
Suaeda vera J.F.Gmel.	chamaephyte (subshrub)	*Chenopodiaceae*						
Teucrium capitatum L.	chamaephyte (subshrub)	*Lamiaceae*	*	*	*	*	*	*
Teucrium gnaphalodes L'Hér.	chamaephyte (subshrub)	*Lamiaceae*			*			
Thapsia villosa L.	hemichryptophyte (perennial herb)	*Umbelliferae*	*		*			
Thymelaea tinctoria (Pourr.) Endl.	nanophanerophyte (shrub)	*Thymelaeaceae*	*					
Thymus vulgaris L.	chamaephyte (subshrub)	*Lamiaceae*	*	*	*	*		
Thymus zygis L.	chamaephyte (subshrub)	*Lamiaceae*	*	*		*	*	*
Trigonella monspeliaca L.	therophyte (annual herb)	*Asteraceae*			*			
Trisetum loeflingianum (L.) C. Presl	therophyte (annual grass)	*Poaceae*		*				

Appendix 1 Characteristics of gypsum plant communities in the study area

Figure A1.1 Overview of gypsum plant communities of the Middle Ebro Valley (Zaragoza). Photos: Ana Foronda

Table A1.2 Site characteristics: elevation, climatic variables and soil chemical properties per study site.

	Site 1	**Site 2**
Geographic coordinates	41º46’29” N 0º35’23” W	41º23’33” N 0º42’18” W
Elevation (m.a.s.l.)	511	394
Climatic variables:		
T (ºC)	13.5	14.7
R (mm·yr-1)	382	302
IdM	16.25	12.23
Soil chemical properties:		
pH	7.92	8.08
EC (mS/cm)	2.19	2.08
P (mg/Kg)	49.35	117.28
TOC (%)	0.89	0.65
TC (%)	2.16	1.81
TN (%)	0.08	0.07

Climatic data (T: average annual temperature and R: average annual rainfall) from “HU05 Lanaja” and “Z02 Belchite” meteorological stations; 2004-2017 period (SIAR-Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente, http://www.siar.es). IdM: de Martonne’s Aridity Index was calculated as IdM=R/(T+10) (the higher value, the lower aridity). Soil chemical properties (pH, EC: electrical conductivity, P: available phosphorous, TOC: total organic carbon, TC: total carbon and N: total nitrogen) were analyzed from five random soil samples at each study site taken from open areas.

Appendices

Chapter 1

Appendix 2 Hypotheses and sampling methods (Chapter 1)

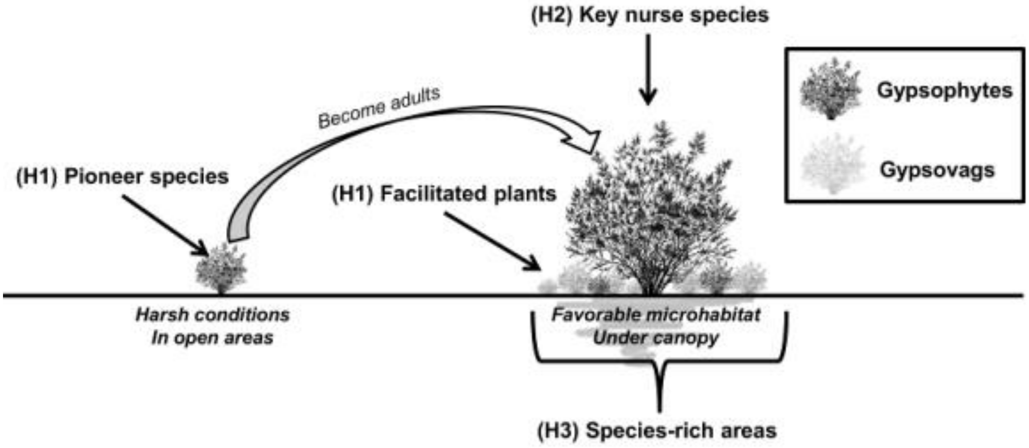


Figure A2.1 Sketch of the hypotheses formulated in the study about the patterns of plant establishment in gypsum plant communities and the role that gypsophytes may have in facilitating plant establishment and structuring plant diversity at community level.

Appendix 2 Hypotheses and sampling methods (Chapter 1)

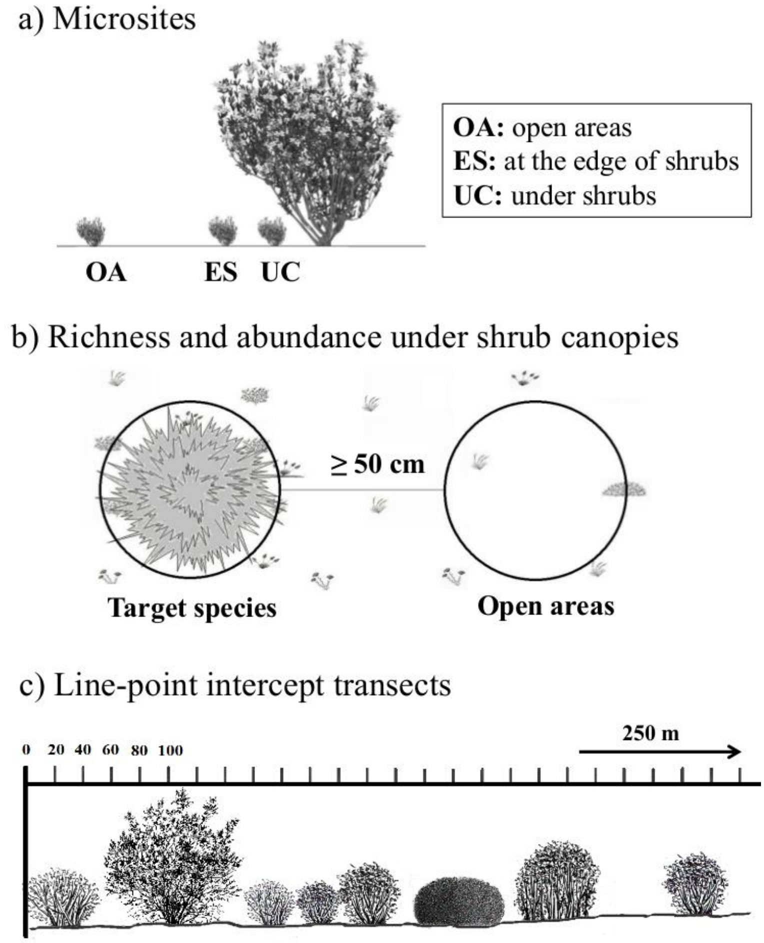
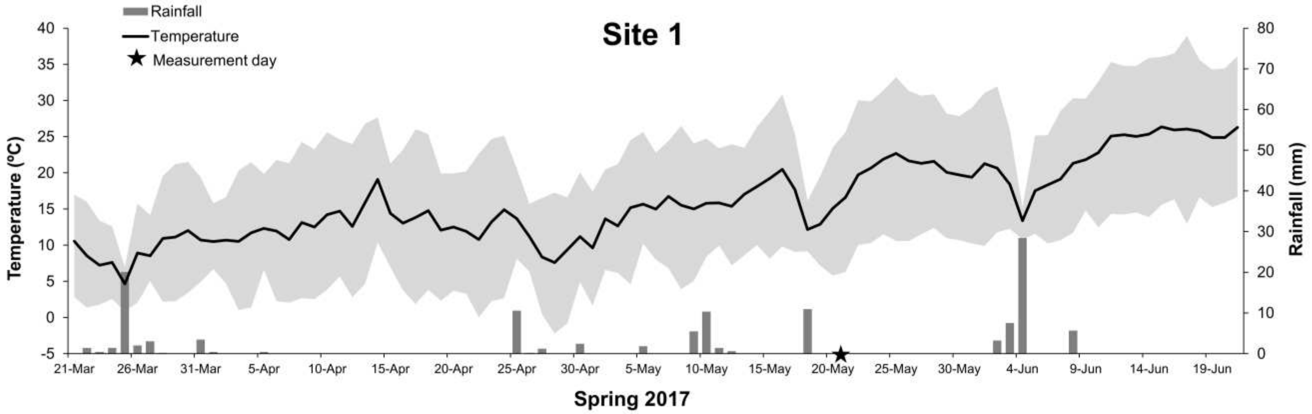


Figure A2.2 Sketch of the sampling methods used in vegetation surveys: **a)** microsites where seedlings and adults were recorded; **b)** arrangement of the rings used to survey richness and abundance under the canopy target species and in paired-rings in open areas at least 50 cm away from the individual of the target species; **c)** line-point-intercept (LPI) transects in which all species contacting with the transect line in points at 20 cm intervals were recorded along 250-m.



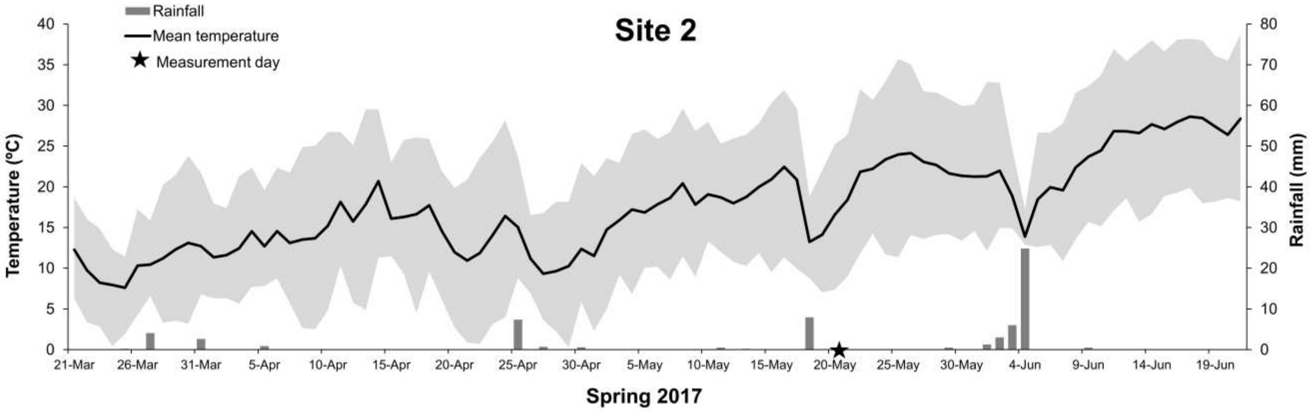


Figure A2.3 Average daily rainfall and average daily temperature (grey shaded envelope represents maximum daily temperature and minimum daily temperature) in spring 2017 (21 March-21 June). Star indicates the measurement day of soil physical properties (21 May 2017).

Appendix 2 Hypotheses and sampling methods (Chapter 1)

Appendix 3 Target species selection (Chapter 1)

Table A3.1 Relative abundance (%) of gypsophyte and gypsovag shrubs recorded at each study site in gypsum plant communities of the Middle Ebro Valley.

			Relative abundance (%)	
Species	**Lifeform**	**Plant height (cm)**	**Site 1**	**Site 2**
Gypsophytes:				
Gypsophila struthium *	Nanophanerophyte	25-75	7.84	6.41
Helianthemum squamatum *	Chamaephyte	10-40	1.88	16.91
Herniaria fruticosa	Chamaephyte	< 30	0.63	11.95
Ononis tridentata *	Nanophanerophyte	< 150	9.72	10.20
Gypsovags:				
Cistus clusii *	Nanophanerophyte		11.60	0.00
Fumana ericoides	Chamaephyte		3.13	0.00
Genista scorpius	Nanophanerophyte	30-200	3.13	3.50
Helianthemum marifolium	Chamaephyte		7.84	0.00
Helianthemum pilosum	Chamaephyte	10-35	0.00	2.04
Helianthemum syriacum	Chamaephyte	5-50	11.29	9.04
Helichrysum stoechas	Chamaephyte	< 70	1.88	7.87
Launea lanifera	Chamaephyte	< 50	0.00	0.58
Linum suffruticosum	Chamaephyte	< 180	3.45	2.04
Lithodora fruticosa	Chamaephyte		0.31	0.00
Plantago albicans	Chamaephyte	6-28	0.00	9.33
Rosmarinus officinalis *	Nanophanerophyte		19.75	3.50
Sedum sediforme	Chamaephyte	< 60	0.00	0.29
Sideritis hirsuta	Chamaephyte	10-69	0.00	3.79
Teucrium capitatum	Chamaephyte	20-35	2.51	2.92
Thymelaea tinctoria	Chamaephyte		1.57	0.00
Thymus vulgaris *	Chamaephyte	10-40	13.48	9.62

* indicate the selected target species. The three most abundant gypsophyte shrubs and the three most abundant gypsovag shrubs (among large shrubs: nanophanerophytes and chamaephytes at least 20 cm tall). Plant height was obtained mainly from “Flora Ibérica” (http://www.anthos.es/), “Herbario de Jaca” (http://floragon.ipe.csic.es/), and “Flora Vascular” (https://www.floravascular.com/). Relative abundance (%) was recorded in 16 plots (5 x 1 m) per study site.

Table A3.2 Average height of the 25 individuals sampled per target species at each study site and length of the shade cast by shrubs.

	Site 1		Site 2	
Species	**H**	**L**	**H**	**L**
Gypsophila struthium	45.6	30.2	37.6	24.9
Ononis tridentata	73.7	48.9	60.0	39.8
Helianthemum squamatum	12.1	8.0	14.4	9.5
Cistus clusii	41.8	27.7	-	-
Rosmarinus officinalis	69.5	46.1	44.8	29.7
Thymus vulgaris	19.9	13.2	23.6	15.6

H = height; L = length of the shade (L = H / tan (α); where α = 56.46º = solar elevation at 3 p.m. on May)

Appendix 4 Microsites where perennial seedlings establish (Chapter 1)

Table A4.1 Summary of the G-test implemented to evaluate the differences between the observed frequencies f and the expected frequencies $f_{(e)}$ of all perennial species (seedlings and adults separately) recorded in **Site 1** at each microsite.

	Seedlings						**Adults**					
Species	**OA [f ($f_{(e)}$)]**	**ES [f ($f_{(e)}$)]**	**UC [f ($f_{(e)}$)]**	**DF**	**G**	**p - value**	**OA [f ($f_{(e)}$)]**	**ES [f ($f_{(e)}$)]**	**UC [f ($f_{(e)}$)]**	**DF**	**G**	**p-value**
Gypsophytes	86(119)*	41(22)*	69(55)*	2	27.631	**<0.001**	34(63)*	56(11)*	13(29)*	2	116.491	**<0.001**
Gypsophila struthium	32(49)*	14(10)	35(22)*	2	15.221	**<0.001**	9(19)*	20(4)*	3(9)*	2	48.990	**<0.001**
Helianthemum squamatum	39(44)	15(9)	19(20)	2	5.627	0.060	15(19)	9(4)*	8(9)	2	7.183	**<0.05**
Herniaria fruticosa	5(4)	1(1)	0(2)	2	3.951	0.139	6(5)	2(1)	1(2)	2	2.030	0.362
Lepidium subulatum	1(1)	1(0)	0(2)	2	2.631	0.268	1(2)	3(0)*	0(1)	2	9.734	**<0.01**
Ononis tridentata	9(21)*	10(4)*	15(9)	2	18.283	**<0.001**	3(16)*	22(3)*	1(7)	2	75.808	**<0.001**
Gypsovags	559(750)*	291(135)*	379(344)*	2	191.223	**<0.001**	565(1046)*	660(189)*	490(480)	2	976.764	**<0.001**
Agropyron cristatum	-	-	-	-	-	-	0(2)	1(0)	2(1)	2	5.687	0.058
Brachypodium retusum	11(13)	1(2)	9(6)	2	2.636	0.268	11(11)	3(3)	9(6)	2	1.694	0.429
Carex halleriana	-	-	-	-	-	-	0(1)	1(0)	0(0)	2	4.415	0.110
Cistus clusii	92(146)*	73(28)*	76(66)	2	79.719	**<0.001**	62(92)*	73(18)*	16(42)*	2	135.966	**<0.001**
Coris monspeliensis	2(5)	4(1)*	3(2)	2	8.178	**<0.05**	9(19)*	6(4)	16(9)*	2	12.986	**<0.01**
Dactylis glomerata	-	-	-	-	-	-	0(1)	1(0)	0(0)	2	4.415	0.110
Dorycnium pentaphyllum	0(1)	1(0)	1(1)	2	4.188	0.123	0(3)*	3(1)*	2(1)	2	11.605	**<0.01**
Fumana ericoides	13(13)	0(3)	9(6)	2	5.998	**<0.05**	11(35)*	27(7)*	19(16)	2	60.150	**<0.001**
Genista scorpius	2(4)	2(1)	2(2)	2	2.715	0.257	3(6)	7(1)*	0(3)	2	21.650	**<0.001**
Helianthemum syriacum	95(109)*	47(21)*	37(49)*	2	32.874	**<0.001**	90(107)*	68(21)*	19(49)*	2	100.886	**<0.001**
Helianthemum marifolium	41(62)*	20(12)*	41(28)*	2	18.567	**<0.001**	90(178)*	103(35)*	101(81)*	2	155.840	**<0.001**
Helichrysum stoechas	49(73)*	37(14)*	35(33)	2	37.786	**<0.001**	18(38)*	23(7)*	21(17)	2	37.185	**<0.001**
Juniperus thurifera	0(1)	0(0)	1(0)	2	2.546	0.280	-	-	-	-	-	-
Koeleria vallesiana	18(14)	4(3)	1(6)*	2	8.910	**<0.05**	81(96)*	46(19)*	31(44)*	2	39.332	**<0.001**
Linum suffruticosum	50(64)*	23(12)*	32(29)	2	12.345	**<0.01**	18(36)*	24(7)*	18(17)	2	38.903	**<0.001**
Lithodora fruticosa	1(1)	0(0)	1(1)	2	0.762	0.683	10(16)*	3(3)	14(7)	2	7.334	**<0.05**
Polygala rupestris	7(16)*	3(3)	16(7)	2	14.035	**<0.001**	7(84)*	28(16)*	104(38)*	2	203.400	**<0.001**
Rosmarinus officinalis	44(55)*	22(11)*	24(25)	2	13.316	**<0.01**	23(61)*	75(12)*	3(28)*	2	227.660	**<0.001**
Stipa lagascae	13(14)	2(3)	8(6)	2	0.548	0.760	18(73)*	45(14)*	58(33)*	2	121.197	**<0.001**
Teucrium capitatum	20(37)*	8(7)	33(17)*	2	21.447	**<0.001**	15(26)*	17(5)*	11(12)	2	24.743	**<0.001**
Thymelaea tinctoria	-	-	-	-	-	-	8(7)	2(1)	2(3)	2	1.008	0.604
Thymus vulgaris	101(118)*	44(23)*	50(54)	2	21.380	**<0.001**	91(145)*	104(28)*	44(66)*	2	163.365	**<0.001**

B = open areas, E = at the edge of adult shrubs, U = under the canopy of adult shrubs. * indicate that the observed frequencies were significantly different to the expected frequencies at each microsite, after pair-wise tests.

Table A4.2 Summary of the G-test implemented to evaluate the differences between the observed frequencies f and the expected frequencies $f_{(e)}$ of all perennial species (seedlings and adults separately) recorded in **Site 2** at each microsite.

	Seedlings						**Adults**					
Species	**OA [f ($f_{(e)}$)]**	**ES [f ($f_{(e)}$)]**	**UC [f ($f_{(e)}$)]**	**DF**	**G**	**p-value**	**OA [f ($f_{(e)}$)]**	**ES [f ($f_{(e)}$)]**	**UC [f ($f_{(e)}$)]**	**DF**	**G**	**p-value**
Gypsophytes	1178(1260)*	284(138)*	264(328)*	2	136.59	**<0.001**	518(716)*	370(79)*	93(186)*	2	682.63	**<0.001**
Gypsophila struthium	19(31)*	4(3)	19(8)*	2	16.18	**<0.001**	7(23)*	21(3)*	4(6)	2	68.17	**<0.001**
Helianthemum squamatum	897(955)*	209(104)*	196(243)*	2	95.98	**<0.001**	390(520)*	240(57)*	79(132)*	2	387.35	**<0.001**
Herniaria fruticosa	255(263)	67(29)*	36(67)*	2	55.55	**<0.001**	112(153)*	89(17)*	8(39)*	2	202.74	**<0.001**
Ononis tridentata	7(18)*	4(2)	13(4)*	2	20.27	**<0.001**	9(23)*	20(2)*	2(6)	2	62.58	**<0.001**
Gypsovags	1629(1900)*	480(208)*	495(495)	2	298.85	**<0.001**	891(1150)*	729(159)*	366(377)	2	1331.45	**<0.001**
Brachypodium retusum	20(20)	4(2)	3(5)	2	2.30	0.318	75(155)*	110(17)*	26(39)*	2	281.89	**<0.001**
Carlina corimbosa	-	-	-	-	-	-	0(1)	1(0)	0(0)	2	5.05	0.080
Carduus sp.	2(1)	0(0)	0(0)	2	1.26	0.533	1(1)	1(0)	0(0)	2	2.91	0.234
Carex halleriana	-	-	-	-	-	-	1(1)	1(0)	0(0)	2	2.91	0.234
Genista scorpius	1(1)	1(0)	0(0)	2	2.91	0.234	6(11)*	8(1)*	1(3)	2	21.04	**<0.001**
Helianthemum syriacum	231(285)*	88(31)*	69(72)	2	80.08	**<0.001**	125(182)*	92(20)*	31(46)*	2	163.71	**<0.001**
Helianthemum pilosum	12(10)	0(1)	2(3)		2.71	0.258	13(18)*	9(2)*	3(5)	2	15.50	**<0.001**
Helichrysum stoechas	529(691)*	202(75)*	211(176)*	2	190.28	**<0.001**	38(119)*	62(13)*	62(30)*	2	194.64	**<0.001**
Koeleria vallesiana	17(15)	3(2)	0(4)*	2	8.95	**<0.05**	36(54)*	32(6)*	5(14)*	2	70.42	**<0.001**
Launea pumila	4(4)	1(0)	0(1)	2	2.57	0.277	0(1)	2(0)*	0(0)	2	10.10	**<0.01**
Linum suffruticosum	25(32)*	11(4)*	8(8)	2	11.83	**<0.01**	6(18)*	10(2)*	9(5)	2	30.34	**<0.001**
Lygeum spartum	1(1)	0(0)	0(0)	2	0.63	0.730	7(12)*	6(1)*	3(3)	2	11.29	**<0.01**
Plantago albicans	477(389)*	34(42)	20(99)*	2	118.05	**<0.001**	434(540)*	202(59)*	100(137)*	2	245.68	**<0.001**
Polygala rupestris	0(1)	1(0)	0(0)	2	5.05	0.080	0(7)*	2(1)	8(2)	2	26.67	**<0.001**
Reseda stricta	5(5)	1(1)	1(1)	2	0.371	0.830	6(7)	2(1)	2(2)	2	1.52	0.468
Rosmarinus officinalis	28(34)	6(4)	12(9)	2	3.30	0.192	6(16)*	15(2)*	1(4)	2	49.61	**<0.001**
Sedum sedifolia	1(1)	1(0)	0(0)	2	2.91	0.234	0(5)*	2(1)	5(1)*	2	18.34	**<0.001**
Sideritis hirsuta	29(40)*	12(4)*	13(10)	2	12.87	**<0.01**	11(27)*	21(3)*	5(7)	2	59.12	**<0.001**
Stipa lagascae	42(70)*	21(8)*	33(18)*	2	38.37	**<0.001**	56(143)*	77(16)*	62(36)*	2	205.22	**<0.001**
Teucrium capitatum	50(90)*	29(10)*	44(23)*	2	59.83	**<0.001**	15(30)*	15(3)*	11(8)	2	32.44	**<0.001**
Thymus vulgaris	155(219)*	65(24)*	79(56)*	2	75.94	**<0.001**	57(109)*	59(12)*	32(28)	2	124.89	**<0.001**

B = open areas, E = at the edge of adult shrubs, U = under the canopy of adult shrubs. * indicate that the observed frequencies were significantly different to the expected frequencies at each microsite, after pair-wise tests.

Appendix 5 Facilitative role of shrubs (Chapter 1)

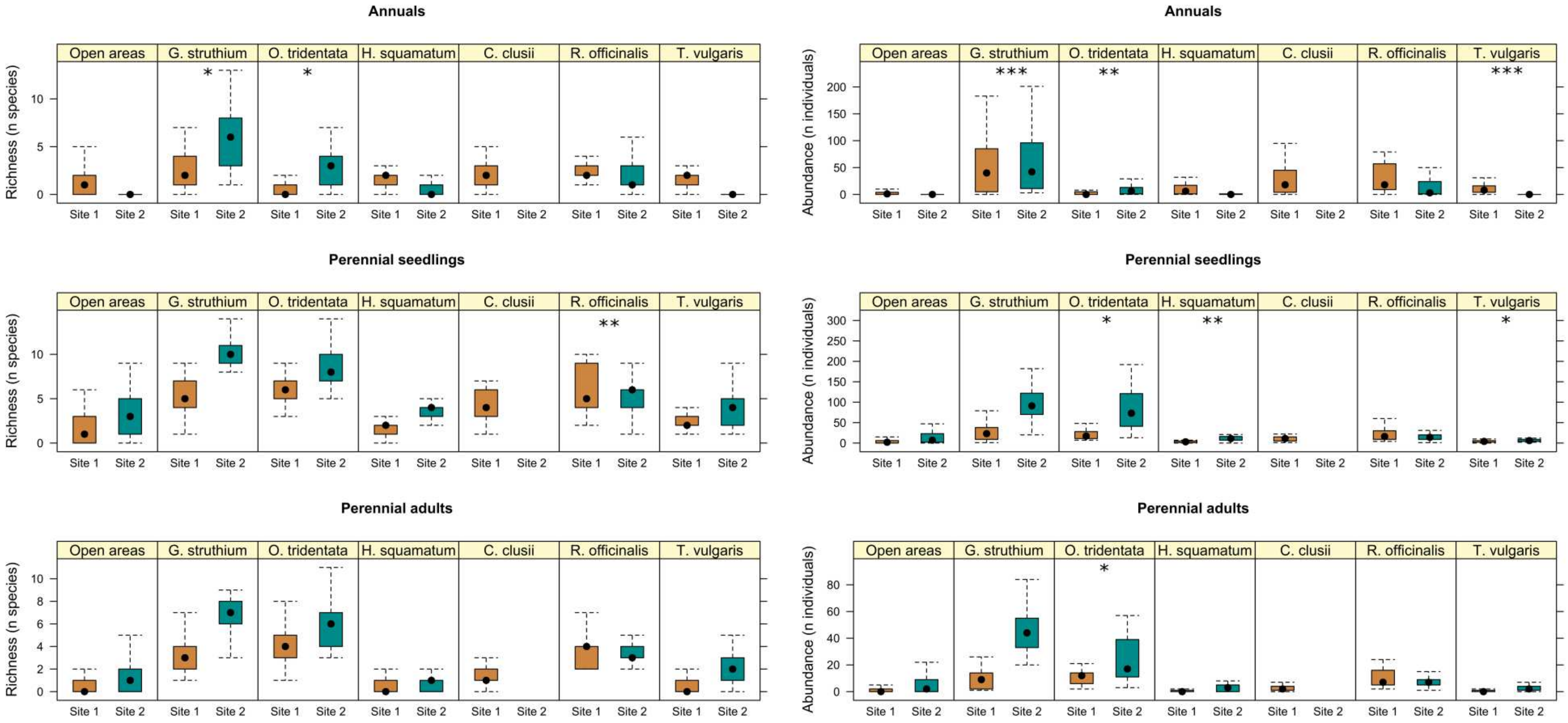


Figure A5.1 Boxplots representing richness and abundance per study site recorded in open areas and under the canopy of each target species, for annuals, perennial seedlings and perennial adults. Significant effects of study site under target species canopies after GLMMs are indicated as * (p≤0.05), ** (p≤0.01) and *** (p≤0.001).

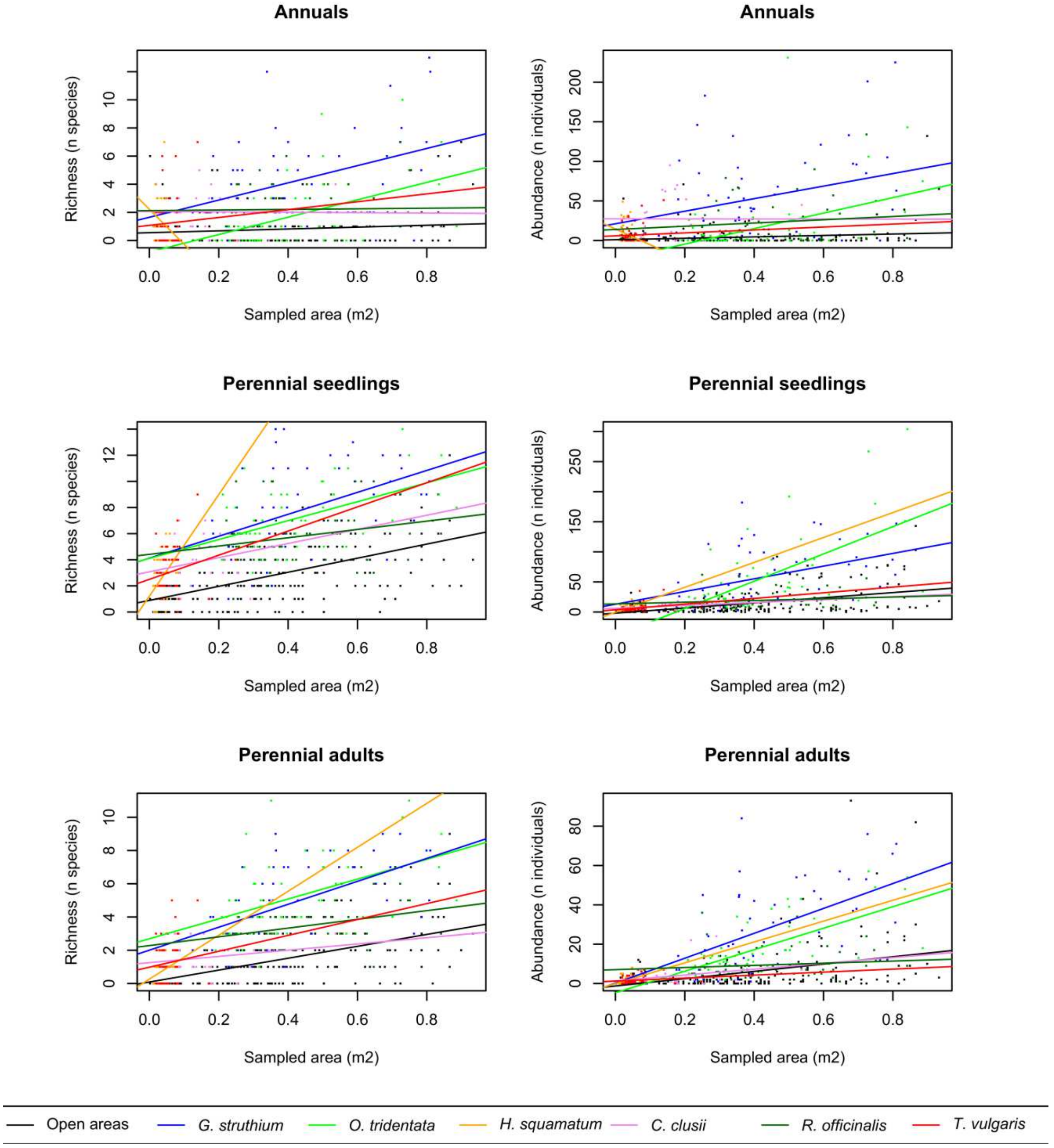


Figure A5.2 Regression lines representing the predictions of the effect of the covariate sampled area (m^2) on richness and abundance in open areas and under the canopy of each target species, for annuals, perennial seedlings and perennial adults.

Table A6.1 Perennials with more than 20 individuals recorded along the line-point intercept transects performed in Site 1, which acted as diversity accumulators (+), diversity repellers (-) and neutrals (0) at different distance intervals (every 40 cm) from the target plant.

Species	N. individuals	0-40	40-80	80-120	120-160	160-200	200-240	240-280	280-320	320-360	360-400
Gysophytes											
*Gypsophila struthium**	206	0	+	+	+	0	0	0	0	0	0
*Ononis tridentata**	141	+	+	0	0	0	0	0	0	0	0
*Helianthemum squamatum**	29	0	0	0	0	0	0	0	0	0	0
Gypsovags											
Brachypodium retusum	271	0	0	0	0	0	0	0	0	0	0
*Cistus clusii **	670	-	0	0	0	0	0	0	0	0	0
Coronilla minima	45	0	0	0	0	0	0	0	0	0	0
Dorycnium pentaphyllum	48	0	0	0	0	0	0	0	0	0	0
Fumana ericacoides	61	0	0	0	0	0	0	0	0	0	0
Genista scorpius	150	0	+	0	0	0	0	0	0	0	0
Helianthemum syriacum	144	-	0	0	0	0	0	0	0	0	0
Helianthemum marifolium	257	+	+	0	0	0	0	0	0	0	0
Koeleria vallesiana	86	-	0	0	0	0	0	0	0	0	0
Linum suffruticosum	61	-	0	0	0	0	0	0	0	0	0
Polygala rupestris	186	+	0	0	0	0	0	0	0	0	0
*Rosmarinus officinalis**	2561	-	0	-	-	+	+	0	0	0	0
Stipa lagascae	132	0	0	0	0	0	0	0	0	0	0
Teucrium capitatum	22	0	0	0	0	0	0	0	0	0	0
Thymelaea tinctoria	29	0	0	0	0	0	0	0	0	0	0
*Thymus vulgaris**	564	0	+	+	0	0	0	0	0	0	0

* indicate a target species in our study.

Table A6.2 Perennials with more than 20 individuals recorded along the line-point intercept transects performed in Site 2, which acted as diversity accumulators (+), diversity repellers (-) and neutrals (0) at different distance intervals (every 40 cm) from the target plant.

Species	**N. individuals**	**0-40**	**40-80**	**80-120**	**120-160**	**160-200**	**200-240**	**240-280**	**280-320**	**320-360**	**360-400**
Gypsophytes											
*Gypsophila struthium**	76	+	+	0	0	0	0	0	0	0	0
*Ononis tridentata**	20	+	+	0	0	0	0	0	0	0	0
*Helianthemum squamatum**	706	-	0	0	0	0	0	0	0	0	0
Herniaria fruticosa	289	0	0	0	0	0	0	0	0	0	0
Gypsovags											
Asphodelus cerasiferus	20	0	0	0	0	0	0	0	0	0	0
Helianthemum syriacum	50	0	0	0	0	0	0	0	0	0	0
Helichrysum stoechas	141	0	0	0	0	0	0	0	0	0	0
Koeleria vallesiana	122	0	0	0	0	0	0	0	0	0	0
Lygeum spartum	495	-	0	0	0	0	0	0	0	0	0
Plantago albicans	580	-	0	+	0	0	0	0	0	0	0
Scorzonera sp.	64	0	0	0	0	0	0	0	0	0	0
Sideritis hirsuta	26	0	0	0	0	0	0	0	0	0	0
Stipa lagascae	538	-	0	0	0	0	0	0	0	0	0
Stipa parviflora	30	0	0	0	0	0	0	0	0	0	0
*Thymus vulgaris**	111	-	0	0	0	0	0	0	0	0	0
Thymus zygis	204	0	0	0	0	0	0	0	0	0	0

* indicate a target species in our study.

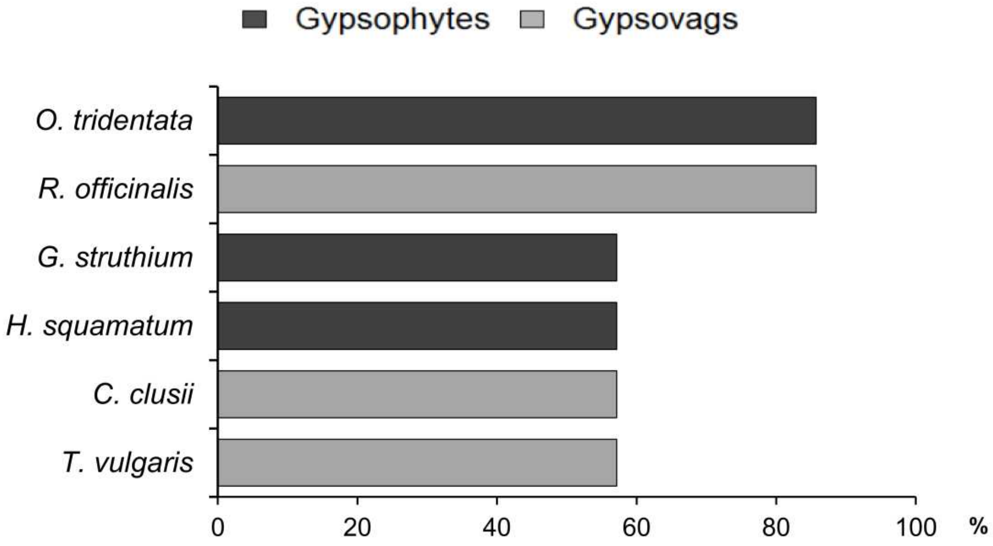


Figure A7.1 Percentage of significant positive effects of each target species, compared to open areas, on microenvironmental and physical-chemical soil conditions (surface mechanical resistance diminishing, humidity increasing, temperature softening and nutrients enrichment).

Appendix 7 Micro-environmental and soil conditions under shrubs (Chapter 1)

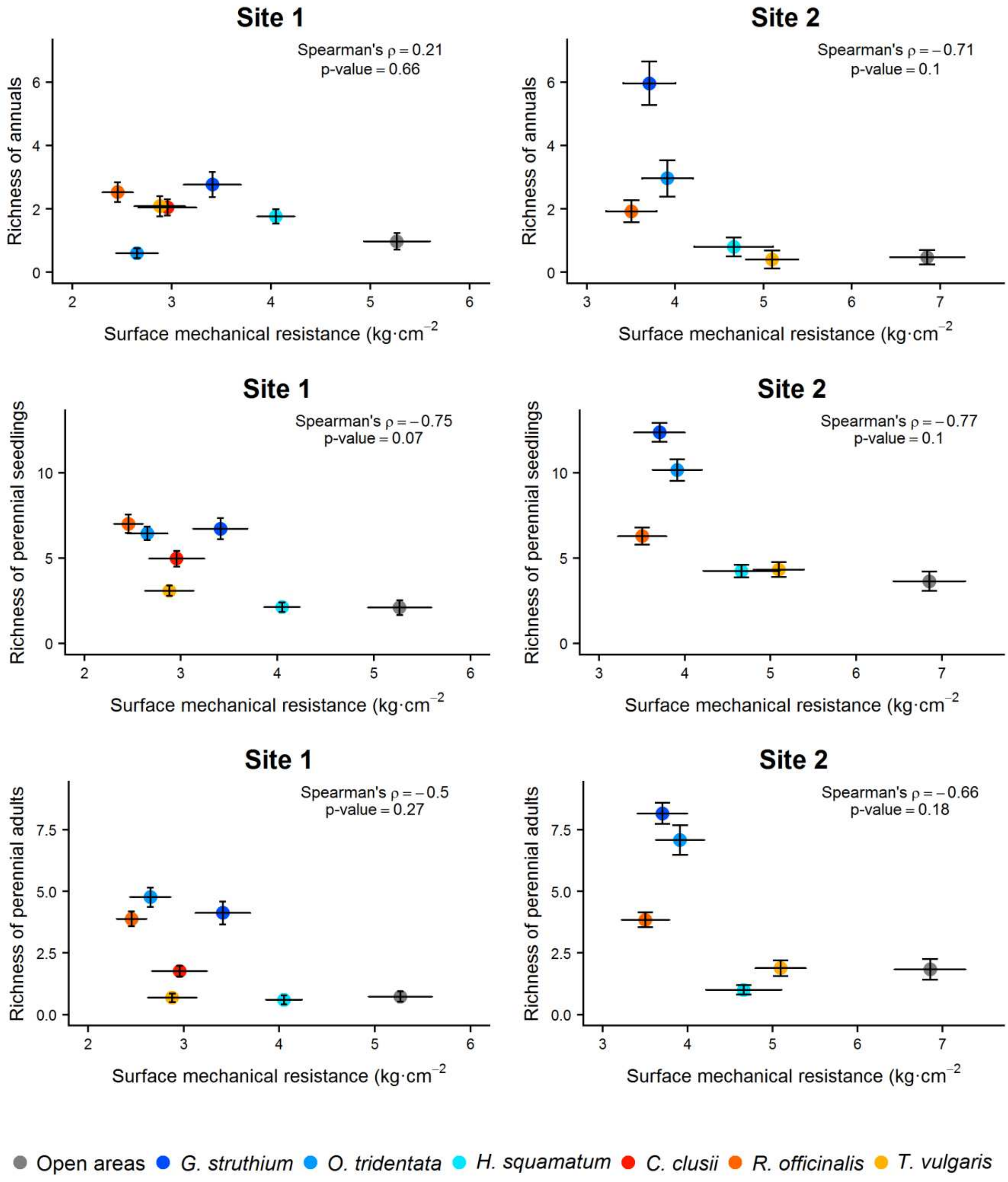


Figure A7.2 Scatterplots showing the correlation between richness (for annuals, perennial seedlings and perennial adults) and surface mechanical resistance per study site. When p-values ≤ 0.05 indicate significant Spearman's rank correlations.

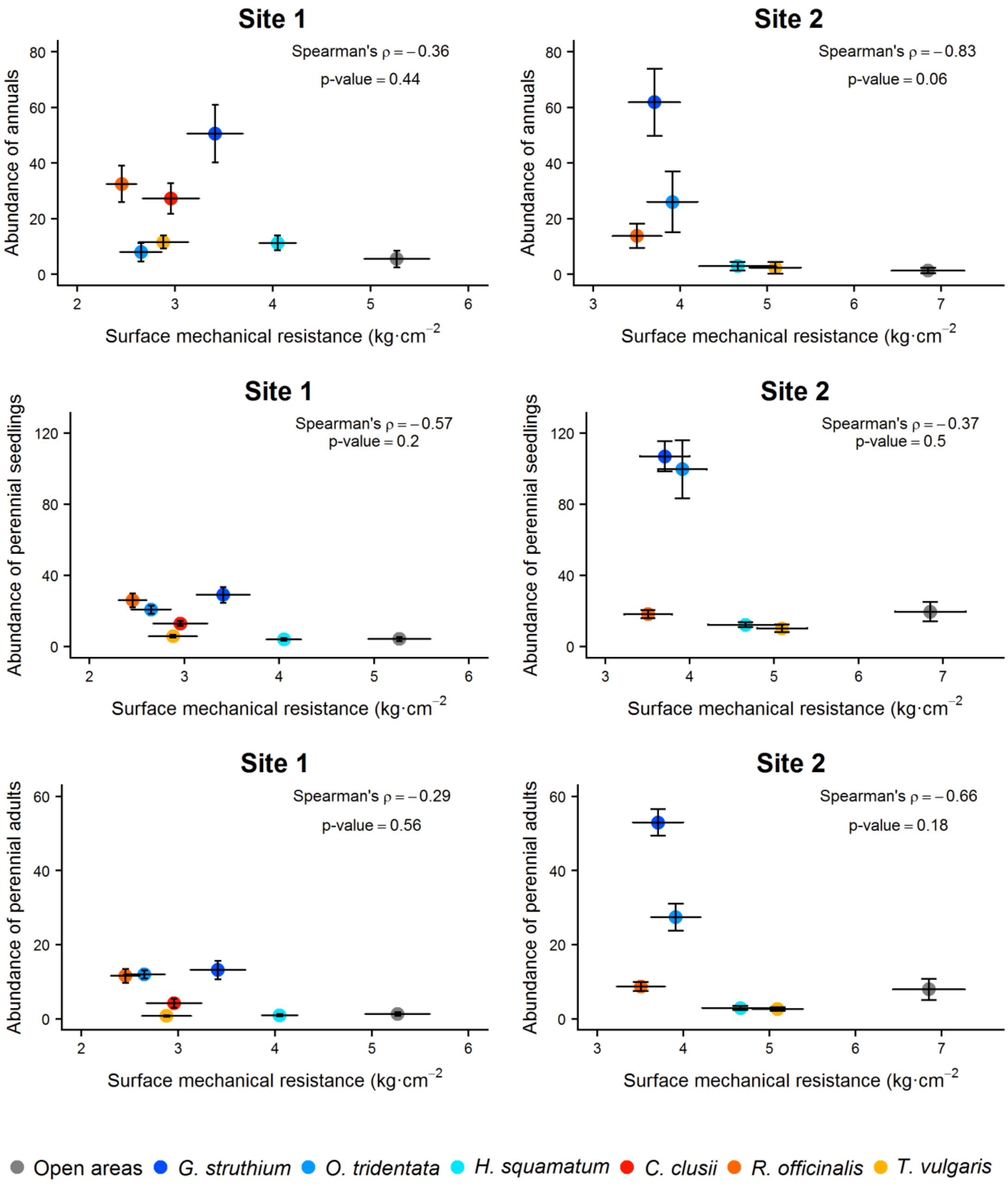


Figure A7.3 Scatterplots showing the correlation between abundance (for annuals, perennial seedlings and perennial adults) and surface mechanical resistance per study site. When p-values ≤ 0.05 indicate significant Spearman's rank correlations.

Appendix 7 Micro-environmental and soil conditions under shrubs (Chapter 1)

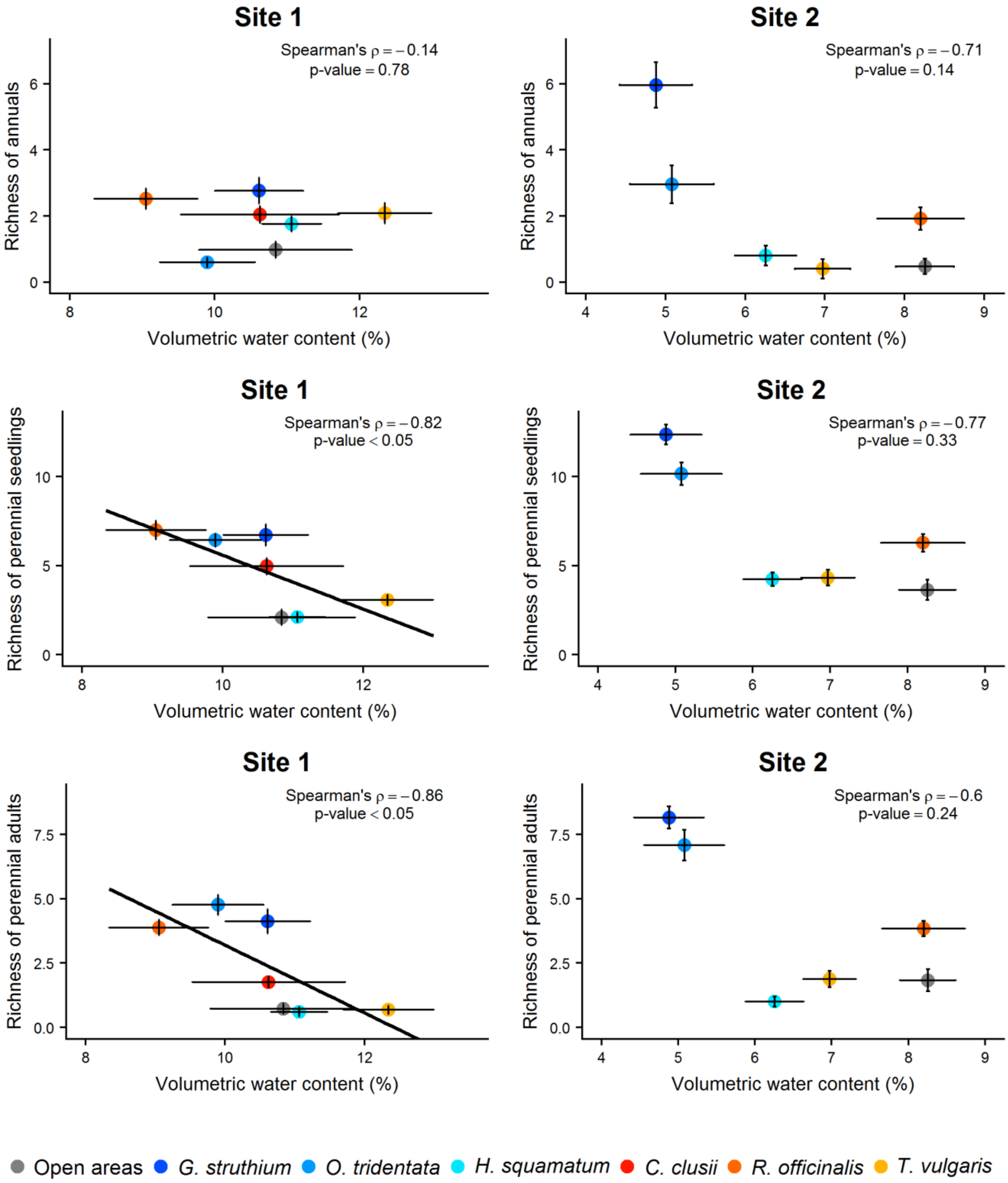


Figure A7.4 Scatterplots showing the correlation between richness (for annuals, perennial seedlings and perennial adults) and volumetric water content in the soil per study site. When p-values ≤ 0.05 indicate significant Spearman's rank correlations.

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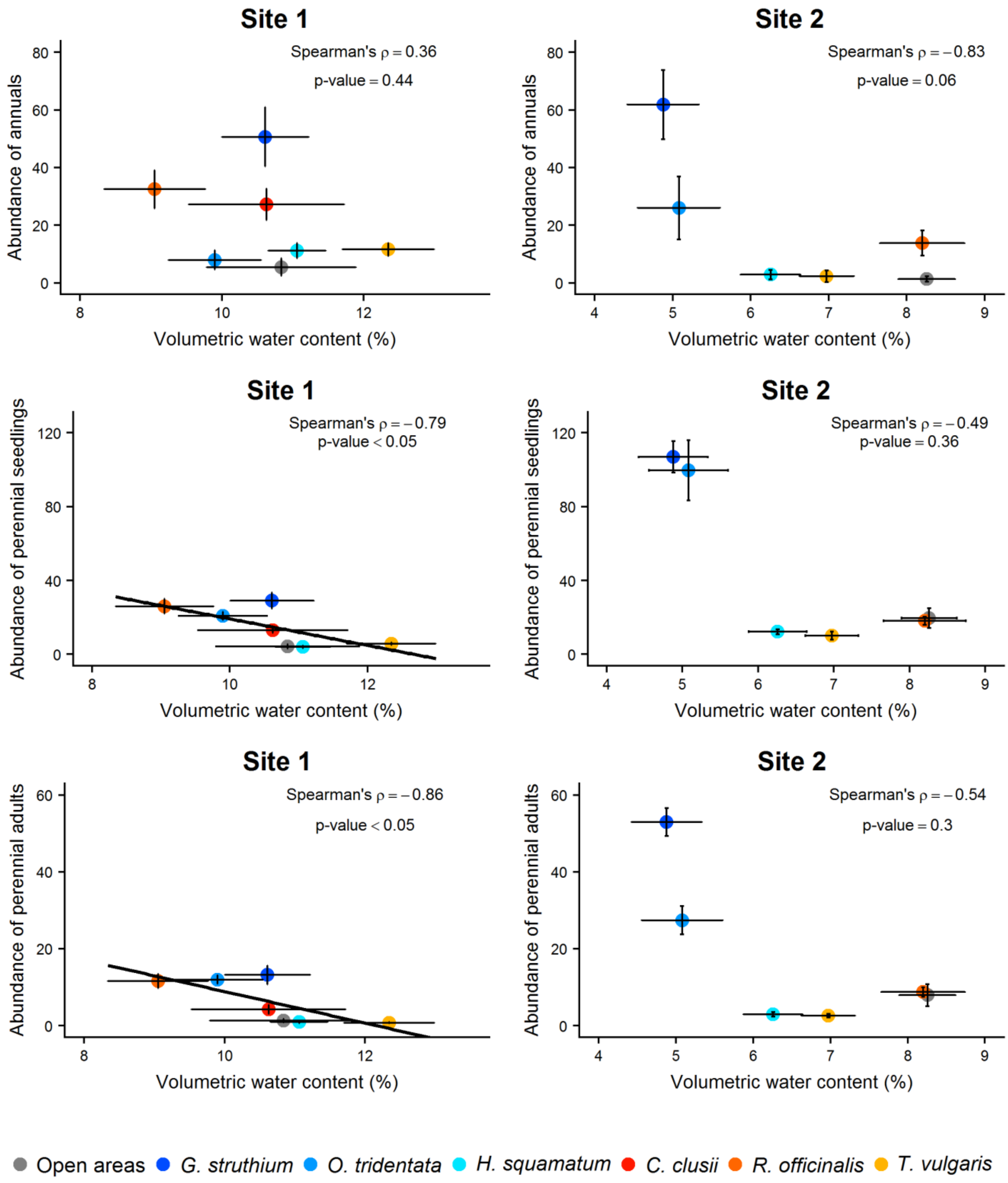

Figure A7.5 Scatterplots showing the correlation between abundance (for annuals, perennial seedlings and perennial adults) and volumetric water content in the soil per study site. When p-values ≤ 0.05 indicate significant Spearman's rank correlations.

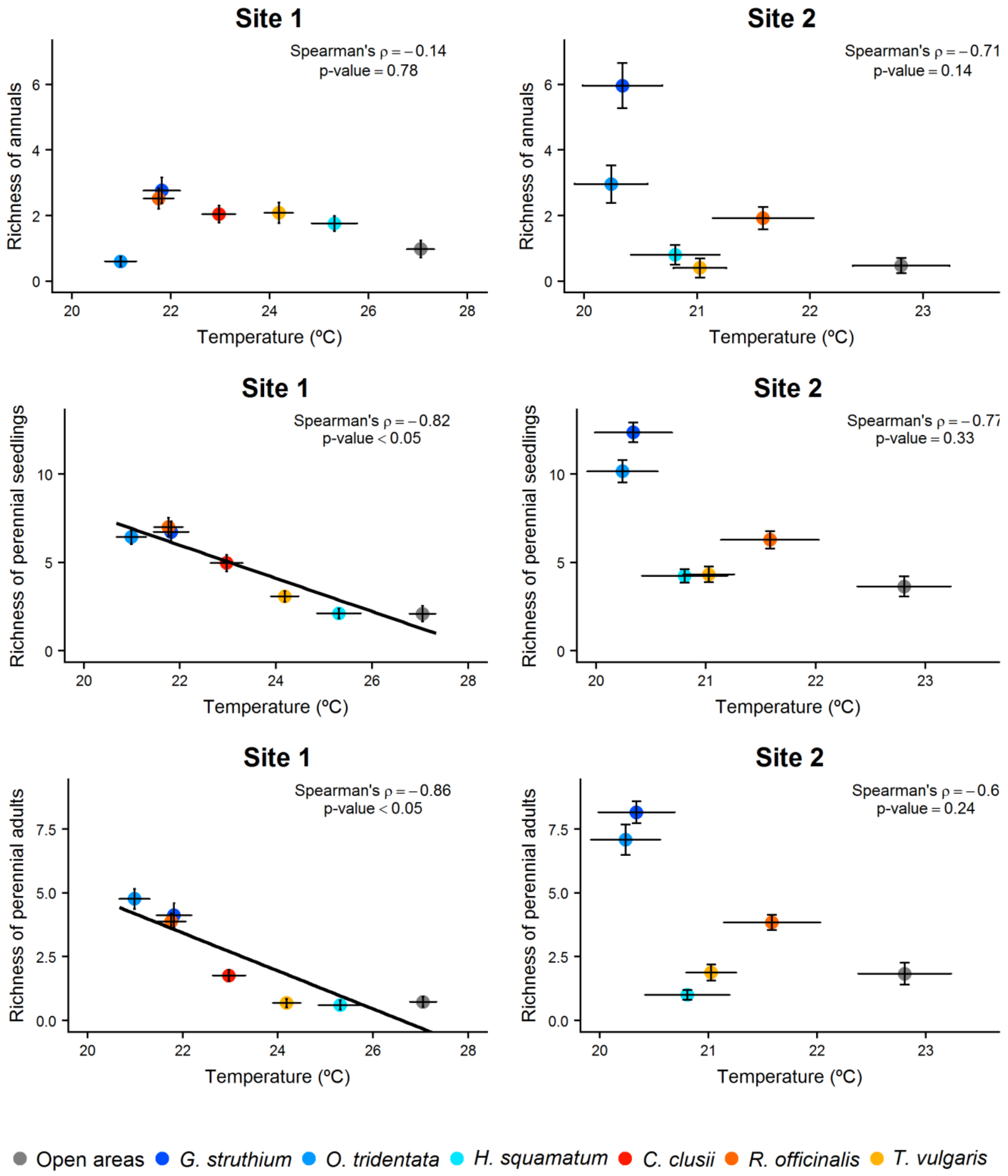

Figure A7.6 Scatterplots showing the correlation between richness (for annuals, perennial seedlings and perennial adults) and soil temperature per study site. When p-values ≤ 0.05 indicate significant Spearman's rank correlations.

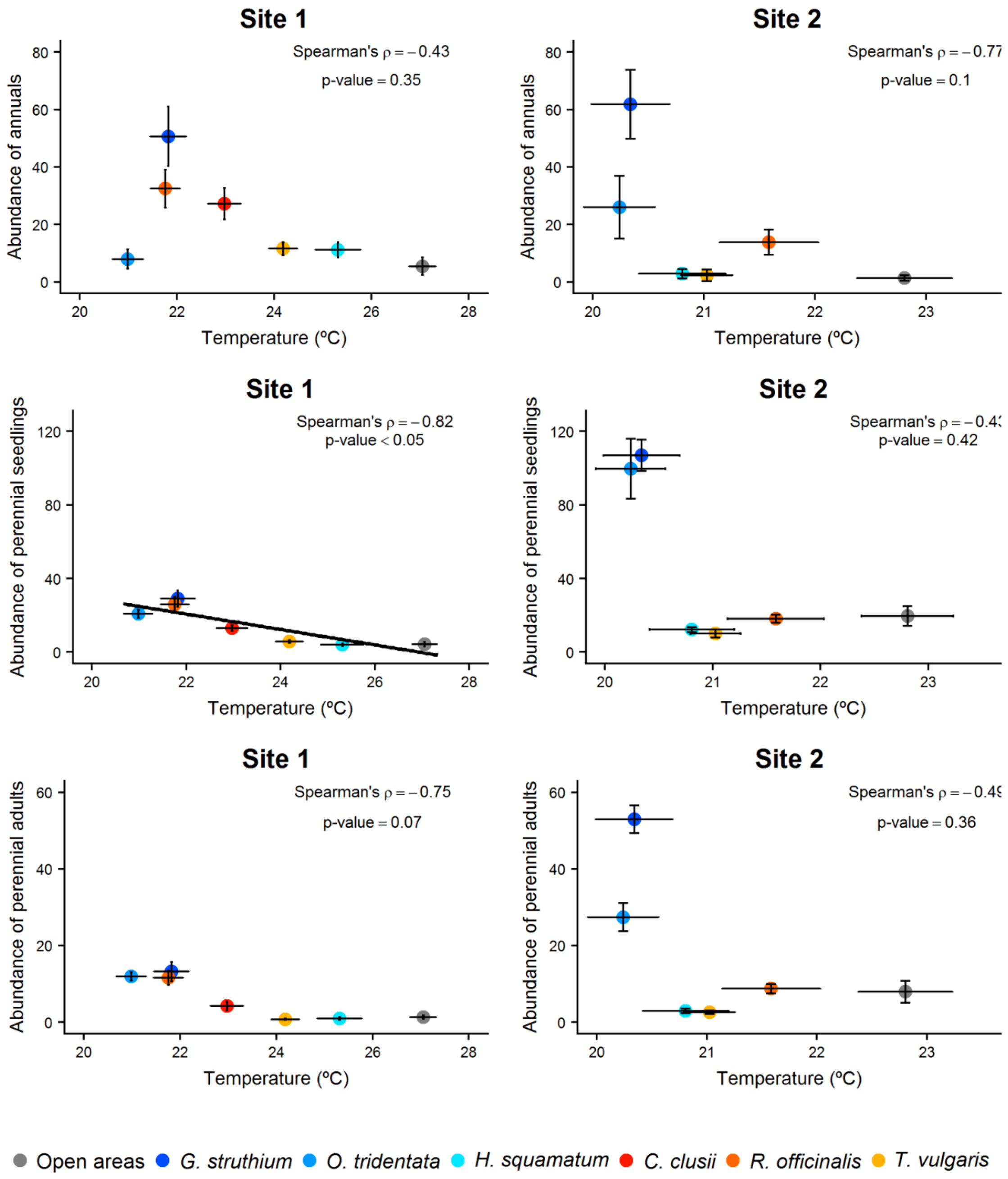

Figure A7.7 Scatterplots showing the correlation between abundance (for annuals, perennial seedlings and perennial adults) and soil temperature per study site. When p-values ≤ 0.05 indicate significant Spearman's rank correlations.

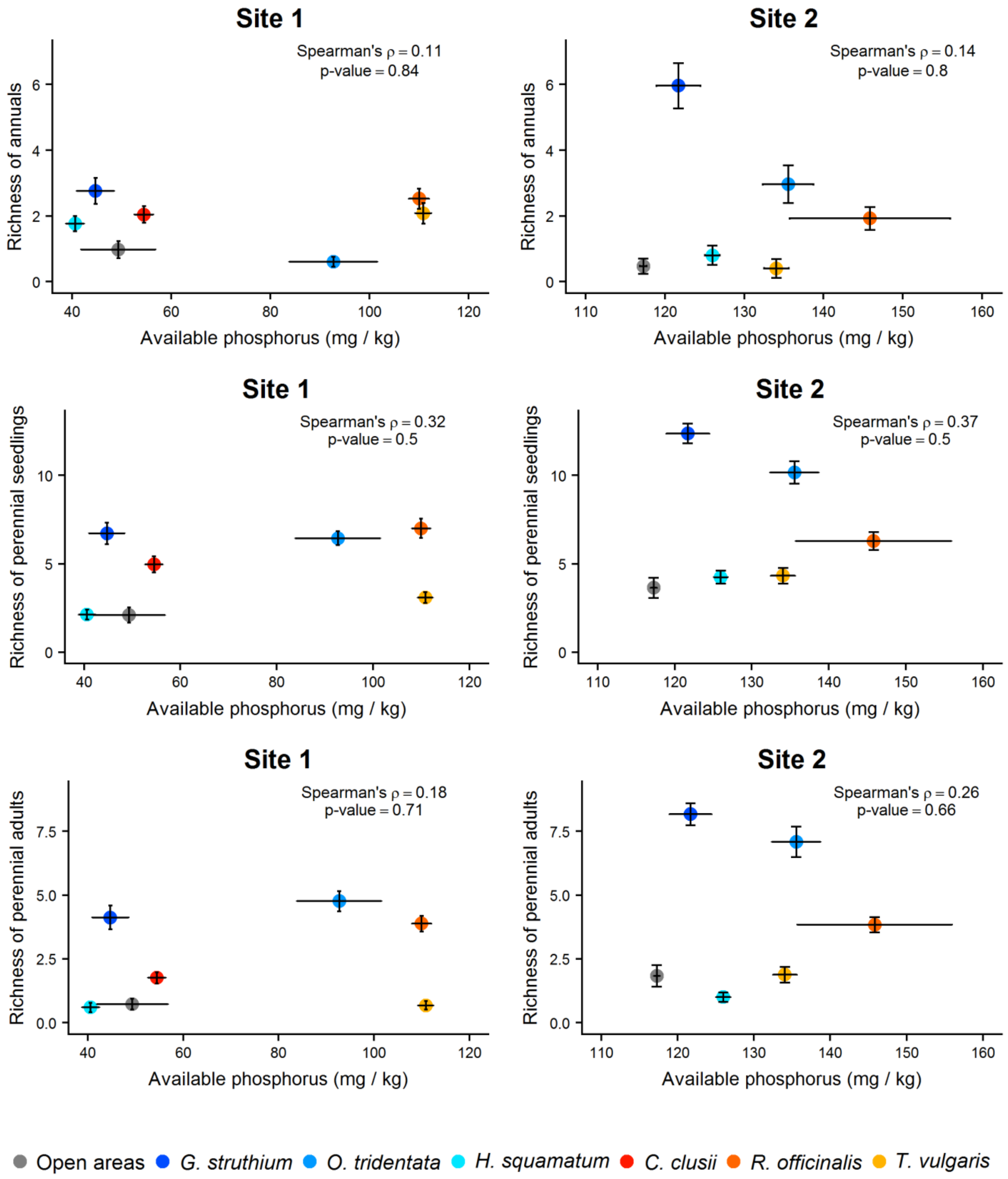

Figure A7.8 Scatterplots showing the correlation between richness (for annuals, perennial seedlings and perennial adults) and available phosphorus in the soil per study site. When p-values ≤ 0.05 indicate significant Spearman's rank correlations.

Appendix 7 Micro-environmental and soil conditions under shrubs (Chapter 1)

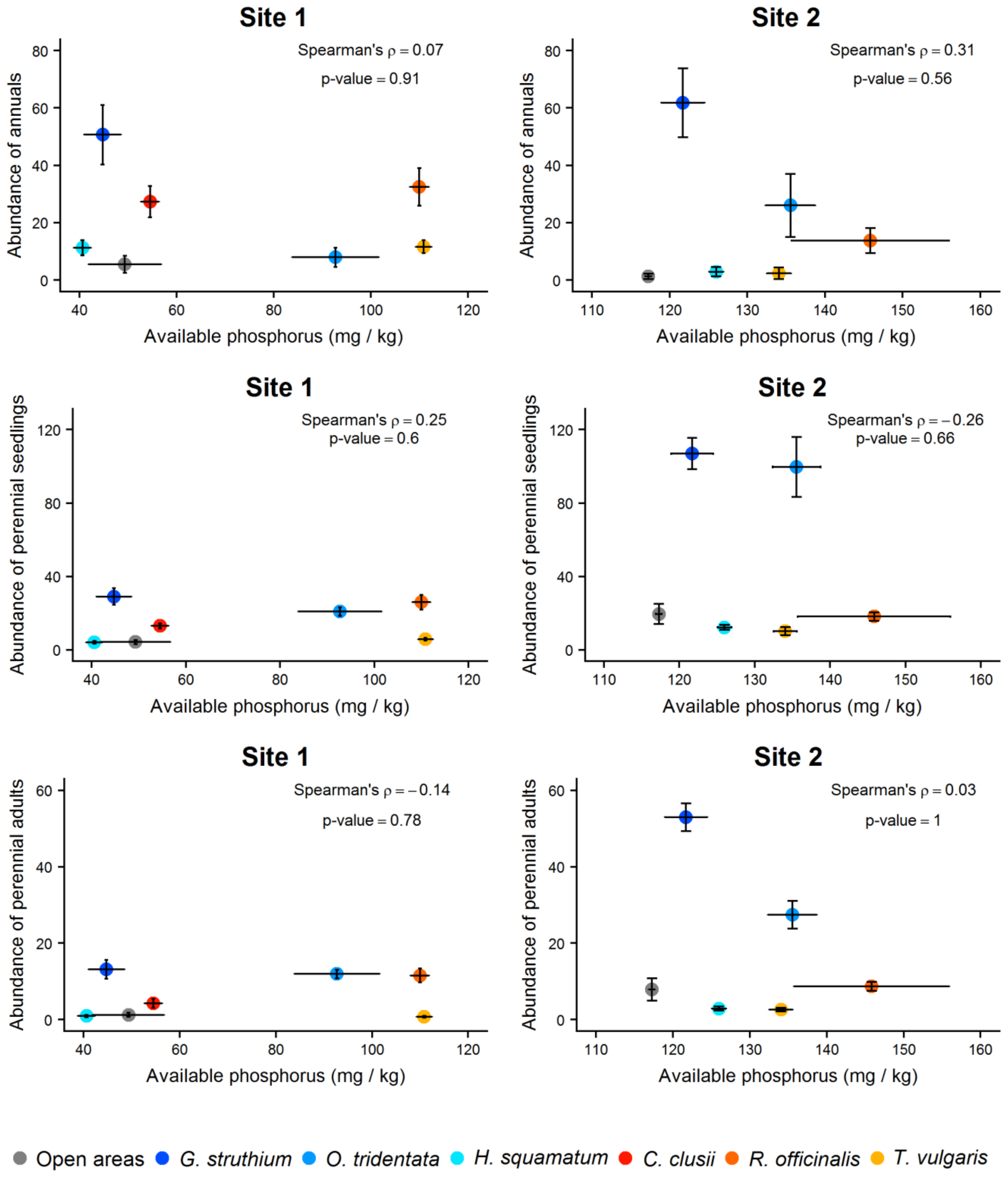

Figure A7.9 Scatterplots showing the correlation between abundance (for annuals, perennial seedlings and perennial adults) and available phosphorus in the soil per study site. When p-values ≤ 0.05 indicate significant Spearman’s rank correlations.

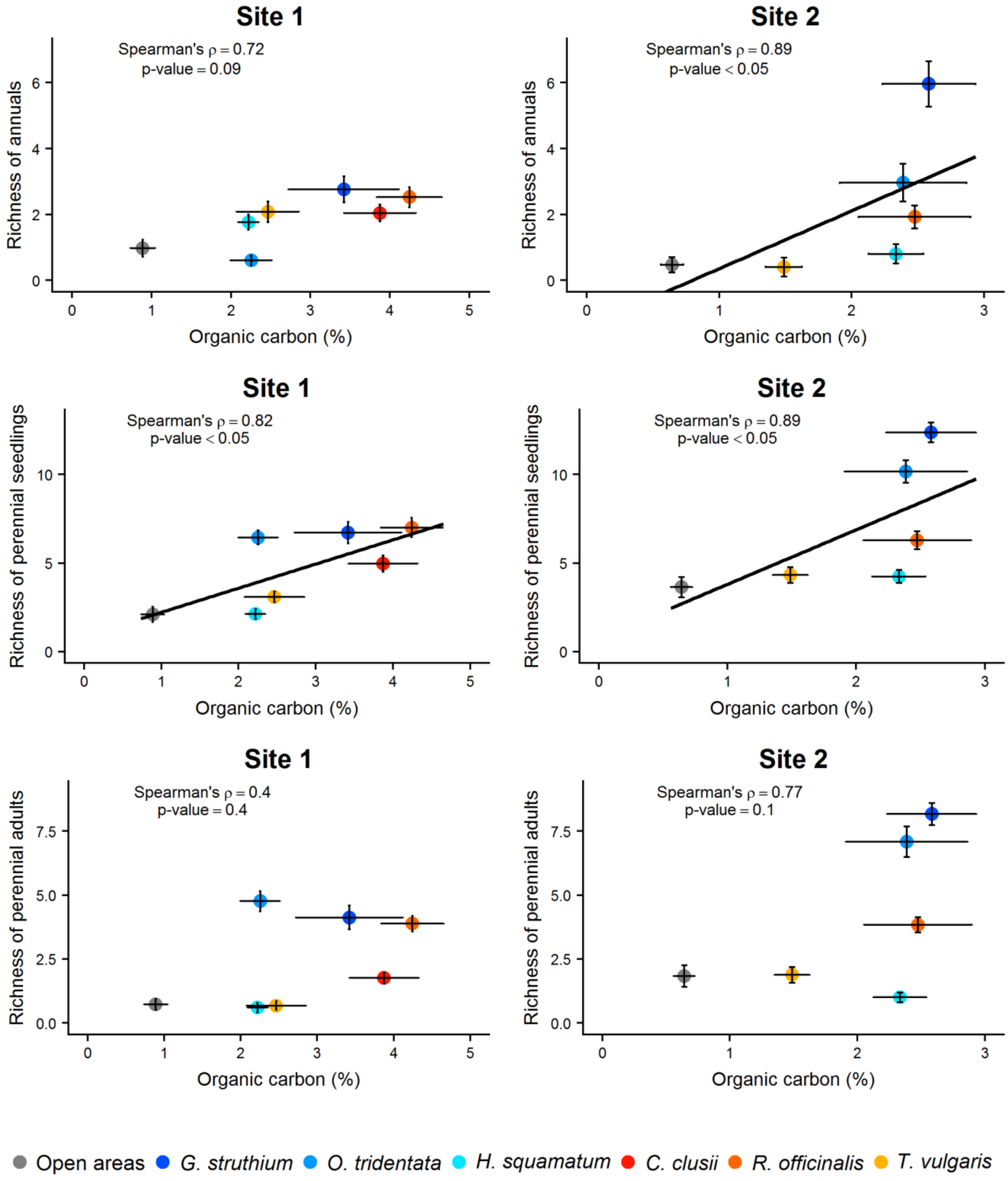

Figure A7.10 Scatterplots showing the correlation between richness (for annuals, perennial seedlings and perennial adults) and organic carbon in the soil per study site. When p-values ≤ 0.05 indicate significant Spearman's rank correlations.

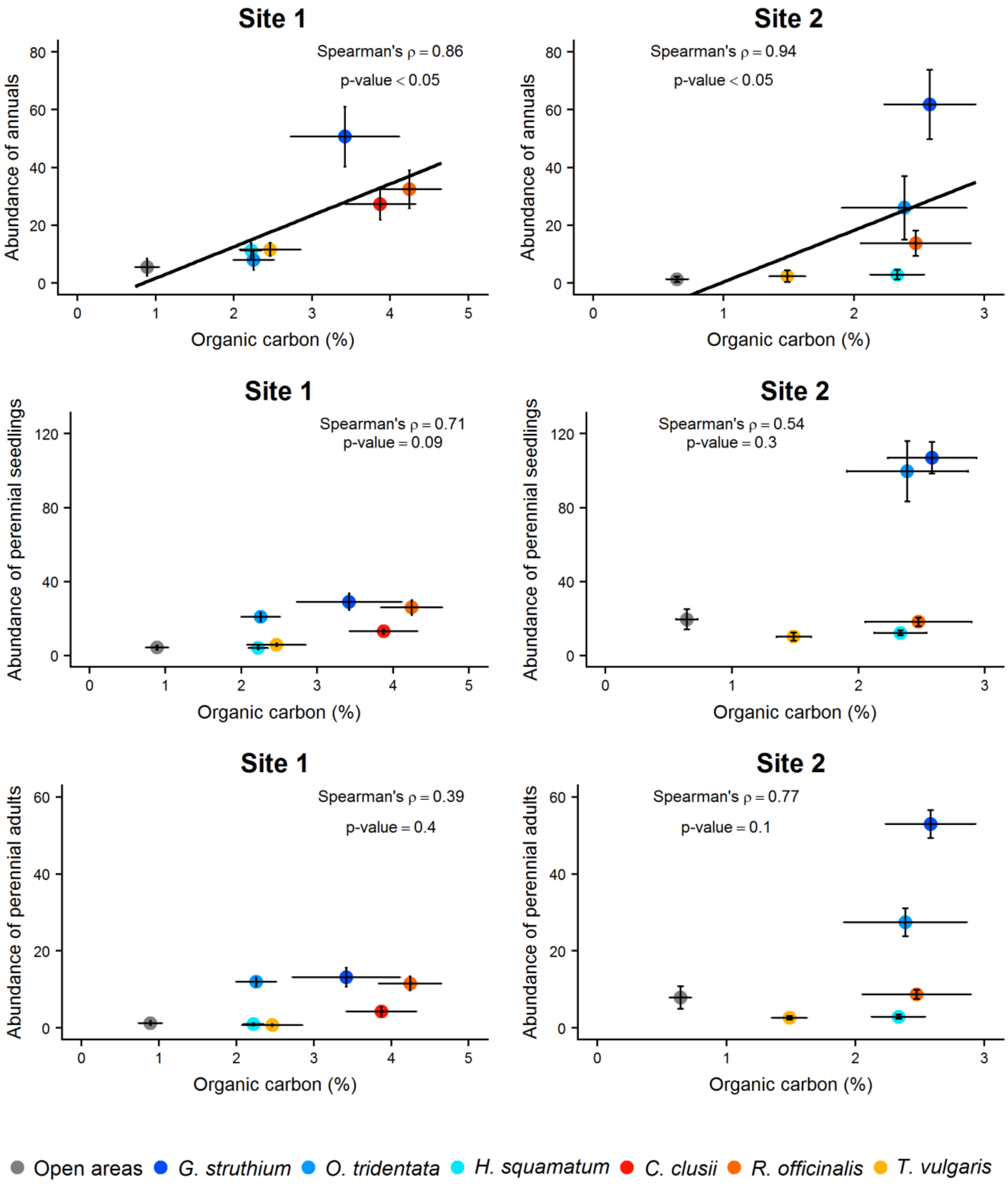

Figure A7.11 Scatterplots showing the correlation between abundance (for annuals, perennial seedlings and perennial adults) and organic carbon in the soil per study site. When p-values ≤ 0.05 indicate significant Spearman's rank correlations.

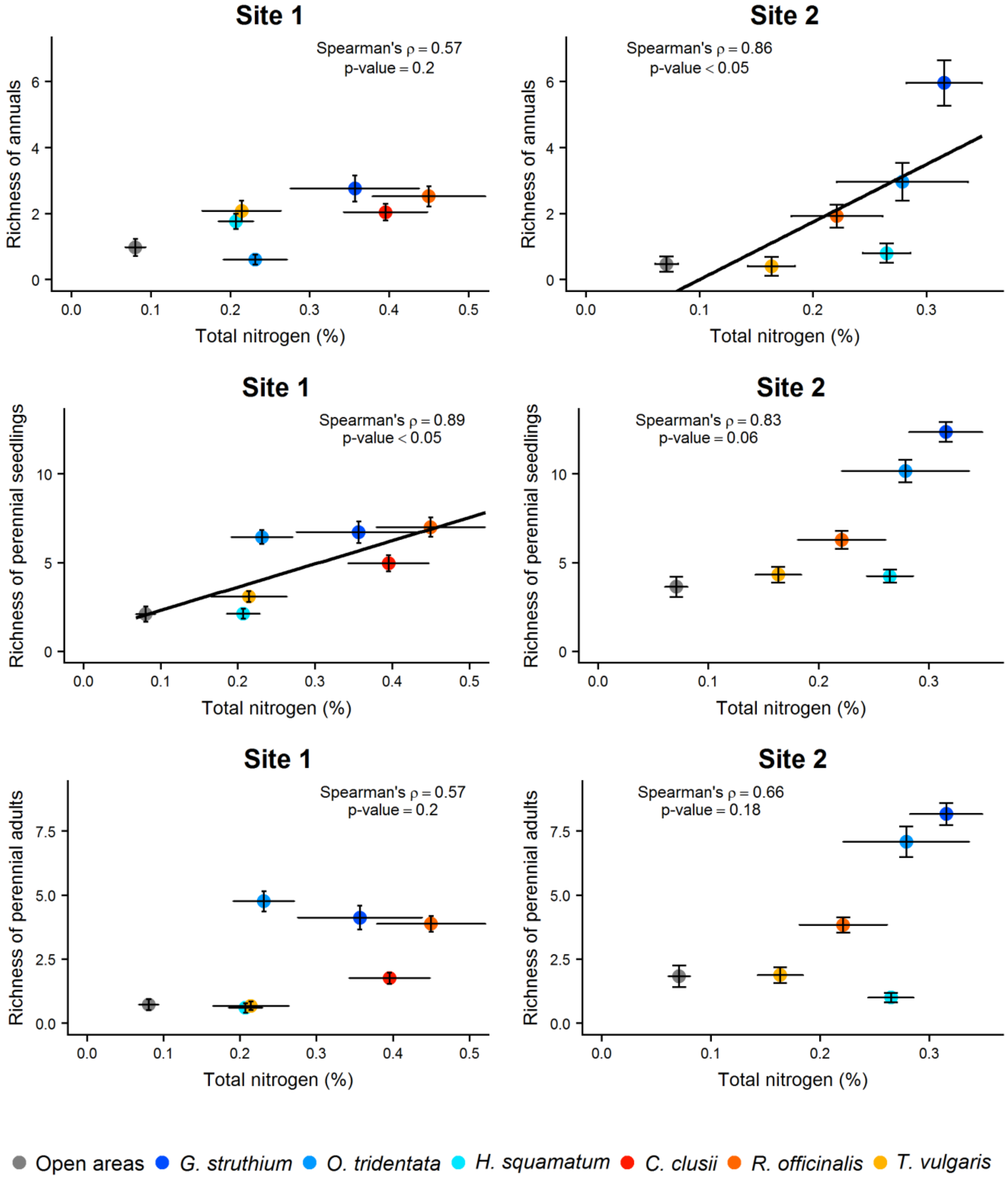

Figure A7.12 Scatterplots showing the correlation between richness (for annuals, perennial seedlings and perennial adults) and total nitrogen in the soil per study site. When p-values ≤ 0.05 indicate significant Spearman’s rank correlations.

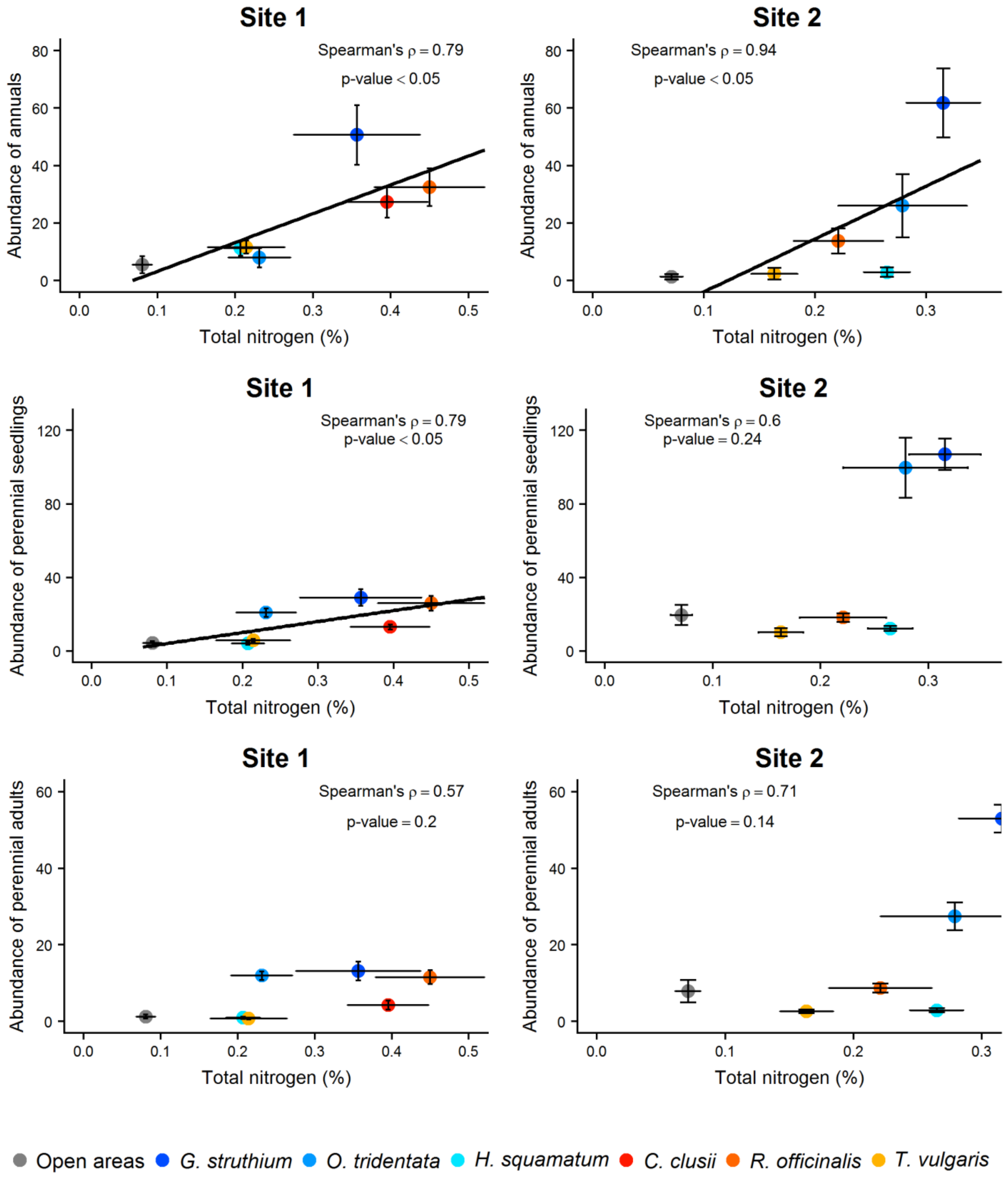

Figure A7.13 Scatterplots showing the correlation between abundance (for annuals, perennial seedlings and perennial adults) and total nitrogen in the soil per study site. When p-values ≤ 0.05 indicate significant Spearman's rank correlations.

Appendix 7 Micro-environmental and soil conditions under shrubs (Chapter 1)

Appendices

Chapter 2

Appendix 8 Species in La Lomaza de Belchite (Chapter 2)

Table A8.1 Species list in the study site (Site 2). For each species, the growth habit, height and seed size are indicated. The abundance of standing individuals and seeds recorded for each species is also indicated.

	Species	Family	Growth habit	Gypsophily	Plant height (cm)	Seed size (mm)	Abundance	Seeds
	Annuals							
1	*Aegilops geniculata* Roth.	*Poaceae*	Graminoid	Gypsovag	15-40	7 x 3	NA	NA
2	*Alyssum alyssoides* (L.) L.	*Brassicaceae*	Forb	Gypsovag	5-15-30)	3-4 x 3-4	NA	NA
3	*Anagallis arvensis* L.	*Primulaceae*	Forb	Gypsovag	(2.5)8-40(70)	0.9-1.4 x 0.6-1	NA	NA
4	*Asterolinon linum-stellatum* (L.) Duby	*Primulaceae*	Forb	Gypsovag	(1)3 - 12-18)	1.2	24	6
5	*Brachypodium distachyon* (L.) P. Beauv.	*Poaceae*	Graminoid	Gypsovag	< 30	1-2 x 3-4	9	NA
6	*Bromus rubens* L.	*Poaceae*	Graminoid	Gypsovag	< 60	NA	11	1
7	*Bupleurum semicompositum* L.	*Umbelliferae*	Forb	Gypsovag	2 - 35	1-1.5 x 0.4-0.7	1	NA
8	*Campanula fastigiata* Dufour ex A. DC	*Campanulaceae*	Forb	Gypsophyte	3.5-6	0.35 x 0.2-0.3	NA	1
9	*Cerastium pumilum* Curtis	*Caryophyllaceae*	Forb	Gypsovag	10	0.5 x 0.5	NA	1
10	*Chaenorrhinum rubrifolium* (Robill. & Castagne ex DC.) Fourr.	*Scrophulariaceae*	Forb	Gypsovag	5-18	0.3-0.5 x 0.25-0.3	NA	195
11	*Clypeola jonthlaspi* L.	*Brassicaceae*	Forb	Gypsovag	3 - 28	2.5-4.5	2	1
12	*Desmazeria rigida* (L.) Tutin	*Poaceae*	Graminoid	Gypsovag	< 40	1.7-2 x 0.5-0.6	16	NA
13	*Diplotaxis ilorcitana* (Sennen) Aedo, Mart.-Laborde & Muñoz Garm.	*Brassicaceae*	Forb	Gypsovag	10-60	0.8-1 x 0.4-0.6	6	NA
14	*Erodium cicutarium* (L.) L'Hér.	*Geraniaceae*	Forb	Gypsovag	< 50	4.5-5.5	13	NA
15	*Eruca vesicaria* (L.) Cav.	*Brassicaceae*	Forb	Gypsovag	20-100	1-1.4 x 0.8-1.1	2	NA
16	*Filago pyramidata* L.	*Asteraceae*	Forb	Gypsovag	< 44	0.5-0.8 x 0.15-0.3	16	24
17	*Galium verrucosum* Huds.	*Rubiaceae*	Forb	Gypsovag	< 50	3-4.5	13	4
18	*Hedypnois cretica* (L.) Dum. Cours.	*Asteraceae*	Forb	Gypsovag	5-40	5-8	2	NA
19	*Helianthemum salicifolium* (L.) Mill.	*Cistaceae*	Forb	Gypsovag	(2)3-25(30)	(0.6)0.8-1(1.2)	2	NA
20	*Hippocrepis ciliata* Willd.	*Fabaceae*	Forb	Gypsovag	5-25(35)	0.5-0.7 x 2.4-2.6	25	NA
21	*Hordeum murinum* L.	*Poaceae*	Graminoid	Gypsovag	(8)15-30(70)	5.7-6.3 x 1.7-2	3	NA
22	*Linaria arvensis* (L.) Desf.	*Scrophulariaceae*	Forb	Gypsovag	1-10	1.1-1.5 x 1.1-1.5	1	NA
23	*Linum strictum* L.	*Linaceae*	Forb	Gypsovag	7-45(55)	1.1-1.6 x 0.8-0.9	33	6
24	*Lithospermum arvense* L.	*Boraginaceae*	Forb	Gypsovag	< 100	1.5-2.5	2	NA
25	*Narduroides salzmannii* (Boiss.) Rouy	*Poaceae*	Graminoid	Gypsovag	< 40	NA	1	NA
26	*Neatostema apulum* (L.) I.M.Johnst.	*Boraginaceae*	Forb	Gypsovag	< 30	1.8-2 x 1.2-1.5	16	NA
27	*Polygala monspeliaca* L.	*Polygalaceae*	Forb	Gypsovag	< 37	2.5-3 x 0.75	3	NA

Appendix 8 Species in La Lomaza de Belchite (Chapter 2)

28	*Reseda stricta* Pers.	*Resedaceae*	Forb	Gypsophyte	30-70(100)	0.9-1.4	NA	3
29	*Scorzonera laciniata* L.	*Asteraceae*	Forb	Gypsovag	(5)15-45(70)	1.5-3 x 15-24	64	NA
30	*Senecio gallicus* Chaix	*Asteraceae*	Forb	Gypsovag	< 67	2-2.5	1	NA
31	*Trigonella monspeliaca* L.	*Fabaceae*	Forb	Gypsovag	3-40	1.2-1.7-0.6-1	NA	NA
32	*Trisetum loeflinngianum* (L.) C. Presl.	*Poaceae*	Graminoid	Gypsovag	NA	NA	5	NA
	Perennials							
33	*Artemisia herba-alba* Asso	*Asteraceae*	Subshrub	Gypsovag	10-50	1-1.2 x 0.5-0.6	12	NA
34	*Asphodelus fistulosus* L.	*Liliaceae*	Forb	Gypsovag	70	4-5.5	20	NA
35	*Astragalus alopecuroides* L.	*Fabaceae*	Forb	Gypsovag	30-80	NA	9	NA
36	*Astragalus incanus* L.	*Fabaceae*	Forb	Gypsovag	NA	2-5	9	NA
37	*Brachypodium retusum* (Pers.) P. Beauv.	*Poaceae*	Graminoid	Gypsovag	(12)40-60(140)	0.2	4	7
38	*Carlina corymbosa* L.	*Asteraceae*	Forb	Gypsovag	10-60	2.5-5	NA	NA
39	*Dipcadi serotiunum* (L.) Medik.	*Liliaceae*	Forb	Gypsovag	<40	5 x 2	10	NA
40	*Echinops ritro* L.	*Asteraceae*	Forb	Gypsovag	(7)22-88	6-8 x 2-2.5	NA	NA
41	*Ephedra fragilis* Desf.	*Ephedraceae*	Shrub	Gypsovag	< 3 (4)	NA	NA	NA
42	*Eryngium campestre* L.	*Umbeliferae*	Subshrub	Gypsovag	(15)20-60	2.5 x 2	1	NA
43	*Genista scorpius* (L.) DC.	*Fabaceae*	Shrub	Gypsovag	30-200	2.1-3.2 x 2-3	NA	NA
44	*Gypsophila struthium* Loefl. subsp. *hispanica* (Willk.) G.López	*Caryophyllaceae*	Shrub	Gypsophyte	(15)25-75(80)	0.5	76	12
45	*Hedysarum boveanum* Bunge ex Basiner	*Fabaceae*	Subshrub	Gypsovag	< 50	2.3-3	2	NA
46	*Helianthemum squamatum* (L.) Pers.	*Cistaceae*	Subshrub	Gypsophyte	10-40	1.3	NA	112
47	*Helianthemum syriacum* (Jacq.) Dum. Cours.	*Cistaceae*	Subshrub	Gypsovag	(2)5-50(85)	1.5	706	21
48	*Helianthemum violaceum* (Cav.) Pers.	*Cistaceae*	Subshrub	Gypsovag	(6)10-35(40)	(1.2)1.5(2)	50	NA
49	*Helichrysum stoechas* (L.) Moench	*Asteraceae*	Subshrub	Gypsovag	< 70	0.4-0.5 x 0.2-0.3	141	109
50	*Herniaria fruticosa* L.	*Caryophyllaceae*	Subshrub	Gypsophyte	< 30	1 x 0.6	289	1107
51	*Koeleria vallesiana* (Honck.) Gaudin	*Poaceae*	Graminoid	Gypsovag	10-40	0.4	122	5
52	*Launaea lanifera* Pau	*Asteraceae*	Subshrub	Gypsovag	< 50	0.5	1	7
53	*Linum suffruticosum* L.	*Linaceae*	Shrub	Gypsovag	< 180	2-3.4-1.1-1.7	3	8
54	*Lygeum spartum* L	*Poaceae*	Graminoid	Gypsovag	NA	10-15 x 5	495	NA
55	*Moricandia arvensis* (L.) DC.	*Cruciferae*	Subshrub	Gypsovag	< 65	1.2 x 0.8	NA	1
56	*Ononis tridentata* L.	*Fabaceae*	Shrub	Gypsophyte	< 150	1.8-2.5(3)	18	NA
57	*Plantago albicans* L.	*Plantaginaceae*	Subshrub	Gypsovag	(4)6-28(70)	2-3.5 x 1-1.5	580	20
58	*Polygala rupestris* Pourr.	*Polygalaceae*	Subshrub	Gypsovag	< 20	3-4 x 1.2-1.5	NA	NA
59	*Salsola vermiculata* L.	*Chenopodiaceae*	Shrub	Gypsovag	< 100	2	9	NA

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60	*Sedum sediforme* (Jacq.) Pau	*Crassulaceae*	Subshrub	Gypsovag	< 60	0.25	4	3
61	*Sideritis hirsuta* L.	*Labiatae*	Subshrub	Gypsovag	10-69	2.3-2.7 x 1.6-2	26	2
62	*Sonchus tenerrimus* L.	*Asteraceae*	Forb	Gypsovag	NA	2.8-3.2 x 0.6-0.9	NA	10
63	*Stipa lagascae* Roem. & Schult.	*Poaceae*	Graminoid	Gypsovag	50-60	4 x 1.5	538	23
64	*Stipa parviflora* Desf.	*Poaceae*	Graminoid	Gypsovag	50-60	2-5 x 0.3-0.5	30	NA
65	*Teucrium capitatum* L.	*Labiatae*	Subshrub	Gypsovag	(10)20-35(45)	1.4-2 x 0.8-1	12	5
66	*Thymus vulgaris* L.	*Labiatae*	Subshrub	Gypsovag	10-40	0.5-0.8	204	89
67	*Thymus zygis* L.	*Labiatae*	Subshrub	Gypsovag	10-30	0.4-0.8	111	NA

Growth habits classification taken from USDA-NRCS (https://plants.usda.gov/). Plant height and seed size were obtained mainly from “Flora Ibérica” (http://www.anthos.es/), “Herbario de Jaca” (http://floragon.ipe.csic.es/), and “Flora Vascular” (https://www.floravascular.com/), but in some cases, when there were not available data, seed size was estimated using graph paper (n=10). Abundance (total number of individuals) was recorded along six paralleled 250-m long transects arranged in the study site in May 2010. Seeds’ column refers to the total number of seeds recorded in the greenhouse for summer samples. NA = Not Available

Appendix 8 Species in La Lomaza de Belchite (Chapter 2)

Table A8.2 Height and canopy area of each of 25 individuals per target shrub randomly selected in the study site.

	G. struthium		***O. tridentata***		***H. squamatum***		***T. vulgaris***	
Individual	Height (m)	Area (m^2)	Height (m)	Area (m^2)	Height (m)	Area (m^2)	Height (m)	Area (m^2)
1	0.39	0.90	0.36	0.21	0.27	0.17	0.33	0.43
2	0.58	1.15	0.70	0.42	0.28	0.08	0.30	0.22
3	0.42	0.67	0.49	0.37	0.20	0.11	0.35	0.06
4	0.52	0.64	0.66	0.52	0.17	0.05	0.29	0.15
5	0.58	0.47	0.60	0.41	0.20	0.06	0.33	0.15
6	0.32	0.68	0.51	0.66	0.14	0.10	0.17	0.08
7	0.37	0.77	0.54	0.24	0.10	0.06	0.25	0.10
8	0.60	0.77	0.43	0.23	0.23	0.15	0.40	0.28
9	0.35	0.41	0.58	0.42	0.18	0.05	0.16	0.09
10	0.60	0.64	0.47	0.32	0.29	0.05	0.24	0.14
11	0.57	1.12	0.55	0.41	0.20	0.11	0.24	0.04
12	0.34	0.62	0.38	0.57	0.29	0.14	0.18	0.06
13	0.48	0.32	0.62	0.29	0.20	0.23	0.32	0.16
14	0.49	0.53	0.32	0.26	0.25	0.12	0.23	0.19
15	0.72	1.80	0.58	0.35	0.15	0.12	0.19	0.09
16	0.57	0.65	0.53	0.44	0.16	0.12	0.31	0.13
17	0.46	0.49	0.37	0.32	0.22	0.12	0.17	0.11
18	0.28	0.62	0.45	0.19	0.26	0.13	0.17	0.10
19	0.35	0.69	0.42	0.18	0.24	0.11	0.16	0.07
20	0.41	0.69	0.50	0.18	0.24	0.08	0.22	0.08
21	0.34	0.51	0.51	0.45	0.12	0.06	0.27	0.02
22	0.25	0.56	0.53	0.52	0.13	0.04	0.22	0.06
23	0.67	1.68	0.70	0.29	0.21	0.11	0.22	0.10
24	0.47	0.69	0.62	0.64	0.18	0.06	0.30	0.07
25	0.67	0.79	0.85	1.14	0.24	0.14	0.23	0.07
Average ± SE	0.47 ± 0.03	0.75 ± 0.07	0.53 ± 0.02	0.40 ± 0.04	0.21 ± 0.01	0.10 ± 0.01	0.25 ± 0.01	0.12 ± 0.02

Appendix 9 Samples germination in the greenhouse (Chapter 2)

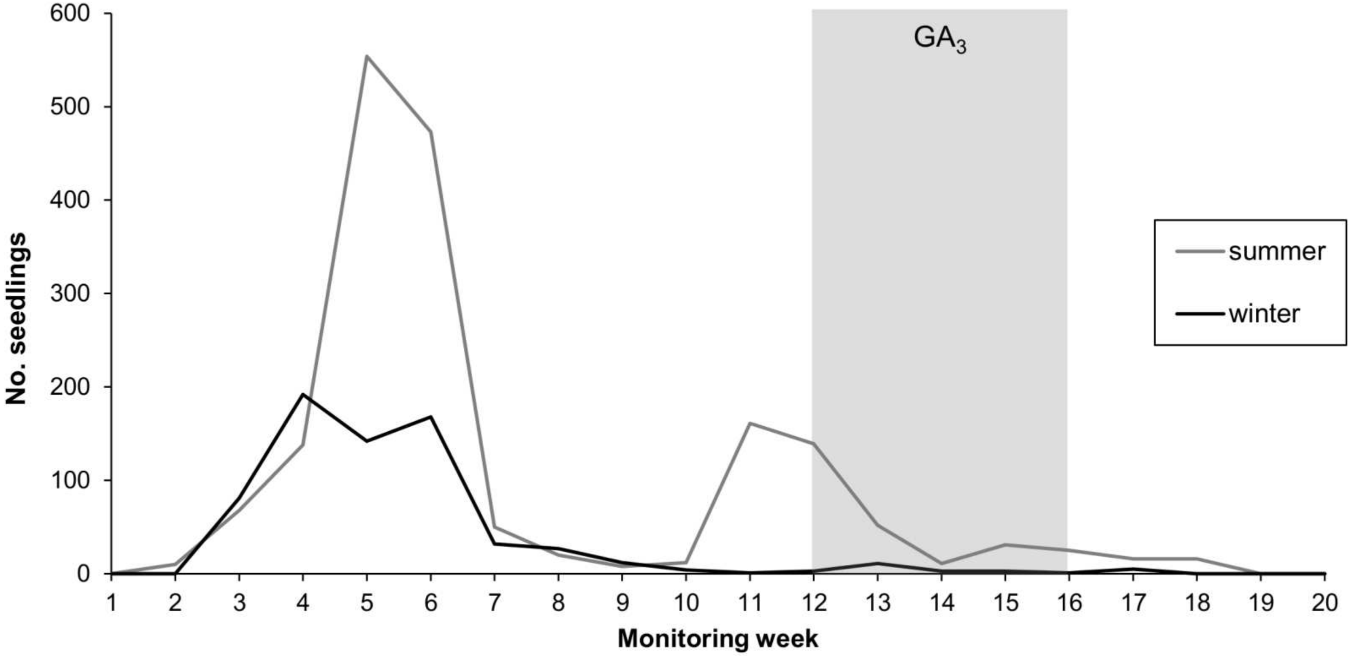

Figure A9.1 Total number of new seedlings (germinated seeds) recorded per monitoring week in winter and summer samples separately. Grey shading represents the period in which trays were irrigated with a gibberellic acid solution (GA$_3$).

Appendix 9 Samples germination in the greenhouse (Chapter 2)

Table A10.1 Results of GLMs to test the effect of the microsite, the target species and the plant height, and the interaction among variables on soil seed bank richness and abundance for annuals and perennials emerged in the persistent soil seed bank.

Response variables	Explanatory variables	DF	Deviance	p-value
Richness of annuals	Microsite	2	9.868	**<0.01**
	Target species	3	3.229	0.357
	Plant height	1	6.551	**<0.05**
	Microsite : Target species	6	9.105	0.168
	Microsite : Plant height	2	0.594	0.743
	Target species : Plant height	3	1.968	0.579
	Microsite : Target species : Plant height	6	0.579	0.997
Richness of perennials	Microsite	2	116.974	**<0.001**
	Target species	3	6.668	0.083
	Plant height	1	0.751	0.386
	Microsite : Target species	6	3.342	0.765
	Microsite : Plant height	2	0.261	0.878
	Target species : Plant height	3	7.935	**<0.05**
	Microsite : Target species : Plant height	6	5.120	0.529
Abundance of annuals	Microsite	2	12.084	**<0.01**
	Target species	3	9.633	**<0.05**
	Plant height	1	8.787	**<0.01**
	Microsite : Target species	6	11.704	0.069
	Microsite : Plant height	2	6.726	**<0.05**
	Target species : Plant height	3	3.431	0.330
	Microsite : Target species : Plant height	6	0.697	0.995
Abundance of perennials	Microsite	2	559.41	**<0.001**
	Target species	3	64.95	**<0.001**
	Plant height	1	9.67	**<0.01**
	Microsite : Target species	6	59.25	**<0.001**
	Microsite : Plant height	2	20.00	**<0.001**
	Target species : Plant height	3	28.02	**<0.001**
	Microsite : Target species : Plant height	6	23.68	**<0.001**

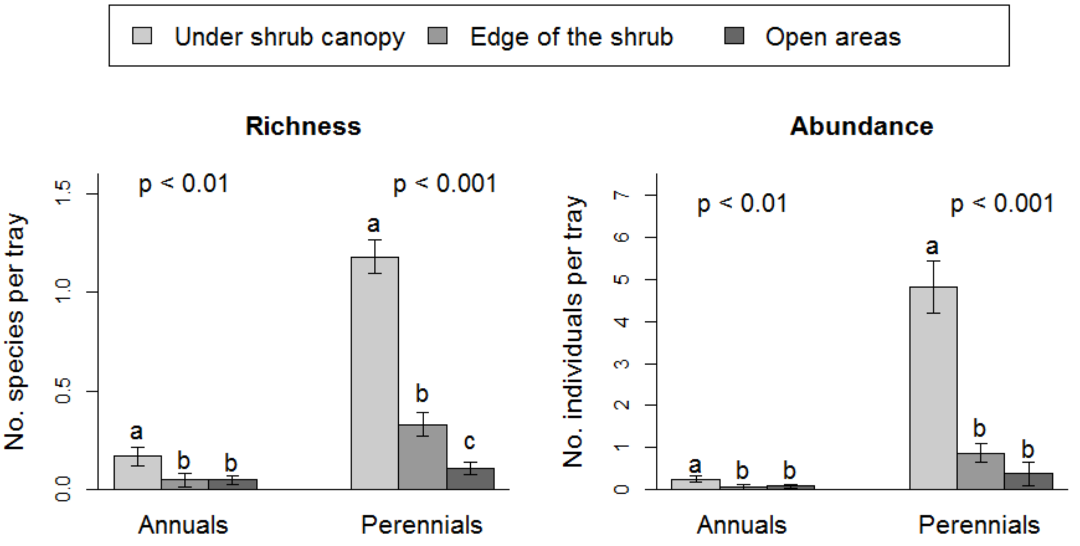

Figure A10.1 Mean richness and mean abundance per tray at each microsite (under shrub canopy, at the edge of the shrub and in open areas) in the persistent seed bank, for annuals and perennials separately. Different letters indicate significant differences after Tukey's multiple comparisons when significant effects of the microsite were found in GLMs with Poisson error distribution ($p \leq 0.05$).

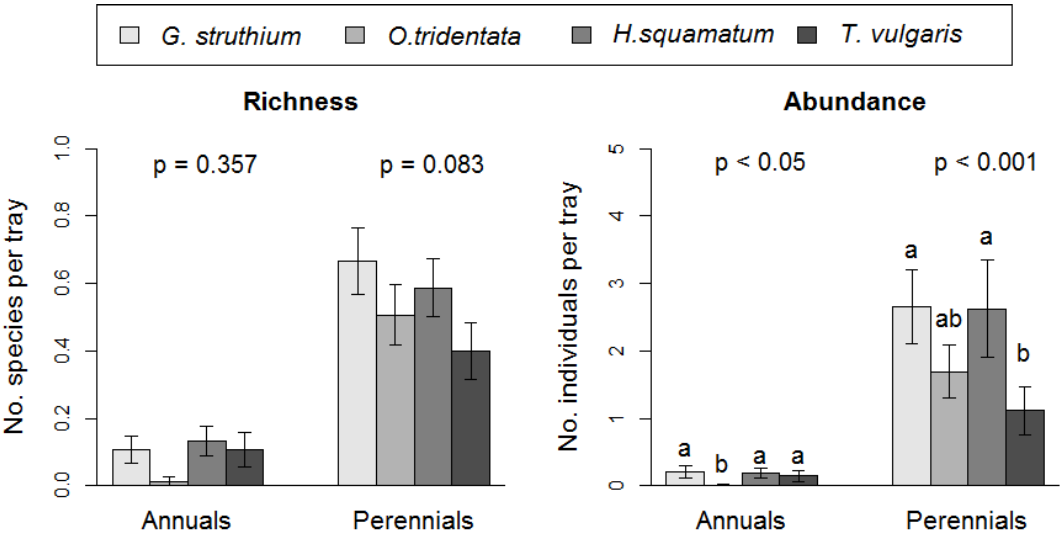

Figure A10.2 Mean richness and mean abundance per tray linked to each target species (*G. struthium O. tridentata*, *H. squamatum* and *T. vulgaris*) in the persistent seed bank, for annuals and perennials separately. Different letters indicate significant differences after Tukey's multiple comparisons when significant effects of the microsite were found in GLMs with Poisson error distribution (p≤0.05).

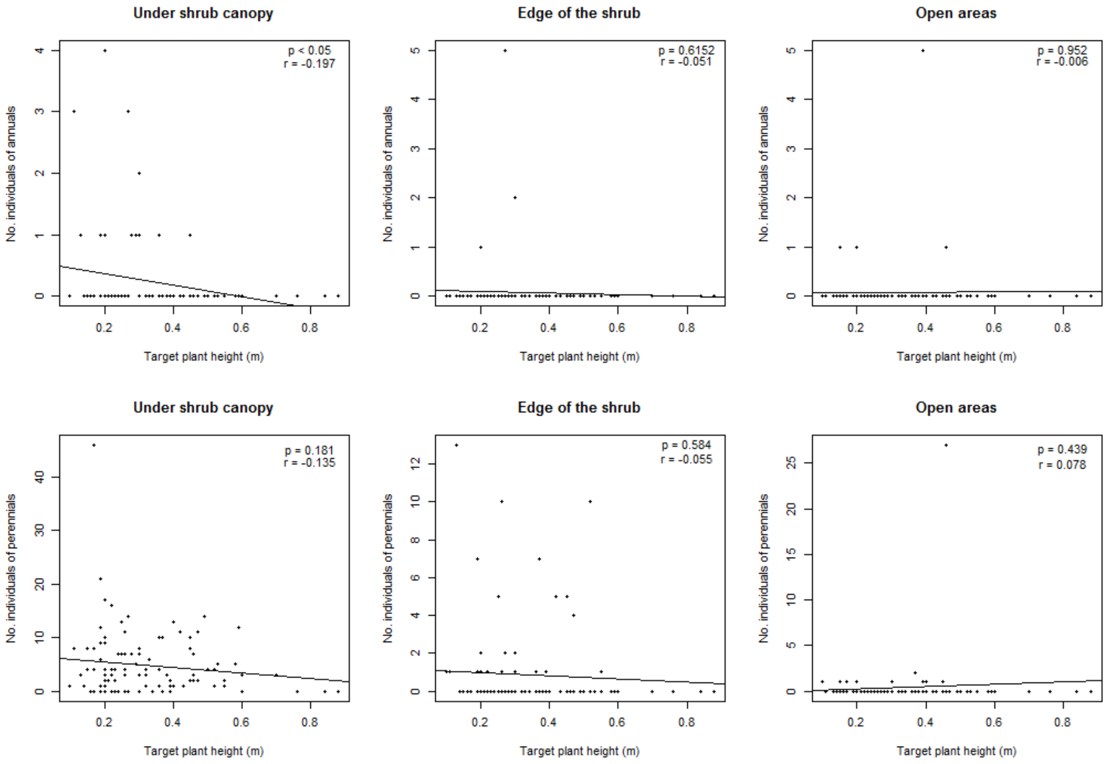

Figure A10.3 Correlations between richness and abundance of annuals and perennials and the plant height (m) per microsite in the persistent seed bank. p≤0.05 indicates significant effects of the plant height (r = Pearson's correlations).

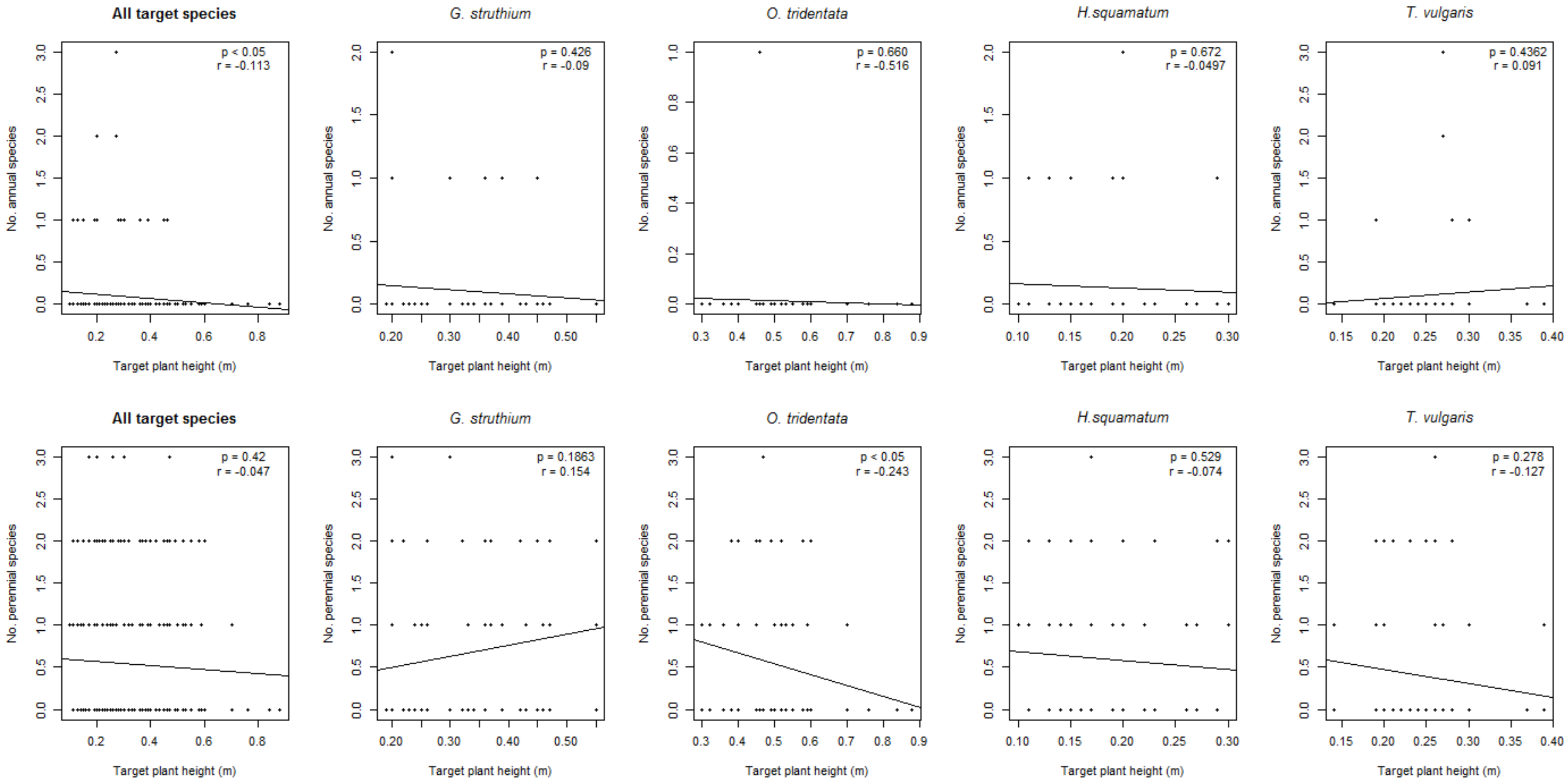

Figure A10.4 Correlations between richness and abundance of annuals and perennials and the plant height (m) per target species (*G. struthium*, *O. tridentata*, *H. squamatum* and *T. vulgaris*) in the persistent seed bank. p≤0.05 indicates significant effects of the plant height (r = Pearson's correlations).

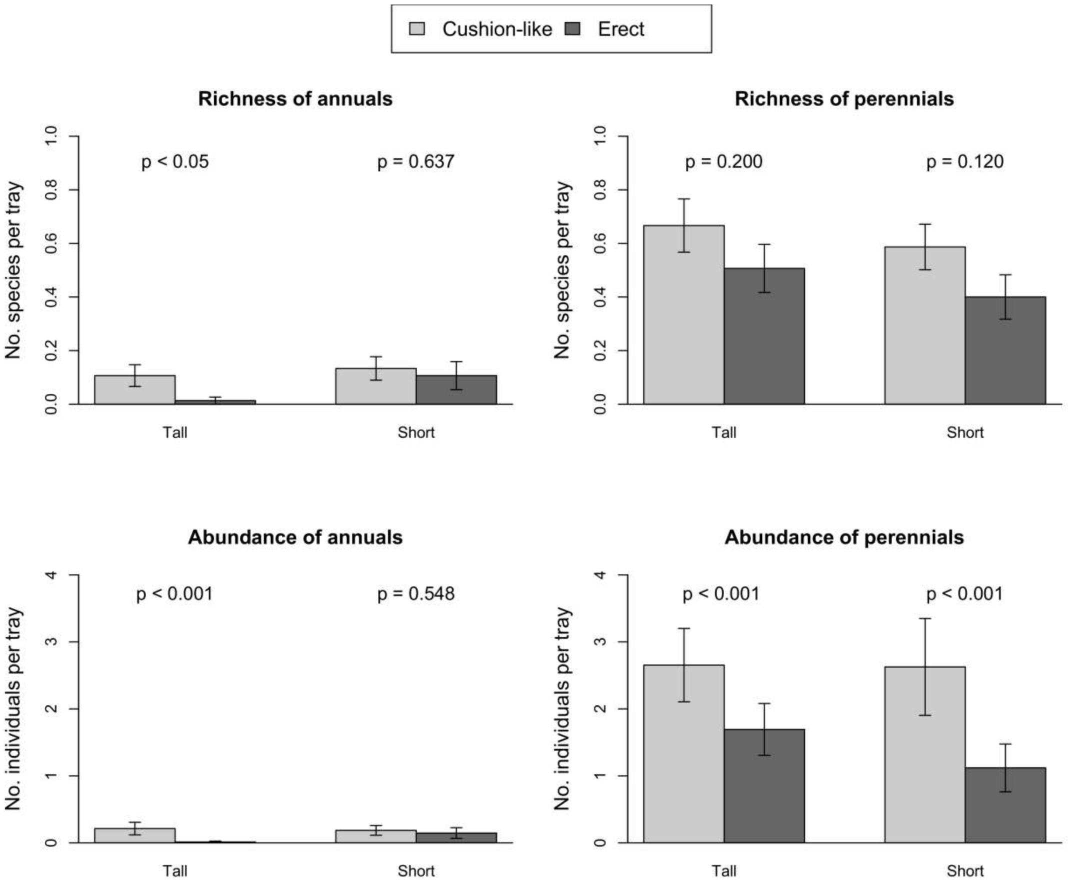

Figure A10.5 Mean richness and abundance per tray of annuals and perennials found in the persistent seed bank in shrubs with different architecture (cushion-like and erect) per size-type (tall and short). $p \leq 0.05$ indicates significant effects of the shrub architecture.

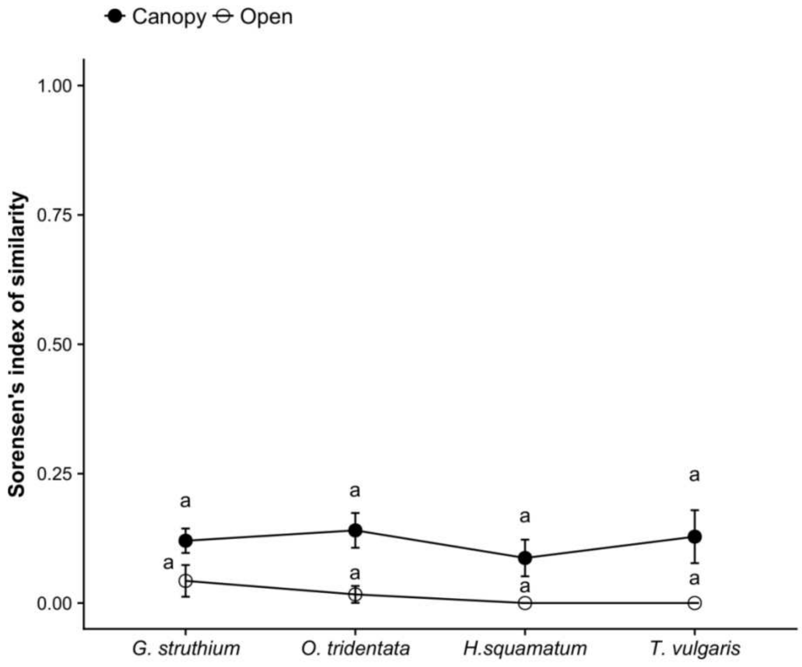

Figure A10.6 Mean Sorensen's index of similarity (SSI) in species composition between the persistent soil seed bank and the aboveground vegetation recorded under target species canopies (*G. struthium*, *O. tridentata*, *H. squamatum* and *T. vulgaris*) and in the paired open areas. We did not find significant differences among target species after Kruskal-Wallis multiple comparisons.

Appendix 10 Persistent soil seed bank (Chapter 2)

Appendix 11 Species in the soil seed bank versus species in above-ground vegetation (Chapter 2)

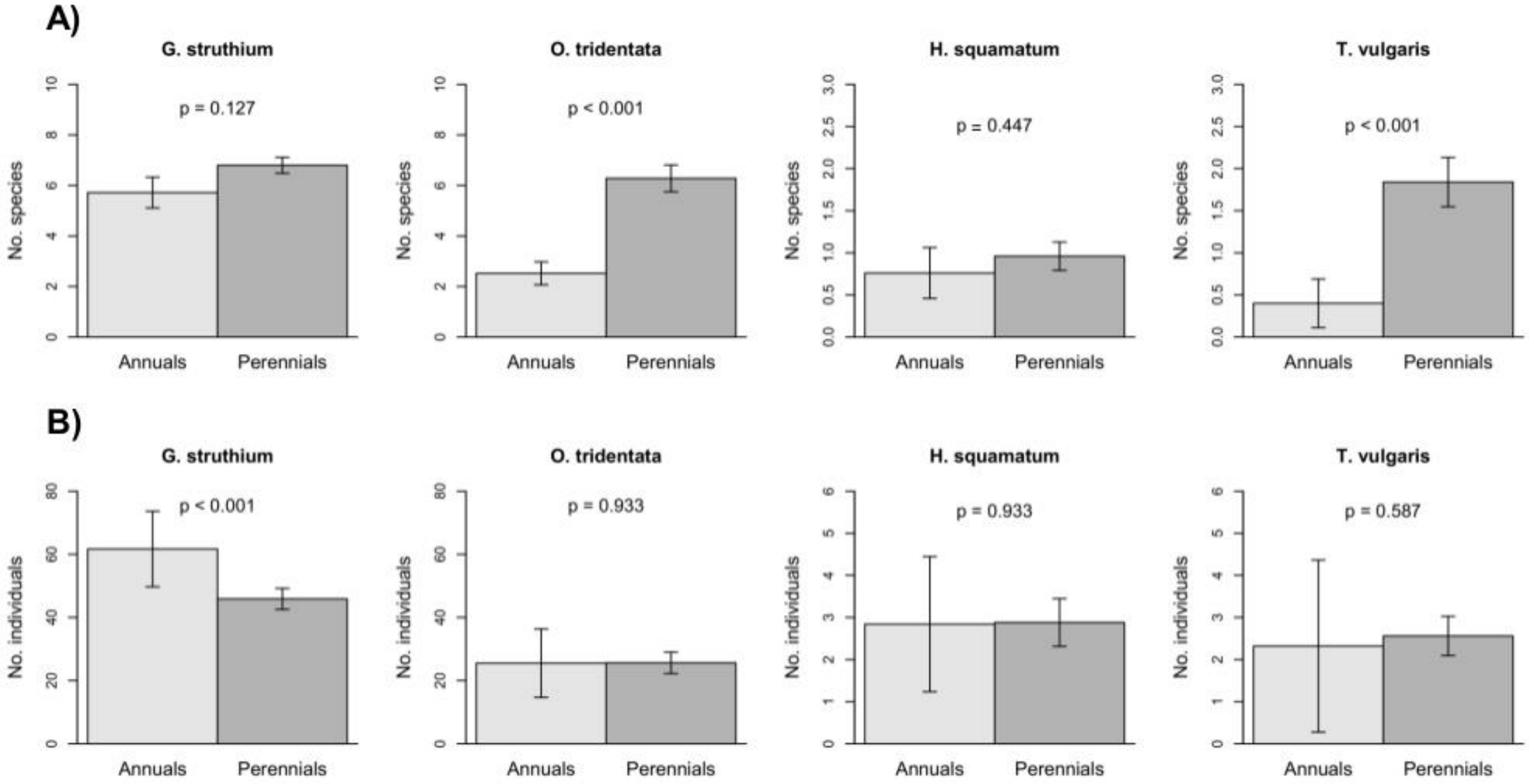

Figure A11.1 Bar plots representing mean A) species richness and B) abundance of annual species and perennial species recorded in the aboveground vegetation surveyed under target shrub canopies (rings survey). Significant differences ($p \leq 0.05$) between annuals and perennials were tested performing GLMs with Poisson error distribution and log link function.

Appendix 11 Species in the soil seed bank versus species in above-ground vegetation (Chapter 2)

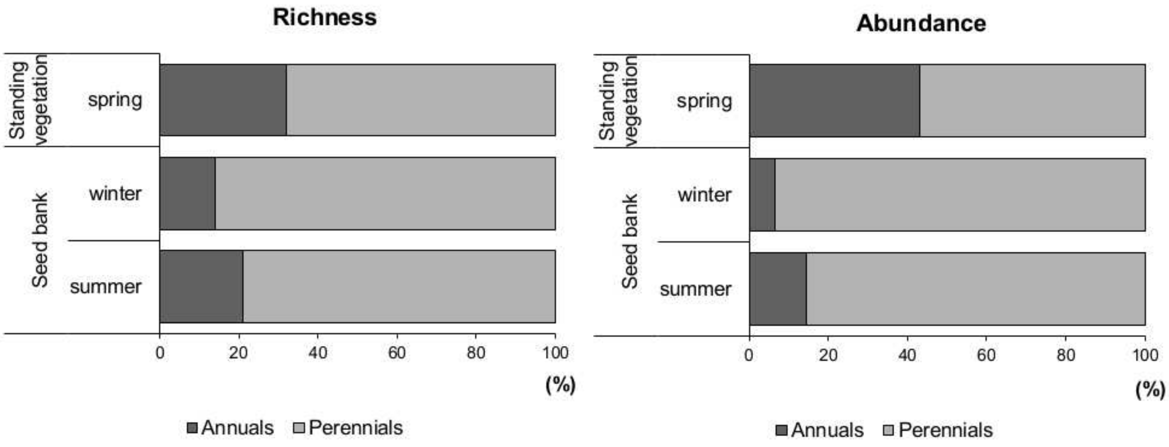

Figure A11.2 Percentage of annuals and perennials (of total richness and abundance) present in the study site as part of the soil seed bank and as part of the aboveground vegetation (rings surveys).

Appendices

Chapter 3

Realistic concentrations of extracts were determined considering the volume of water from rainfall that would pass through a surface area of live material equivalent to the area of a tray used in the greenhouse experiment (Orr et al. 2005). Water volume received in that surface (60 cm x 40 cm = 2400 cm^2) was calculated considering average annual rainfall in the study site (367 $mm \cdot year^{-1}$; Zuera 'Aspasa' meteorological station, 1973-2012 period; source: Gobierno de Aragón, http://opendata.aragon.es). Then, fresh leaves covering 2400 cm^2 of soil surface in the field were collected from five individuals of *C. clusii* and consequently fresh biomass was weighed using a 0.01 g precision balance. Thus, leaf extract concentration was determined using mean biomass of leaves per individual (81.19 g; Table S1) and mean water volume passing through a surface of 2400 cm^2 in the field (88.08 l). The resulting mass concentration of leaf extracts under natural conditions was approximately 1 $g \cdot l^{-1}$. We formulated root extracts to the same volumetric ratio as leaf extracts, considering that below-ground material occupies the same surface than above-ground material in *C. clusii* (Guerrero-Campo 1998). Roots were lighter than leaves, therefore, the resulting mass concentration in root extracts was lower than in leaf extracts.

T**able A12.1** Fresh weight of leaves collected from five individuals of *C. clusii* covering in natural communities.

Individual	**Plant height (cm)**	**Leaves biomass (g)**
Cistus 1	54	48.34
Cistus 2	72	99.83
Cistus 3	60	91.44
Cistus 4	45	62.67
Cistus 5	43	103.66
Mean	54.8	81.19

Appendix 13 Amelioration of micro-environmental conditions under the canopies of *Cistus clusii* and *Gypsophila struthium* ssp. *hispanica* (Chapter 3)

We measured soil compaction, soil humidity, and soil temperature under the canopy of 15 random individuals of *C. clusii*, 15 random individuals of *G. struthium* and at 15 random points in open patches (n=45) in spring. Soil compaction was measured as surface mechanical resistance ($kg \cdot cm^{-2}$) with a force gauge equipped with a compression plate with a diameter of 2 cm (MECMESIN Basic Force Gauge 500N). Soil humidity (6 cm maximum depth) was measured as the soil volumetric water content (%) with a ML3 ThetaProbe soil moisture sensor (Delta-T Devices). Finally, surface soil temperature (6 cm deep) was measured with a T-bar digital stem thermometer (ATM Ltd ST-9265A). In order to check the influence of plant size on soil physical properties, volume of all individuals was measured.

In addition, we collected soil samples under the canopy of five random individuals of *C. clusii*, five random individuals of *G. struthium* and at five random points in open patches (n=15). Soil samples were dried and sieved over a 2 mm mesh sieve before the analyses. We analyzed available phosphorous (P), total organic carbon (TOC), total carbon (C) and total nitrogen (N) in all soil samples. Available phosphorous of samples extracted with Bray nº1 reagent (Bray and Kurtz 1945) was estimated using a spectrometer (UNICAM 8625 UV/Vis Spectrometer) with the absorbance at 430 nm. To analyze total organic carbon, samples were first submitted to a chromatic acid digestion (Heanes 1984), and then this was estimated using a spectrometer (UNICAM 8625 UV/Vis Spectrometer) with the absorbance at 590 nm. Finally, soil samples were ground to a fine powder and then total carbon and total nitrogen were measured in a Vario MAX CN analyzer (Elementar Vario MAX CN).

To test the effect of the microsite on physical-chemical soil properties, ANCOVAs were performed considering plant volume as a covariate. Pairwise comparisons among target species were performed with Bonferroni correction for all the soil properties analyzed. Statistical analyses were performed in R (R Core Team 2017)

Appendix 13 Amelioration of micro-environmental conditions under the canopies of *Cistus clusii* and *Gypsophila struthium* ssp. *hispanica* (Chapter 3)

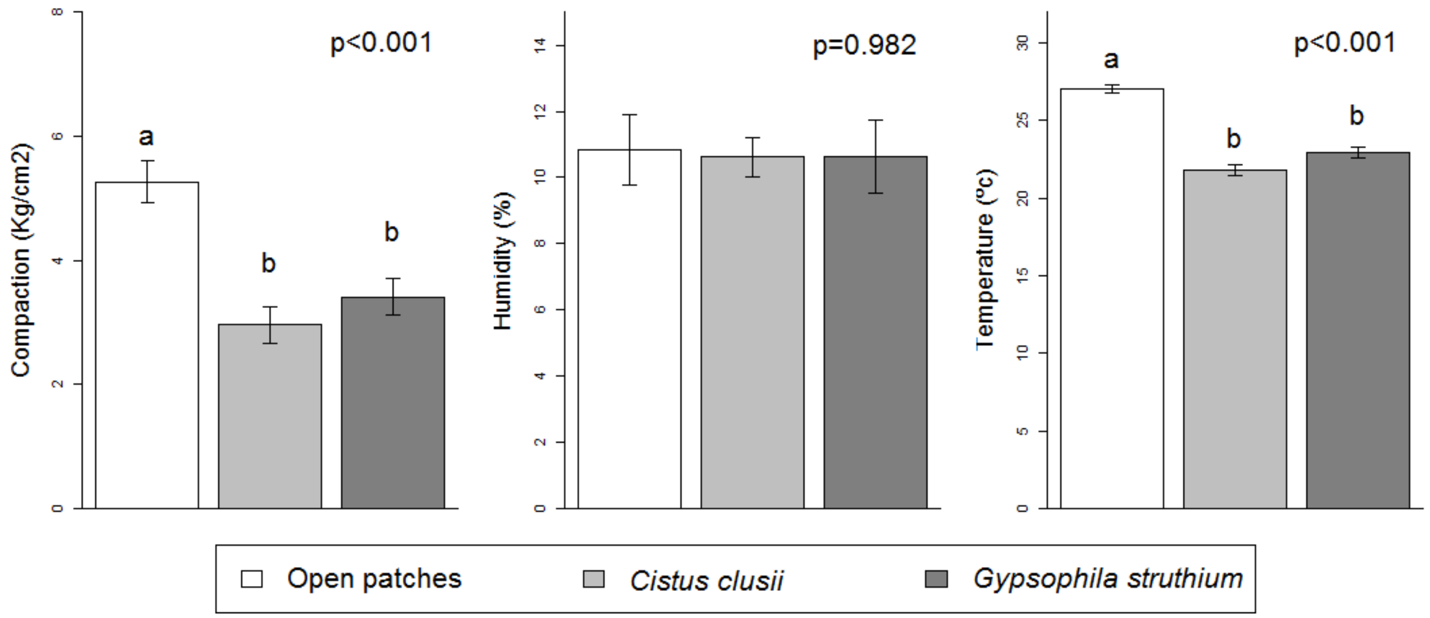

Figure A13.1. Mean soil compaction (Kg·cm^{-2}), soil humidity (% volumetric water content) and soil temperature (ºC) measured in different microsites: in open patches, under the canopy of *C. clusii* and under the canopy of *G. struthium*. The microsite effect was significant when $p<0.05$ in ANOVAs. Different letters indicate statistically significant differences among microsites after pairwise comparisons with Bonferroni correction.

Appendix 13 Amelioration of micro-environmental conditions under the canopies of *Cistus clusii* and *Gypsophila struthium* ssp. *hispanica* (Chapter 3)

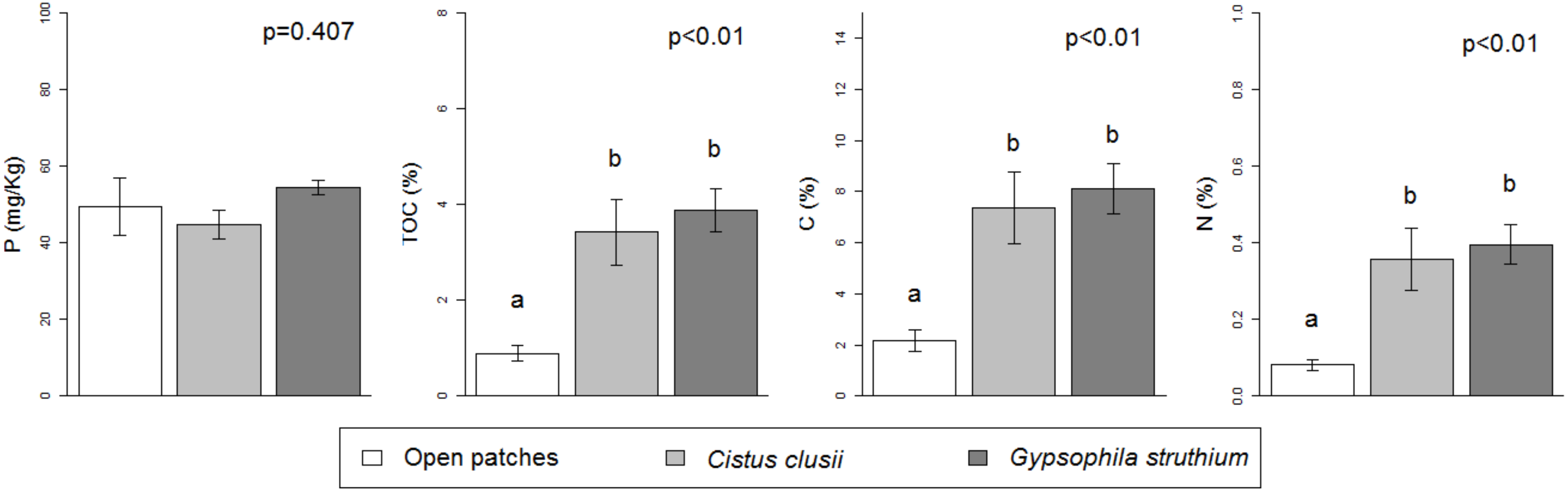

Figure A13.2. Mean values of available phosphorous (P), total organic carbon (TOC), total carbon (C) and total nitrogen (N) of soil samples collected from each microsite: in open patches, under the canopy of *C. clusii* and under the canopy of *G. struthium*. The microsite effect was significant when $p<0.05$ in ANOVAs. Different letters indicate statistically significant differences among target species after pairwise comparisons with Bonferroni correction.

Appendix 13 Amelioration of micro-environmental conditions under the canopies of *Cistus clusii* and *Gypsophila struthium* ssp. *hispanica* (Chapter 3)

Appendix 14 Effects of extract treatments on seedling biomass (Chapter 3)

Table A14.1. Mean values ± SE per extract treatment (C, L, R, RL) of the total biomass of the seedlings of the test species in the greenhouse experiment and summary of the LMMs implemented to test the effects of the extract treatments, the time since the germination time and the interaction among these factors on total biomass.

	Mean values ± SE				**Treatments**			**Time since germination**			**Treatments x time since germination**		
Test species	**C**	**L**	**R**	**RL**	**DF**	**F**	**p-value**	**DF**	**F**	**p-value**	**DF**	**F**	**p-value**
G. struthium	73.27 ± 6.58	110.69 ± 10.26	135.53 ± 14.14	90.19 ± 10.77	3	1.65	0.217	1	82.58	**<0.001**	3	2.23	0.086
H. squamatum	4.51 ± 0.85	3.92 ± 0.59	9.91 ± 3.33	5.12 ± 1.31	3	1.65	0.219	1	110.20	**<0.001**	3	1.40	0.256
H. syriacum	4.87 ± 0.55	8.35 ± 0.91	8.96 ± 1.54	6.78 ± 1.01	3	0.88	0.471	1	43.67	**<0.001**	3	0.02	0.995
T. vulgaris	6.25 ± 1.86	16.04 ± 2.18	21.72 ± 5.14	17.11 ± 5.44	3	0.88	0.473	1	46.99	**<0.001**	3	1.44	0.245
H. stoechas	6.85 ± 1.24	15.22 ± 2.57	13.75 ± 4.41	10.86 ± 4.45	3	1.24	0.332	1	2.29	0.134	3	0.99	0.407
L. suffruticosum	3.94 ± 0.73	5.38 ± 0.63	7.97 ± 2.24	4.44 ± 0.62	3	1.07	0.389	1	41.77	**<0.001**	3	2.52	0.066
S. lagascae	13.15 ± 7.96	10.34 ± 4.41	10.72 ± 3.57	2.46 ± 0.21	3	0.64	0.601	1	172.65	**<0.001**	3	0.23	0.875
C. clusii	3.61 ± 0.67	5.01 ± 0.80	8.52 ± 1.99	4.24 ± 1.28	3	0.33	0.805	1	374.91	**<0.001**	3	1.39	0.248

C: control, L: leaf extract, R: root extract, RL: root and leaf extract

Appendix 14 Effects of extract treatments on seedling biomass (Chapter 3)

Table A14.2 Mean values ± SE per extract treatment (C, L, R, RL) of the below-ground/above-ground biomass ratio of the seedlings of the test species in the greenhouse experiment and summary of the LMMs implemented to test the effects of the extract treatments, the time since the germination time and the interaction among these factors on below-ground/above-ground biomass ratio.

	Mean values ± SE				**Treatments**			**Time since germination**			**Treatments x time since germination**		
Test species	**C**	**L**	**R**	**RL**	**DF**	**F**	**p-value**	**DF**	**F**	**p-value**	**DF**	**F**	**p-value**
G. struthium	0.19 ± 0.01	0.17 ± 0.01	0.19 ± 0.02	0.18 ± 0.01	3	0.41	0.752	1	1.87	0.173	3	1.91	0.130
H. squamatum	0.18 ± 0.02	0.20 ± 0.04	0.22 ±0.02	0.21 ± 0.02	3	1.65	0.219	1	110.20	**<0.001**	3	1.40	0.256
H. syriacum	0.28 ± 0.02	0.26 ± 0.02	0.24 ± 0.01	0.25 ± 0.02	3	0.88	0.470	1	43.67	**<0.001**	3	0.02	0.995
T. vulgaris	0.34 ± 0.04	0.27 ± 0.03	0.23 ± 0.02	0.27 ± 0.03	3	0.88	0.473	1	46.99	**<0.001**	3	1.44	0.245
H. stoechas	0.26 ± 0.02	0.23 ± 0.02	0.24 ± 0.02	0.25 ± 0.04	3	1.24	0.332	1	2.29	0.138	3	0.99	0.407
L. suffruticosum	0.36 ± 0.03	0.30 ± 0.02	0.31 ± 0.03	0.29 ± 0.04	3	1.07	0.389	1	41.76	**<0.001**	3	2.52	0.066
S. lagascae	0.48 ± 0.03	0.51 ± 0.03	0.43 ± 0.03	0.46 ± 0.03	3	0.64	0.601	1	172.65	**<0.001**	3	0.23	0.875
C. clusii	0.26 ± 0.02	0.23 ± 0.01	0.25 ± 0.02	0.26 ± 0.02	3	0.33	0.805	1	374.91	**<0.001**	3	1.39	0.248

C: control, L: leaf extract, R: root extract, RL: root and leaf extract

Appendix 15 Chemical analyses of leaf and root extracts (Chapter 3)

Chemical compositions of *C. clusii* leaves and roots were analysed by gas chromatography coupled with mass spectrometry on a Shimadzu GCMS-QP2010 with auto-sampler. The extracts were prepared by individually soaking 0.003 g of chopped leaves and 0.003 g of chopped roots in 1 ml of methanol for 24 hours at room temperature in darkness. Methanol was used as polar solvent to extract water-soluble compounds (Sokmen et al. 1999), which might be present in the aqueous extracts used in the experiment.

The GC-MS was equipped with a Supelco Omegawax 320 capillary column (length 30 m, inner diameter 0.32 mm, film thickness of 0.25 µm; Sigma Aldrich, Stockholm, Sweden). Samples (1 µl) were injected in split mode with a split ratio of 10:1. Helium was used as a carrier gas with a total flow of 22.7 $ml \cdot min^{-1}$ and a column flow at 1.79 $ml \cdot min^{-1}$. The injection temperature was 220°C and the oven programmed as follows: initial temperature of 50 °C for 2 min and then increased to 240 °C with a rate of 20 $°C \cdot min^{-1}$. The mass spectrometer was operated in electron ionization mode at 70 eV. The ion source temperature was 250 °C. Individual compounds were identified by comparing the obtained mass spectra results to known standards.

Appendix 15 Chemical analyses of leaf and root extracts (Chapter 3)

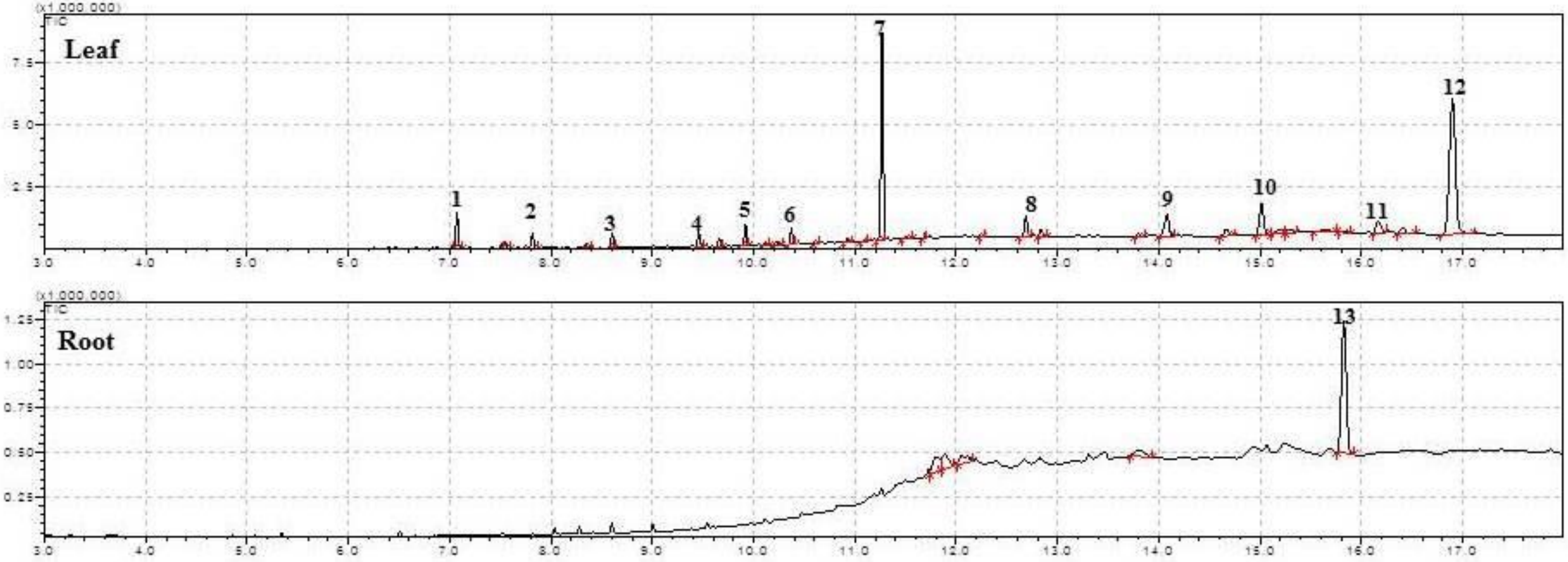

Figure A15.1 Chromatograms of the compounds from *C. clusii* leaves and roots extracted with methanol analyzed by GC-MS. Only the biggest peaks are represented: (1) Bornyl acetate; (2) Linderol; (3) Ionene; (4) Caryophyllene oxide; (5) Viridiflorol; (6) Cubenol; (7) Manoyl oxide; (8) 1-Bromotriacontane; (9) n-Tetratetracontane; (10) Oxomanoyl oxide; (11) Rhododendrol; (12) Pregnane-3,17,20-triol, (3 alpha-5 beta, 20S); (13) 2,3-Oxidosqualene.

Appendix 15 Chemical analyses of leaf and root extracts (Chapter 3)

Table A15.1 List of compounds extracted from *C. clusii* leaves and roots. The molecular formula, molecular weight, the nature of the compound, the retention time, and the peak area are shown for each compound.

Plant part	Name of the compound	Formula	Mol. weight	Nature of the compound	Ret. time	Peak area
Leaf	Bornyl acetate	$C_{12}H_{20}O_2$	196	Terpene	7.1	490,915
	Linderol	$C_{10}H_{18}O$	154	Terpene	7.8	382,273
	Ionene	$C_{13}H_{18}$	174	Terpene	8.6	276,425
	Caryophyllene oxide	$C_{15}H_{24}O$	220	Terpene	9.5	63,540
	Viridiflorol	$C_{15}H_{26}O$	222	Terpene	9.9	82,184
	Cubenol	$C_{15}H_{26}O$	222	Terpene	10.4	85,235
	Manoyl oxide	$C_{20}H_{34}O$	290	Terpene	11.3	1,227,866
	1-Bromotriacontane	$C_{30}H_{61}Br$	500	Haloalkane	12.7	178,859
	n-Tetratetracontane	$C_{44}H_{90}$	618	Aliphatic alkane	14.1	295,447
	Oxomanoyl oxide	$C_{20}H_{32}O_2$	304	Terpene	15.0	316,269
	Rhododendrol	$C_{10}H_{14}O_2$	166	Phenolic compound	16.2	376,334
	Pregnane-3,17,20-triol, (3α, 5β, 20S)	$C_{21}H_{36}O_3$	336	Steroid	16.9	1,716,398
Root	2,3-Oxidosqualene	$C_{30}H_{50}O$	426	Terpene	15.8	837,645

Table A15.2 Potential phytotoxic effects of the compounds extracted from *C. clusii* leaves and roots. Phytotoxic effects are not attributable to the compound in isolation, but to the extracts containing the compound.

Name of the compound	Potential phytotoxic effects	Found in	References
Bornyl acetate	Inhibit germination and control seedling growth	*Cistus ladanifer* L.	Verdeguer et al. (2012)
Linderol	Not tested	*Thymus vulgaris* L.	TSAI et al. (2011)
Ionene	Affect germination and initial radical elongation	*Salvia multicaulis* Vahl. var. *simplicifolia* Boiss.	Mancini et al. (2009)
Caryophyllene oxide	Inhibit radicle elongation	*Anisomeles indica* (L.) Kuntze	Batish et al. (2012)
Viridiflorol	Inhibit germination and control seedling growth	*Cistus ladanifer* L.	Verdeguer et al. (2012)
Cubenol	Inhibit germination and growth of the roots	*Schinus molle* L.	Simionatto et al. (2011)
Manoyl oxide	Not tested	*Cistus salvifolius* L., *C. creticus* L.	Mastino et al. (2017)
1-Bromotriacontane	Not tested	*Jatropha curcas* L.	Mahalakshmi et al. (2016)
n-Tetratetracontane	Not tested	*Leea indica* (Burm. f.) Merr.	Srinivasan et al. (2008)
Oxomanoyl oxide	Not tested	*Pinus banksiana* Lamb.	Conner and Rowe (1977)
Rhododendrol	Inhibit germination and seedling growth	*Rhododendron catawbiense* Michx., *R. maximum* L.	Gant (1978)
Pregnane-3,17,20-triol, (3α, 5β, 20S)	-	-	-
2,3-Oxidosqualene	Not tested	*Arabidopsis thaliana* (L.) Heynh.	Husselstein–Muller et al. (2001)

Appendices

Chapter 4

Appendix 16 Study sites characteristics (Chapter 4)

Table A16.1 Species list recorded from the field surveys carried out in May 2015 in undisturbed gypsum plant communities in the surroundings of the restored gypsum mine.

Species	**Family**
Aegilops geniculata Roth.	*Poaceae*
Agropyron cristatum (L.) Gaertn.	*Poaceae*
Asphodelus fistulosus L.	*Liliaceae*
Asterolinon linum-stellatum (L.) Duby	*Primulaceae*
Bromus rubens L.	*Poaceae*
Centaurea melitensis L.	*Asteraceae*
Cerastium pumilum Curtis	*Caryophyllaceae*
Chaenorrhinum rubrifolium (Robill. & Castagne ex DC.) Fourr.	*Scrophulariaceae*
Cistus clusii Dunal	*Cistaceae*
Coris monspeliensis L.	*Primulaceae*
Desmazeria rigida (L.) Tutin	*Poaceae*
Echium vulgare L.	*Boraginaceae*
Ephedra fragilis Desf	*Ephedraceae*
Erodium cicutarium (L.) L'Hér.	*Geraniaceae*
Eruca vesicaria (L.) Cav.	*Brassicaceae*
Filago pyramidata L.	*Asteraceae*
Fumana ericoides (Cav.) Gand.	*Cistaceae*
Galium verrucosum Huds.	*Rubiaceae*
Genista scorpius (L.) DC.	*Fabaceae*
Gypsophila struthium Loefl. subsp. *hispanica* (Willk.) G.López	*Caryophyllaceae*
Helianthemum marifolium (L.) Mill.	*Cistaceae*
Helianthemum squamatum (L.) Pers.	*Cistaceae*
Helianthemum syriacum (Jacq.) Dum. Cours.	*Cistaceae*
Helianthemum violaceum (Cav.) Pers.	*Cistaceae*
Helichrysum stoechas (L.) Moench	*Asteraceae*
Herniaria fruticosa L.	*Caryophyllaceae*
Hippocrepis ciliata Willd.	*Fabaceae*
Hordeum murinum L.	*Poaceae*
Koeleria vallesiana (Honck.) Gaudin	*Poaceae*
Launaea pumila (Cav.) Kuntze	*Asteraceae*
Launea lanifera Pau	*Asteraceae*
Lepidium subulatum L.	*Brassicaceae*
Linum strictum L.	*Linaceae*
Linum suffruticosum L.	*Linaceae*
Lithodora fruticosa (L.) Griseb.	*Boraginaceae*
Lygeum spartum L.	*Poaceae*
Matthiola fruticulosa (L.) Maire	*Brassicaceae*
Peganum harmala L.	*Zygophyllaceae*
Polygala rupestris Pourr.	*Polygalaceae*
Reseda stricta Pers.	*Resedaceae*
Rosmarinus officinalis L.	*Lamiaceae*
Scorzonera laciniata L.	*Asteraceae*
Senecio auricula Bourg. ex Coss.	*Asteraceae*
Sonchus tenerrimus L.	*Asteraceae*
Stipa parviflora Desf.	*Poaceae*
Teucrium capitatum L.	*Lamiaceae*
Teucrium gnaphalodes L'Hér.	*Lamiaceae*
Thapsia villosa L.	*Umbelliferae*
Thymus vulgaris L.	*Lamiaceae*
Trigonella monspeliaca L.	*Asteraceae*

Appendix 16 Study sites characteristics (Chapter 4)

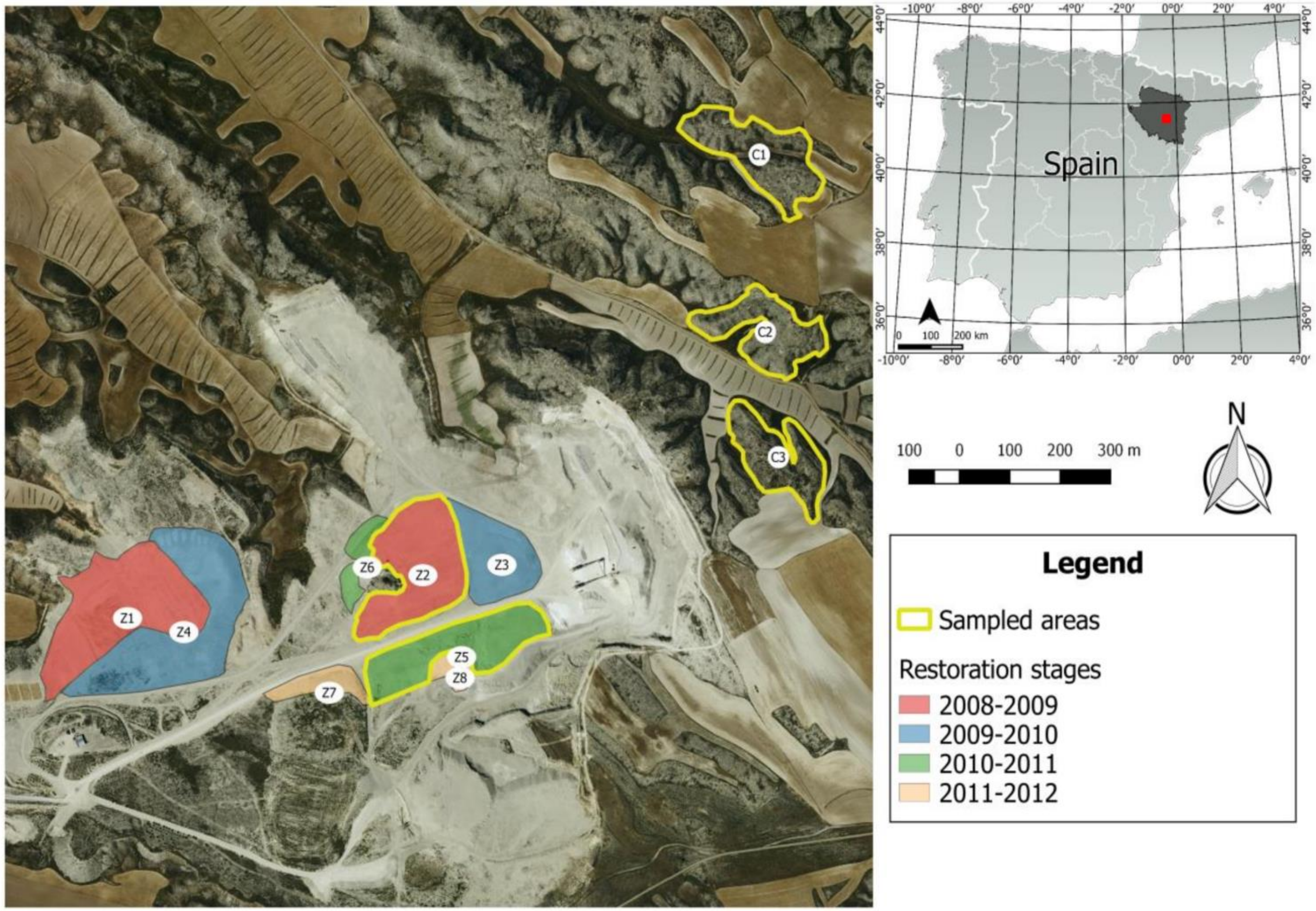

Figure A16.1 Location of the restored gypsum mine and detail of the plots restored in different phases. The surveyed plots are highlighted with the contour in yellow.

Figure A16.2 General view of the experimental plantation in the gypsum spoil dump. The shrub is *Gypsophila struthium*

Appendix 16 Study sites characteristics (Chapter 4)

Appendix 17 Species spatial associations with *G. struthium* (Chapter 4)

Table A17.1 Total frequency of each species recorded in the restored plots (R1 and R2) and the undisturbed surrounding habitat (C) and the observed and expected frequencies in which the species appeared associated either with *G. struthium* or with other shrubs

		f_t			***G. struthium*** f_o (f_e)			**Other shrubs** f_o (f_e)		
Species		**R1**	**R2**	**C**	**R1**	**R2**	**C**	**R1**	**R2**	**C**
G. struthium		720	1458	127	-	-	-	-	-	-
Other shrubs*		94	17	348						
Perennial species										
Agropyron cristatum (L.) Gaertn.	Grass	24	4	72	3(2.6)	1(0.9)	0(1.4)	1(0.3)	0(0)	0(3.7)$^{-}$
Avenula bromoides (Gouan) H. Scholz	Ruderal grass	48	0	0	2(5.1)	0(0)	0(0)	3(0.7)$^{+}$	0(0)	0(0)
Brachypodium retusum (Pers.) P. Beauv.	Grass	2	1	0	0(0.2)	1(0.2)	0(0)	0(0)	0(0)	0(0)
Carduus tenuiflorus Curtis	Ruderal herb	6	6	0	0(0.6)	1(1.3)	0(0)	0(0.1)	0(0)	0(0)
Cistus clusii Dunal	Shrub	0	0	26	0(0)	0(0)	0(0.5)	0(0)	0(0)	1(1.3)
Convolvulus arvensis L.	Ruderal herb	0	9	0	0(0)	0(1.9)	0(0)	0(0)	0(0)	0(0)
Convolvulus lineatus L.	Ruderal herb	6	14	0	1(0.6)	0(3.0)$^{-}$	0(0)	0(0.1)	0(0)	0(0)
Coris monspeliensis L.	Dwarf shrub	7	67	394	0(0.7)	12(14.5)	5(7.4)	0(0.1)	0(0.2)	15(20.3)$^{-}$
Dittrichia viscosa (L.) Greuter	Ruderal herb	396	43	0	39(42.2)	6(9.3)	0(0)	1(5.5)$^{-}$	0(0.1)	0(0)
Echium vulgare L.	Ruderal herb	2	9	1	0(0.2)	1(1.9)	0(0)	0(0)	1(0)$^{+}$	0(0.1)
Ephedra fragilis Desf.	Shrub	0	0	25	0(0)	0(0)	1(0.5)	0(0)	0(0)	0(1.3)
Fumana ericoides (Cav.) Gand.	Dwarf shrub	0	0	1	0(0)	0(0)	0(0)	0(0)	0(0)	1(0.1)$^{+}$
Genista Scorpius (L.) DC.	Shrub	10	6	169	2(1.1)	0(1.3)	0(3.2)$^{-}$	0(0.1)	0(0)	0(8.7)$^{-}$
Helianthemum marifolium (L.) Mill.	Dwarf shrub	0	0	3	0(0)	0(0)	0(0.1)	0(0)	0(0)	0(0.2)
Helianthemum squamatum (L.) Pers.	Dwarf shrub	72	168	773	7(7.7)	21(36.3)$^{-}$	13(14.5)	2(1.0)	0(0.4)	42(39.8)$^{+}$
Helianthemum syriacum (Jacq.) Dum. Cours.	Dwarf shrub	26	48	474	2(2.8)	8(10.4)	25(8.9)$^{+}$	1(0.4)	0(0.1)	25(24.4)
Helianthemum violaceum (Cav.) Pers.	Dwarf shrub	5	2	13	0(0.5)	0(0.4)	0(0.2)	0(0.1)	0(0)	0(0.7)
Helichrysum stoechas (L.) Moench.	Dwarf shrub	42	28	467	3(4.5)	5(6.0)	7(8.8)	0(0.6)	1(0.1)	21(24.1)
Herniaria fruticosa L.	Dwarf shrub	125	73	518	11(13.3)	7(15.8)$^{-}$	4(9.7)$^{-}$	2(1.7)	0(0.2)	23(26.7)
Koeleria vallesiana (Honck.) Gaudin	Grass	6	0	144	1(0.6)	0(0)	4(2.7)	0(0.1)	0(0)	10(7.4)
Launaea lanífera Pau	Dwarf shrub	47	25	10	2(5.0)	3(5.4)	0(0.2)	1(0.7)	0(0.1)	0(0.5)
Launaea pumila (Cav.) Kuntze	Dwarf shrub	0	0	31	0(0)	0(0)	0(0.6)	0(0)	0(0)	3(1.6)
Lepidium subulatum L.	Dwarf shrub	175	1360	57	11(18.7)$^{-}$	91(293.5)$^{-}$	1(1.1)	6(2.4)$^{+}$	1(3.4)	7(2.9)$^{+}$
Limonium sp.	Dwarf shrub	0	1	0	0(0)	0(0.2)	0(0)	0(0)	0(0)	0(0)
Linum suffruticosum L.	Dwarf shrub	0	0	3	0(0)	0(0)	0(0.1)	0(0)	0(0)	0(0.2)

Appendix 17 Species spatial associations with *G. struthium* (Chapter 4)

Lithodora fruticosa (L.) Griseb.	Dwarf shrub	9	9	247	0(1.0)	1(1.9)	2(4.6)	0(0.1)	0(0)	12(12.7)
Lolium perenne L.	Ruderal grass	299	112	0	22(31.9)$^{-}$	31(24.2)$^{+}$	0(0)	7(4.2)	3(0.3)$^{+}$	0(0)
Lygeum spartum L.	Grass	45	534	272	1(4.8)$^{-}$	47(115.3)$^{-}$	4(5.1)	1(0.6)	0(1.3)	2(14)$^{-}$
Matthiola fruticulosa (L.) Maire	Dwarf shrub	6	13	10	2(0.6)	2(2.8)	0(0.2)	0(0.1)	0(0)	0(0.5)
Onopordum nervosum Boiss.	Ruderal herb	4	10	0	1(0.4)	5(2.2)$^{+}$	0(0)	0(0.1)	0(0)	0(0)
Peganum harmala L.	Dwarf shrub	0	0	9	0(0)	0(0)	0(0.2)	0(0)	0(0)	0(0.5)
Piptatherum miliaceum (L.) Coss.	Ruderal grass	41	24	0	5(4.4)	13(5.2)$^{+}$	0(0)	1(0.6)	0(0.1)	0(0)
Plantago albicans L.	Dwarf shrub	22	4	0	0(2.3)	1(0.9)	0(0)	0(0.3)	0(0)	0(0)
Polygala rupestris Pourr.	Dwarf shrub	0	1	40	0(0)	0(0.2)	5(0.8)$^{+}$	0(0)	0(0)	10(2.1)$^{+}$
Reseda stricta Pers.	Herb	63	7	14	7(6.7)	2(1.5)	0(0.3)	0(0.9)	0(0)	2(0.7)
Rosmarinus officinalis L.	Shrub	0	0	128	0(0)	0(0)	0(2.4)	0(0)	0(0)	1(6.6)$^{-}$
Salsola genistoides Juss. ex Poir.	Shrub	2	0	0	0(0.2)	0(0)	0(0)	0(0)	0(0)	0(0)
Salsola vermiculata L.	Shrub	82	11	0	2(8.7)$^{-}$	0(2.4)	0(0)	0(1.1)	0(0)	0(0)
Salvia lavandulifolia Vahl	Dwarf shrub	0	1	0	0(0)	0(0.2)	0(0)	0(0)	0(0)	0(0)
Scorzonera laciniata L.	Ruderal herb	145	76	4	6(15.5)$^{-}$	13(16.4)	0(0.1)	0(2)	0(0.2)	2(0.2)$^{+}$
Senecio auricula Bourg. ex Coss.	Herb	0	0	27	0(0)	0(0)	1(0.5)	0(0)	0(0)	0(1.4)
Sonchus tenerrimus L.	Ruderal herb	974	504	1	148(103.8)$^{+}$	178(108.8)$^{+}$	0(0)	23(13.6)$^{+}$	3(1.3)	0(0.1)
Stipa parviflora Desf.	Grass	194	402	723	11(20.7)$^{-}$	87(86.8)$^{+}$	29(13.6)$^{+}$	4(2.7)	0(1)	59(37.2)$^{+}$
Teucrium capitatum L.	Dwarf shrub	11	17	96	1(1.2)	2(3.7)	3(1.8)	0(0.2)	0(0)	3(4.9)
Teucrium gnaphalodes L'Hér.	Dwarf shrub	0	0	3	0(0)	0(0)	1(0.1)$^{+}$	0(0)	0(0)	0(0.2)
Thapsia villosa L.	Ruderal herb	0	1	5	0(0)	0(0.2)	1(0.1)	0(0)	0(0)	0(0.3)
Thymus vulgaris L.	Dwarf shrub	2	0	523	0(0.2)	0(0)	16(9.8)$^{+}$	0(0)	0(0)	26(26.9)
Annual species										
Aegilops geniculata Roth.	Grass	0	0	8	0(0)	0(0)	1(0.2)	0(0)	0(0)	0(0.4)
Ajuga chamaepitys (L.) Schreb.	Herb	1	0	0	0(0.1)	0(0)	0(0)	0(0)	0(0)	0(0)
Anacyclus clavatus (Desf.) Pers.	Ruderal herb	20	3	0	1(2.1)	0(0.6)	0(0)	0(0.3)	0(0)	0(0)
Asphodelus fistulosus L.	Ruderal herb	0	0	2	0(0)	0(0)	0(0)	0(0)	0(0)	0(0.1)
Asterolinon linum-stellatum (L.) Duby	Herb	11	0	35	0(1.2)	0(0)	1(7)	0(0.2)	0(0)	1(1.8)
Astragalus stella Gouan	Herb	5	2	0	0(0.5)	0(0.4)	0(0)	0(0.1)	0(0)	0(0)
Bromus diandrus Roth.	Ruderal grass	56	0	0	13(6.0)$^{+}$	0(0)	0(0)	4(0.8)$^{+}$	0(0)	0(0)
Bromus Rubens L.	Ruderal grass	1247	50	7	89(132.9)$^{-}$	13(10.8)	1(0.1)	20(17.4)	0(0.1)	1(0.4)
Carthamus lanatus L.	Ruderal herb	1	0	0	0(0.1)	0(0)	0(0)	0(0)	0(0)	0(0)
Centaurea melitensis L.	Ruderal herb	9	2	4	1(1.0)	0(0.4)	0(0.1)	1(0.1)	0(0)	0(0.2)
Cerastium pumilum Curtis	Herb	2	12	1	1(0.2)	0(2.6)	0(0)	0(0)	0(0)	0(0.1)
Chaenorrhinum rubrifolium (Robill. & Castagne ex	Herb	0	0	62	0(0)	0(0)	0(1.2)	0(0)	0(0)	0(3.2)$^{-}$

Appendix 17 Species spatial associations with *G. struthium* (Chapter 4)

DC.) Fourr.										
Desmazeria rigida (L.) Tutin	Ruderal grass	6	5	4	0(0.6)	0(1.1)	0(0.1)	0(0.1)	0(0)	0(0.2)
Diplotaxis erucoides DC.	Ruderal herb	6	21	0	0(0.6)	0(4.5)$^{-}$	0(0)	0(0.1)	0(0.1)	0(0)
Diplotaxis ilorcitana (Sennen) Aedo, Mart.-Laborde & Muñoz Garm.	Ruderal herb	7	4	0	0(0.7)	0(0.9)	0(0)	0(0.1)	0(0)	0(0)
Erodium cicutarium (L.) L'Hér.	Ruderal herb	5	10	1	0(0.5)	3(2.2)	0(0)	0(0.1)	0(0)	0(0.1)
Eruca vesicaria (L.) Cav.	Ruderal herb	46	26	2	4(4.9)	0(5.6)$^{-}$	0(0)	0(0.6)	0(0.1)	0(0.1)
Euphorbia falcata L.	Ruderal herb	2	0	0	0(0.2)	0(0)	0(0)	0(0)	0(0)	0(0)
Filago pyramidata L.	Herb	33	25	4	2(3.5)	5(5.4)	0(0.1)	3(0.5)$^{+}$	0(0.1)	0(0.2)
Galium verrucosum Huds.	Herb	6	7	32	1(0.6)	6(1.5)$^{+}$	3(0.6)$^{+}$	0(0.1)	0(0)	2(1.6)
Hedypnois cretica (L.) Dum. Cours.	Ruderal herb	4	0	0	0(0.4)	0(0)	0(0)	0(0.1)	0(0)	0(0)
Hippocrepis ciliata Willd.	Ruderal herb	148	89	4	10(15.8)$^{-}$	13(19.2)$^{-}$	0(0.1)	1(2.1)	0(0.2)	0(0.2)
Hordeum murinum L.	Ruderal grass	833	14	6	47(88.8)$^{-}$	5(3.0)	0(0.1)	18(11.6)$^{+}$	0(0)	1(0.3)
Linum strictum L.	Herb	8	0	76	2(0.9)	0(0)	11(1.4)$^{+}$	0(0.1)	0(0)	3(3.9)
Malcolmia africana (L.) R. Br.	Herb	1	1	0	0(0.1)	0(0.2)	0(0)	0(0)	0(0)	0(0)
Medicago littoralis Rohde ex Loisel.	Ruderal herb	2	0	0	0(0.2)	0(0)	0(0)	0(0)	0(0)	0(0)
Medicago sativa L.	Ruderal herb	0	1	0	0(0)	0(0.2)	0(0)	0(0)	0(0)	0(0)
Neatostema apulum (L.) I.M. Johnst.	Ruderal herb	15	14	0	2(1.6)	0(3.0)$^{-}$	0(0)	0(0.2)	0(0)	0(0)
Plantago coronopus L.	Ruderal herb	96	13	0	10(10.2)	2(2.8)	0(0)	2(1.3)	0(0)	0(0)
Scabiosa stellata L.	Ruderal herb	0	1	0	0(0)	1(0.2)	0(0)	0(0)	0(0)	0(0)
Senecio gallicus Chaix	Ruderal herb	4	0	0	0(0.4)	0(0)	0(0)	0(0.1)	0(0)	0(0)
Spergularia diandra (Guss.) Boiss.	Herb	0	2	0	0(0)	0(0.4)	0(0)	0(0)	0(0)	0(0)
Sphenopus divaricatus (Gouan) Rchb.	Grass	0	2	0	0(0)	0(0.4)	0(0)	0(0)	0(0)	0(0)
Tragopogon dubius Scop.	Ruderal herb	13	1	0	1(1.4)	1(0.2)	0(0)	0(0.2)	0(0)	0(0)
Trigonella monspeliaca L.	Ruderal herb	18	3	2	3(1.9)	1(0.6)	0(0)	0(0.3)	0(0)	0(0.1)
Unknown species										
Unknown 1		3	0	0	0(0.3)	0(0)	0(0)	1(0)$^{+}$	0(0)	0(0)
Unknown 2		2	0	0	0(0.2)	0(0)	0(0)	0(0)	0(0)	0(0)
Unknown 3		0	3	0	0(0)	0(0.6)	0(0)	0(0)	0(0)	0(0)
Unknown 4		0	0	3	0(0)	0(0)	0(0.1)	0(0)	0(0)	0(0.2)
Unknown 5		5	1	0	0(0.5)	0(0.2)	0(0)	0(0.1)	0(0)	0(0)
Unknown 6		3	5	0	0(0.3)	3(1.1)	0(0)	0(0)	0(0)	0(0)

* Other shrubs are the nanophanerophytes *(C. clusii, E. fragilis, G. scorpius, R. officinalis, S. genistoides* and *S. vermiculata).*

f_t = total frequency of the species; f_o = observed frequency of the species associated either with *G. struthium* or other shrubs; f_e = expected frequency of the species associated either with *G. struthium* or other shrubs; (+) positive significant spatial association; (-) negative significant spatial association.

Appendix 17 Species spatial associations with *G. struthium* (Chapter 4)

Appendix 18 *Gypsophila struthium* as ecosystem engineer (Chapter 4)

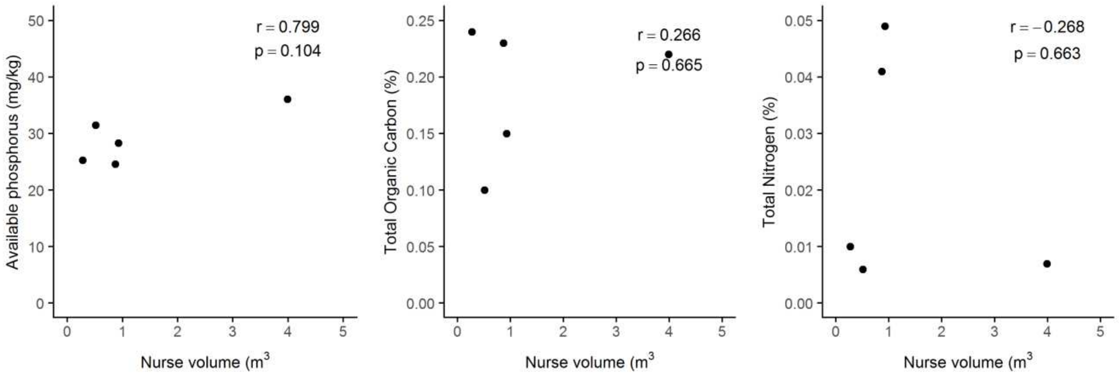

Figure A18.1 Pearson's correlations between the nurse volume and the nutrients content in the soil under canopy (available phosphorus, total organic carbon and total nitrogen). Significant effect of the nurse volume when p-value ≤ 0.05.

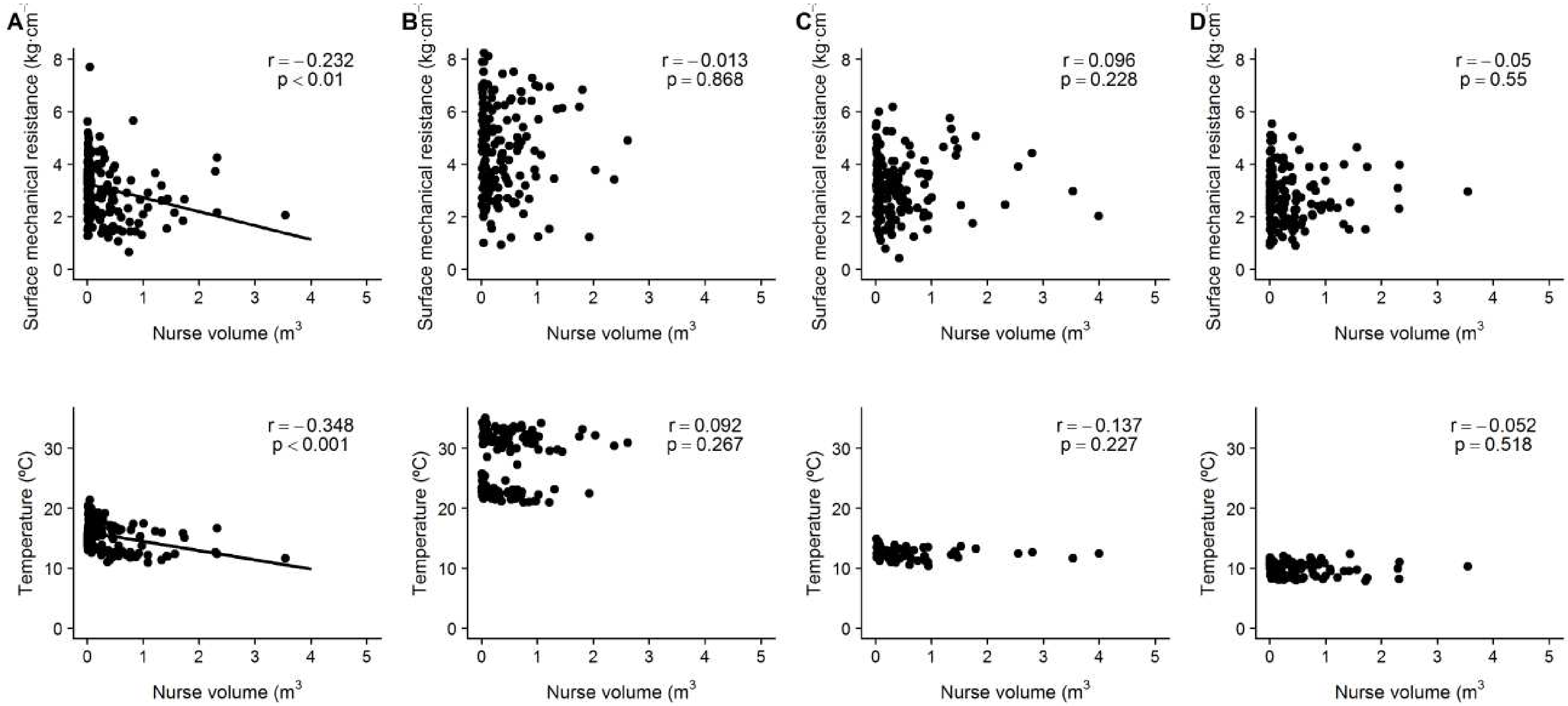

Figure A18.2 Pearson's correlations between the nurse volume and surface mechanical resistance and soil temperature under *G. struthium* canopy, per season: (A) spring, (B) summer, (C) autumn and (D) winter. Significant effect of the nurse volume when p-value ≤ 0.05.

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