



Tesis doctoral

Universidad de Almería

Nuria Pistón Caballero

Diversidad filogenética e interacciones entre plantas:
consecuencias para la dinámica de comunidades vegetales



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Tesis Doctoral



**DIVERSIDAD FILOGENÉTICA E INTERACCIONES ENTRE
PLANTAS: CONSECUENCIAS PARA LA DINÁMICA DE
COMUNIDADES VEGETALES**

Memoria presentada por Nuria Patricia Pistón Caballero para optar al Grado de Doctora en Ciencias Aplicadas y Medioambientales por la Universidad de Almería. Esta tesis ha sido dirigida por Francisco I. Pugnaire de Iraola, Profesor de Investigación de la Estación Experimental de Zonas Áridas (EEZA-CSIC) y codirigida por Cristina Armas Kulik, Postdoctoral Ramón y Cajal en la EEZA, y por Christian Schöb, Postdoctoral SNSF Ambizione de la Universidad de Zúrich.

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A mi familia, por ser mi inspiración

*“It is pleasant
to know you can do anything
so difficult. It is good when you
have mastered it, and you are
really in competition with yourself.”*

Robert Lax en *Circus Days & Nights*

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INDEX

SUMMARY	1
INTRODUCCIÓN	5
JUSTIFICACIÓN E HIPÓTESIS	15
OBJETIVOS.....	17

CHAPTER I: Complementarity among shrub species enhances community richness and phylogenetic diversity along an environmental gradient

1	
Abstract.....	21
Introduction.....	23
Methods	25
Field sites, species and data collection	25
Micro-environmental conditions along the gradient	27
Sign and intensity of plant interactions	28
Assessing species diversity along the environmental gradient.....	28
Community level species richness and phylogenetic diversity	29
Results	30
Discussion.....	36
Differences in microhabitat conditions.....	37
Shrub effects on subordinate richness and overall abundance along the gradient	38
Differences in subordinate community composition among shrub species.....	39
Environmental context-dependence of complementarity effects .	39
Conclusions	41
Appendix A. Supplementary data.....	42

CHAPTER II: Phylogenetic distance among beneficiary species in a cushion plant species explains interaction outcome

51

Abstract.....	53
Introduction.....	55
Methods	57
Field sites, species and data collection	57
Effect of cushions on species biomass.....	59
Contribution of cushions to phylogenetic diversity.....	60
Contribution of cushions to species richness.....	61
Results	61
Discussion.....	63
Relationship between phylogenetic relatedness and plant-plant interactions.....	64
The influence of interactions on community assemblage.....	65
Conclusion	66
Appendix B. Supplementary data	68
CHAPTER III: Differences in facilitation and its feedback effects between two phenotypes of a legume shrub	73
Abstract.....	75
Introduction.....	77
Methods	79
Field site and species	79
Observational study	80
Canopy removal experiment.....	81
Beneficiary removal experiment.....	82
Statistical analysis.....	83
Results	84
Discussion.....	90
Functional traits, environment and community composition	90
Reciprocal effects	91

Cost of facilitation	91
Conclusion	93
Appendix C. Supplementary material.....	94
CHAPTER IV: Ontogenetic shifts and phylogenetic relatedness in annual plants interactions in a semi-arid community	97
Abstract.....	99
Introduction.....	101
Methods	103
Field site, species and experimental setup.....	103
Net interaction outcome and phylogenetic relatedness	106
Assessing trait variation through time	107
Statistical analysis.....	107
Results	108
Discussion.....	110
Plant performance and survival	111
Trait plasticity	111
Conclusion	112
Appendix D. Supplementary material.	113
GENERAL CONCLUSIONS.....	115
REFERENCIAS.....	121
Otras aportaciones científicas derivadas de la Tesis Doctoral.....	135
Índice de figuras y tablas.....	137

SUMMARY

Plant-plant interactions are a major mechanism governing plant communities and their responses to environmental changes. However, it remains unclear how phylogenetic similarity affects interactions and how they influence the resulting community structure. Thus, our aim with this Thesis was to clarify fundamental aspects of plant interaction, their dependence on phylogenetic similarity, and the consequences for the dynamics of communities. We wanted to test i) whether there are complementary effects of foundation species increasing species richness and phylogenetic diversity; ii) if phylogenetic similarity among species influences plant interactions; iii) how these interactions change along an environmental severity gradient; iv) whether phenotypic variability in a foundation species modulates species richness and phylogenetic diversity, and whether it affects facilitation costs; and finally, under homogeneous environmental conditions, v) we expect ontogenetic shifts towards more negative species interactions for closely related species and towards less negative interactions for distantly related species. For these purposes we made various observations and experiments in the field supported by a series of physiological and functional analyses of plant communities.

In the first chapter we analyzed the effects of seven potential facilitator species with contrasting morphologies on subordinate plant communities along an environmental gradient, linking their effects to conditions under their canopies. We were also interested in analyzing complementary effects of co-occurring shrub species to community-level species richness and phylogenetic diversity, and whether the effects of potential facilitator species differed along the gradient. For this, we used ecological and phylogenetic data of alpine plant communities along two altitudinal gradients on opposing aspects of the Sierra Nevada Mountains (Spain). Composition of subordinate communities was different among shrubs

and among sites and was correlated with relative humidity under shrubs along the environmental gradient, indicating the dependence of subordinate species on micro-environmental conditions created by the shrubs. Positive effects of shrubs on overall plant abundance and species richness prevailed in the most severe parts of the gradient while under relatively milder conditions shrub effects were mostly neutral or negative. In addition, we observed that such effects differed with shrub identity along the gradient. Thus, in sites where microhabitat differences were more extreme and where there was at least one shrub species showing a positive effect on richness and abundance, shrub species had complementary effects on other plant species, therefore promoting whole-community species richness and phylogenetic diversity. However, this complementary effect was absent at sites of low environmental severity where individual shrub species had non-significant or negative effects on species richness and/or overall abundance.

In the second chapter we wanted to investigate factors that influence cushion-plant subordinate community depending on environmental conditions. The phylogenetic limiting similarity hypothesis states that closely related species tend to compete stronger than distantly related species, although evidence is inconclusive. We used ecological and phylogenetic data on alpine plant communities along an environmental severity gradient in Sierra Nevada to assess the importance of phylogenetic relatedness in affecting the interaction between cushion plants and the whole community, and how these interactions may affect community assemblage and diversity. We first measured species richness and individual biomass of species growing within and outside the nurse cushion species, *Arenaria tetraquetra* ssp. *amabilis*. We then assembled the phylogenetic tree of species present in both communities and calculated the phylogenetic distance between the cushion species and its beneficiary species, as well as the phylogenetic community structure. We also estimated changes in species richness at the local level due to cushions presence. The effects of cushions on closely related species

changed from negative to positive as environmental conditions became more severe, while the interaction with distantly related species did not change along the environmental gradient. Overall, we found environmental context-dependence in patterns of phylogenetic similarity, as the interaction outcome between nurses and their close- and distantly-related species showed an opposite pattern with environmental severity.

In the third chapter we wanted to further explore factors influencing the dynamics of plant subordinate communities. Foundation species with different phenotypes may modify the microhabitat differently, could differ in subordinate community composition, and consequently receive different feedbacks from this subordinate plant community. We explored whether tight and loose canopy types of the species *Cytisus galianoi* are associated with differences in microhabitat conditions and subordinate community composition. We also wanted to experimentally test reciprocal effects between the most frequent subordinate species, *Festuca indigesta*, and the foundation species trying to evidence phenotypic differences in the bi-directional interaction. We performed an observational and a removal experiment in an alpine plant community in Sierra Nevada. Both *C. galianoi* phenotypes apparently did affect understory microhabitats differently and hosted differentiated subordinate communities. There were differences regarding community composition and biomass between the two phenotypes, as well as differences in the physiological status of subordinate species. The tight *C. galianoi* phenotype showed the highest facilitation effect and received a negative feedback. By contrast, the loose phenotype showed higher species richness, higher plant abundance, and more phylogenetic diversity than the tight phenotype, but no negative feedback. Our results suggest that negative feedback effects of the subordinate species on the tight phenotype might cause the phenotype change from tight to loose.

For a more detailed study of plastic changes throughout the life cycle of plants, in the fourth chapter we wanted to highlight whether -and to what

extent- early stages and survival, as well as the sign and intensity of species interactions, varied with phylogenetic relatedness and how these interactions influenced the long-term dynamics of plant communities. We removed environmental heterogeneity but test competitive abilities outdoor. We analyzed interaction outcome between annual species growing in intraspecific interaction, and between closely-, medium- and distantly-related species across plant life stages in a semiarid community in SE Spain. Competition was less intense between distantly related species, which resulted in higher survival than close relatives and conspecifics. Moreover, we found a high variance in the interaction regarding number of leaves between conspecifics compared with the rest of relatives differing in degree of relatedness, suggesting that this trait responded plastically to competition. Ontogenetic changes in species interactions depend on their phylogenetic relatedness. Thus, our data highlights the need to incorporate multiple life stages when assessing factors contributing to individual survival and species coexistence.

This information will be relevant to predict the effects of global change on plant communities in extreme environments particularly in alpine environments, which harbor unique communities allowing us to anticipate impacts and, therefore, helping to improve their management and conservation.

INTRODUCCIÓN

Las interacciones planta-planta son una parte principal de los mecanismos que gobiernan la respuesta de las especies y comunidades vegetales a los cambios ambientales (Goldberg, 1996; Brooker, 2006), existiendo un balance entre las interacciones positivas o negativas. Si el balance neto es positivo la interacción es de facilitación, y si este es negativo, de competencia (Armas & Pugnaire, 2005). Aunque durante mucho tiempo la teoría ecológica y los modelos de interacción entre plantas han estado centrados en la competencia, hoy en día se reconoce la importancia ecológica de las interacciones positivas entre plantas (Pugnaire *et al.*, 1996a; Lortie *et al.*, 2004; Brooker & Callaway, 2009), las cuales toman gran relevancia en zonas de ambientes extremos tales como los ecosistemas alpinos o los semiáridos (Pugnaire *et al.*, 1996b; Callaway *et al.*, 2002; Kikvidze *et al.*, 2005; Tirado & Pugnaire, 2005; Armas *et al.*, 2011; Butterfield *et al.*, 2013; Cavieres *et al.*, 2014). Sin embargo, aún no está claro el papel de las interacciones planta-planta bajo unas nuevas condiciones ambientales, ni cómo las interacciones se verán alteradas en respuesta a estos cambios (Brooker *et al.*, 2008). En este contexto, es preciso esclarecer la relación entre las interacciones entre plantas y los gradientes ambientales, la importancia de la especificidad de esas interacciones y cuál es su influencia en el funcionamiento y estructura de las comunidades vegetales (Choler *et al.*, 2001; Callaway & Howard, 2007).

La facilitación entre plantas puede alterar profundamente la distribución de especies en una comunidad (Callaway *et al.*, 2002; Kikvidze *et al.*, 2005). Bajo las extremas condiciones ambientales en zonas de alta montaña, son de especial interés las plantas nodriza caracterizadas por ser perennes y de forma pulvinular. Estas plantas facilitan el establecimiento y crecimiento de otras mediante el mejoramiento de las condiciones físicas bajo sus copas (Cavieres *et al.*, 2005; Reid *et al.*, 2010), siendo un mecanismo clave para aumentar la productividad del ecosistema (Callaway, 1995). En

ambientes extremos, varios estudios han señalado que protegen a la comunidad de plantas (i.e., subordinadas) de los altos niveles de irradiación y temperatura (Körner, 2003; Anthelme *et al.*, 2014), contribuyen al aumento de la disponibilidad hídrica por acción de la sombra o del levantamiento hidráulico (Prieto *et al.*, 2010) e incrementan la disponibilidad de nutrientes debido a la acumulación de materia orgánica (Rodríguez-Echeverría & Pérez-Fernández, 2003). Por ejemplo, en varios de sus estudios, Badano & Cavieres (2006); Badano *et al.* (2006); Cavieres & Badano (2009) encontraron que el efecto de la facilitación sobre la riqueza y diversidad de plantas subordinadas en comunidades de alta montaña en los Andes es muy importante. Esta facilitación se debía principalmente al aumento en la disponibilidad de agua y, sobre todo, debido al amortiguamiento de las temperaturas extremas bajo sus copas. La intensidad e importancia de esta facilitación varió dependiendo de la especie facilitadora y del clima local, pero muchas especies subordinadas se encontraron exclusivamente bajo las copas de estas plantas y no en zonas sin su protección. Por tanto, los efectos ecológicos de las plantas nodriza sobre el resto de la comunidad son importantes, y esa importancia puede verse incrementada en el futuro por el aumento de las temperaturas a nivel global y la disminución de la disponibilidad hídrica para las plantas debido a una disminución de las precipitaciones (Guisan & Theurillat, 2000; Engler *et al.*, 2011).



Fig. II. Imagen de la planta nodriza *Arenaria tetraquetra* subsp. *amabilis* en la cara Norte del Parque Nacional de Sierra Nevada. Fotografía: C. Schöb.

Los ecosistemas alpinos son zonas de estudio ecológico ideales por presentar gradientes altitudinales acusados que reproducen cambios similares a los que ocurren a lo largo de gradientes latitudinales, pero de forma más rápida y a una escala espacial menor; son refugios de flora y fauna relictas y son escenarios muy sensibles a cambios ambientales por los frágiles equilibrios entre los componentes del sistema. Por consiguiente, es fundamental estudiar cómo las interacciones planta-planta van a verse afectadas por estos cambios ambientales, o cómo se pueden mitigar estos cambios aumentando la resiliencia del ecosistema (Kikvidze *et al.*, 2011). En este contexto, hemos de tener en cuenta que en el caso de las montañas secas con escasa precipitación, como Sierra Nevada, España, pueden existir dos gradientes opuestos de temperatura y humedad a lo largo del gradiente altitudinal (Schöb *et al.*, 2013). Este gradiente cruzado puede generar un fenómeno de estrés hídrico en las plantas a altitudes bajas que no se ve en otros sistemas montañosos más húmedos (Callaway *et al.*, 2002). En cambio, en las cotas altas de estas montañas secas, la temperatura en verano se suaviza, la nieve permanece más tiempo y el deshielo se produce más tarde, lo

que en conjunto proporciona una mayor disponibilidad de agua para las plantas (Cavieres *et al.*, 2005).

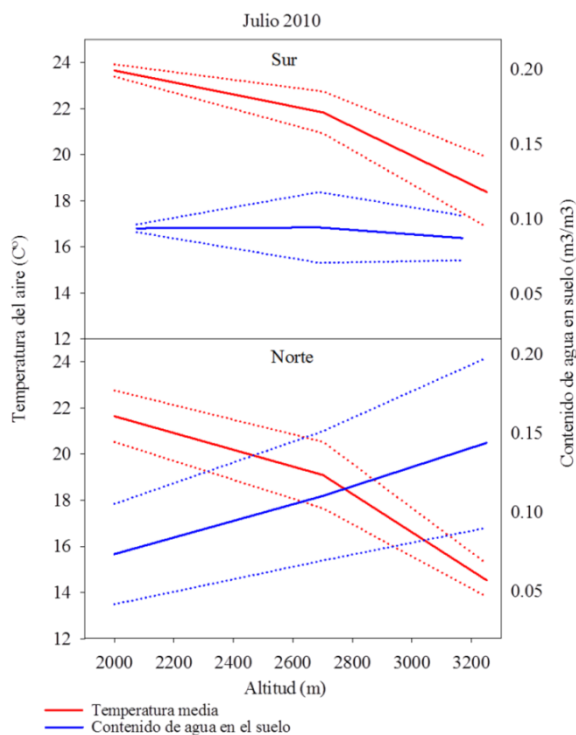


Figura I2. Gradiente opuesto de temperatura del aire y humedad del suelo de Sierra Nevada.

De acuerdo con la hipótesis del gradiente de estrés (en inglés SHG; Bertness & Callaway, 1994), la frecuencia y la importancia del efecto facilitador de las plantas nodriza aumentarán con un incremento de la severidad ambiental. Esta hipótesis ha sido apoyada ampliamente (Armas *et al.*, 2011; He *et al.*, 2013; Pugnaire *et al.*, 2015) aunque existe cierta controversia (Maestre *et al.*, 2005; Michalet, 2006; Kawai, 2007; Maestre *et al.*, 2009). Estos últimos ejemplos predicen que el resultado de las interacciones no siempre aumentará con la severidad ambiental, sino que podría disminuir en el extremo más severo dependiendo de la estrategia de las especies interactuantes (i.e., si son especies tolerantes al estrés o si son especies competidoras, sensu Grime (1977)) y de si el factor principal que

causa el estrés es un recurso o no (Maestre *et al.*, 2009). Es necesario tener en cuenta que las interacciones planta-planta son altamente específicas (Callaway, 1998), lo cual induce a pensar que los diferentes rasgos ecológicos de las especies que interactúan (Choler *et al.*, 2001; Gómez-Aparicio, 2009; Gross *et al.*, 2009) o su similitud filogenética (Cavender-Bares *et al.*, 2009; Valiente-Banuet & Verdú, 2013) son factores fundamentales que afectan al resultado de estas interacciones.

Para entender los procesos que rigen la coexistencia de especies en la última década se ha desarrollado el uso de patrones de similitud filogenética entre especies (Webb *et al.*, 2002; Pausas & Verdu, 2010). La filogenia de comunidades se inició como un enfoque útil debido a la complejidad del uso de rasgos funcionales (Ackerly, 1997; Cavender-Bares *et al.*, 2009). Asumiendo que muchos rasgos ecológicos están conservados a lo largo de la evolución (Blomberg *et al.*, 2003; Wiens & Graham, 2005; pero ver Mayfield & Levine, 2010), incluyendo aquellos que influyen en las interacciones entre especies (Violle *et al.*, 2011), la dominancia de las interacciones competitivas en una comunidad producirá la coexistencia de especies con rasgos diferentes que permitan la segregación de nichos ecológicos y su coexistencia; por tanto, encontraremos un patrón filogenético más disperso (Webb *et al.*, 2002). En cambio, si los filtros abióticos son de mayor importancia en una comunidad, se espera que el conjunto de especies que la formen se caracterice por una mayor similitud en sus rasgos ecológicos, que correspondería a adaptaciones morfológicas y fisiológicas a las condiciones ambientales y por tanto, en estas comunidades encontraremos un patrón filogenético más agregado (Verdú & Pausas, 2007). No obstante, la presencia de especies nodriza y la dominancia de la facilitación en una comunidad podría cambiar estos patrones filogenéticos siendo capaces de crear comunidades más diversas comparadas con las zonas de claro sin estas plantas nodriza (Butterfield *et al.*, 2013). Bajo esta asunción de la conservación de rasgos a lo largo de la historia evolutiva se formuló la hipótesis de la similitud filogenética limitada (Darwin, 1859;

MacArthur & Levins, 1967), la cual parece confirmarse en la mayoría de casos estudiados (Valiente-Banuet *et al.*, 2006; Valiente-Banuet & Verdú, 2007, 2008, 2013; Castillo *et al.*, 2010). De estos estudios se concluye que la relación evolutiva es clave modulando el resultado de la interacción entre especies. Sin embargo, como ha sido discutido anteriormente, hay que tener en cuenta la importancia de las condiciones ambientales para definir el resultado de estas interacciones. Además, aún hay resultados contradictorios (Cahill *et al.*, 2008; Losos, 2008; Burns & Strauss, 2011; HilleRisLambers *et al.*, 2012; Bennett *et al.*, 2013; Godoy *et al.*, 2014), lo que enfatiza la necesidad de realizar más estudios sobre cómo interactúan ambos factores. Estos trabajos ayudarían a mejorar nuestras interpretaciones sobre los procesos reinantes en el ensamblaje de una comunidad a partir del estudio de los patrones filogenéticos.

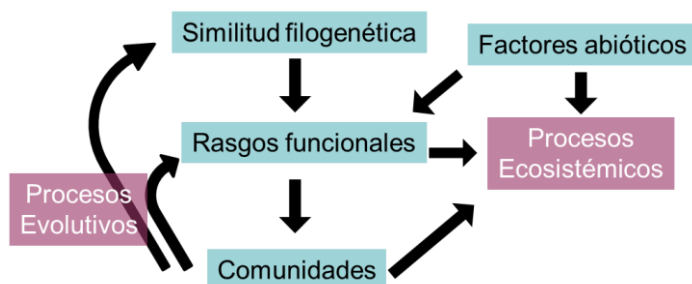


Figura I3. Esquema mostrando las relaciones entre los distintos factores que afectan al ensamblaje de la comunidad: los rasgos funcionales tienden a ser similares entre especies con un ancestro común. Para las plantas en particular, los rasgos fisiológicos y el ensamblaje de especies con rasgos variados influirán en los procesos ecosistémicos. Por tanto, estos rasgos serán un vínculo importante por el cual la historia evolutiva influye en los procesos ecológicos. Las interacciones bióticas dentro de las comunidades también influirán en los rasgos, provocando una retroalimentación entre los procesos ecológicos y evolutivos. Además, los factores abióticos modularán dichos rasgos reflejando así su origen geográfico a la vez que influirán directamente en los procesos ecosistémicos.

Un punto importante a tener en cuenta es que, hasta la fecha, la mayoría de los estudios que se han centrado en facilitación, se han realizado utilizando solamente una única especie nodriza por comunidad a pesar de que

se sabe que en ambientes severos coexisten distintas especies de arbustos potencialmente facilitadores (Pugnaire *et al.*, 2004). Debido a la variedad existente en la forma de las copas entre especies de arbustos (Aubert *et al.*, 2014), y sabiendo que las interacciones entre plantas van a depender de su morfología y de su estado fisiológico (Armas & Pugnaire, 2011; Schöb *et al.*, 2013; Bråthen & Ravolainen, 2015), dicha diversidad funcional podría influir en la capacidad de modificar su micro-hábitat y así, facilitar distintas comunidades de subordinadas. Los nichos ecológicos que crean las especies nodriza raramente serán idénticos, por lo que estas probablemente crearán comunidades únicas aumentando en conjunto la riqueza y la diversidad filogenética a nivel de toda la comunidad. A pesar de la gran importancia que tiene este efecto combinado de distintas especies nodriza, prácticamente no existen estudios sobre ello (pero ver Zhang *et al.*, 2011; Amat *et al.*, 2015), especialmente en zonas tan severas y vulnerables como las alpinas (Cavieres *et al.*, 2007; Wang *et al.*, 2008; Chu *et al.*, 2009). Este efecto se conoce como complementariedad, i.e., el proceso por el cual una comunidad diversa de plantas nodriza aumenta más el rendimiento de una comunidad entera que el efecto producido por una única especie de nodriza (Huston, 1997). A través de la complementariedad, varias especies de nodriza generarán en conjunto un nicho mayor y crearán una mayor heterogeneidad ambiental que la originada por una única especie nodriza (Harper, 1977; Jones *et al.*, 1994b; Odling-Smee *et al.*, 1996). Esta posible no-redundancia entre especies nodriza y su efecto complementario podría ser crítico en el funcionamiento del ecosistema, incluso aumentando su productividad (Loreau & Hector, 2001; Cadotte, 2013).

Además de las diferencias morfológicas entre especies, las diferencias dentro de cada especie e incluso a lo largo del ciclo de vida la planta (i.e., ontogenia), también juegan un papel fundamental en la dinámica de las interacciones planta-planta (Armas & Pugnaire, 2005; Soliveres *et al.*, 2010; Schöb *et al.*, 2012; Trinder *et al.*, 2012, 2013). Por ello, si en una misma

comunidad existen plantas nodriza de una misma especie con distintos fenotipos y difieren en la calidad de su micro-hábitat, el efecto facilitador también podría variar. Por ejemplo, Michalet *et al.* (2011), estudiando dos fenotipos de la planta nodriza *Geum rosii* en una comunidad alpina en Arizona, encontró que los fenotipos de copa “abierta” (i.e., escasa cobertura de ramas) tenían un mayor efecto facilitador sobre la comunidad de subordinadas que los fenotipos de copa “densa” (i.e., cobertura de ramas compacta). Es importante destacar que la fuente de variación fenotípica en estas plantas nodriza puede cambiar entre especies y entornos, y ser debida a la variabilidad genética, la plasticidad del medio o incluso, que sean distintos estados ontogénicos (Pugnaire *et al.*, 1996b; Schöb *et al.*, 2013; Al Hayek *et al.*, 2014). Hasta la fecha pocos estudios han intentado cuantificar el impacto de las distintas fuentes de variación fenotípica sobre las interacciones entre plantas, a pesar de que los resultados serían de vital importancia para entender el contexto de dichas interacciones (Chen *et al.*, 2015). Incluso a lo largo del ciclo de vida, las mismas condiciones ambientales que son beneficiosas para las plántulas pueden resultar negativas para plantas más adultas (Schupp, 1995), lo que hace que la facilitación pueda cambiar a competencia a medida que las plantas avanzan en su desarrollo (Callaway & Walker, 1997; Armas & Pugnaire, 2005, 2009; Miriti, 2006; Schiffers & Tielbörger, 2006). La inmensa mayoría de los estudios que evalúan cambios ontogénicos en las interacciones planta-planta se centran en ventanas temporales concretas a lo largo del desarrollo de las especies facilitadas (Valiente-Banuet & Verdú, 2008; Armas & Pugnaire, 2009). Sin embargo, estas aproximaciones no dan una visión global del tema, ya que, la interacción que observamos a largo plazo va a ser el resultado de la dinámica en las interacciones a corto-medio plazo; por lo tanto, el resultado de los estudios de interacciones podría depender en gran medida de la etapa vital de las plantas estudiadas (Trinder *et al.*, 2012), afectando incluso a la estructura de las comunidades en los procesos de sucesión cíclica (Armas *et al.*, 2009).

El balance entre los procesos de facilitación, neutralidad y competencia a corto y largo plazo es un determinante fundamental de la dinámica de las comunidades vegetales (Aguilar & Sala, 1999). Por tanto, un mayor conocimiento sobre la importancia relativa de las interacciones en la composición y estructura de las comunidades, así como un entendimiento de los condicionantes para que se de uno u otro signo en la interacción, son fundamentales para poder entender el ensamblaje de las especies, la dinámica y el funcionamiento de estos ecosistemas (Callaway, 2007; Brooker *et al.*, 2008; Pugnaire *et al.*, 2011; Valiente-Banuet & Verdú, 2013; Soliveres *et al.*, 2014). Por tanto, es primordial dar un enfoque que unifique los distintos factores que afectan a las interacciones entre plantas para dilucidar la importancia y el impacto sobre la dinámica de las comunidades.

JUSTIFICACIÓN E HIPÓTESIS

Las interacciones planta-planta son una parte principal de los mecanismos que gobiernan la formación de comunidades vegetales y las respuestas a los cambios ambientales. Sin embargo, aún no está claro el papel que juega la similitud filogenética entre plantas. Así, con esta tesis doctoral pretendemos aclarar aspectos fundamentales relacionados con la interacción entre plantas dependiendo de la similitud filogenética entre ellas y sus consecuencias para la dinámica de comunidades. Pretendemos comprobar, i) si existen efectos complementarios del conjunto de plantas nodriza aumentando la riqueza de especies y la diversidad filogenética a nivel de toda la comunidad, ii) si la similitud filogenética influye en las interacciones entre las nodriza y la comunidad de plantas subordinadas, iii) como varían dichas interacciones a lo largo de un gradiente de severidad ambiental, iv) si la variación fenotípica en una especie nodriza modula la riqueza y diversidad filogenética bajo su copa y si hay diferencias en el coste de facilitación que sufren ambos fenotipos, y por último, v) si la competencia entre especies cercanas en la filogenia es el resultado de cambios en el signo de la interacción a lo largo del ciclo de vida de las plantas. Esta información será relevante de cara a realizar predicciones del efecto del cambio global en las comunidades de ambientes extremos. Estas zonas son excepcionales debido a la particularidad de albergar una diversidad única, permitiéndonos así anticipar las respuestas ante estos cambios y, por tanto, mejorar su gestión y conservación.

Las hipótesis generales que nos planteamos para responder estos objetivos son las siguientes:

1. Cada una de las especies nodriza albergará comunidades de plantas subordinadas únicas. Ello es debido a que el conjunto de las especie nodriza, tendrá efectos complementarios aumentando la riqueza y diversidad filogenética a nivel de toda la comunidad.

2. En los procesos de interacción entre plantas existe un efecto de la similitud filogenética entre especies dependiente del ambiente que modula la estructura de las comunidades de vegetales.
3. La variación fenotípica en una especie nodriza modulará el resultado de las interacciones nodriza-subordinadas, afectando también a la riqueza y diversidad filogenética de la comunidad de subordinadas. A su vez, dicha comunidad podría conducir a cambios fenotípicos en las nodriza a través de un efecto de retroalimentación negativa.
4. Las interacciones entre plantas modularán su respuesta a lo largo de su ciclo de vida dependiendo de la similitud filogenética entre ellas.

OBJETIVOS

Determinar qué papel juega la similitud filogenética modulando las interacciones entre plantas y cuál es la estructura y composición de la comunidad resultante teniendo en cuenta la interdependencia con otros factores como el clima, la complementariedad entre especies de arbustos de una misma comunidad, los cambios fenotípicos y la ontogenia.

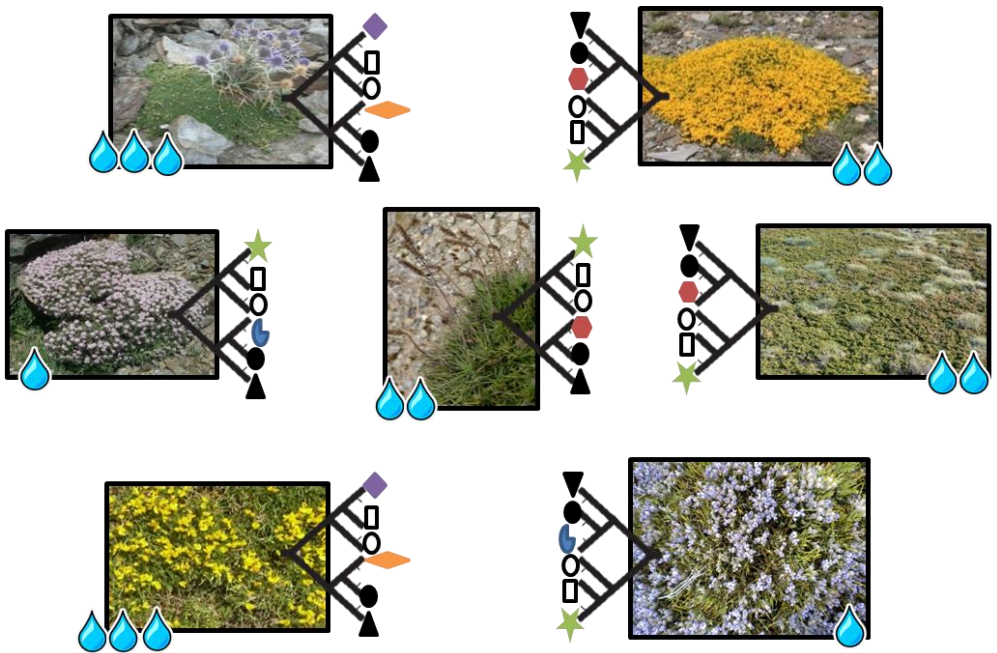
Específicamente, los objetivos que se abordarán en los diferentes capítulos de esta tesis son:

1. Analizar los efectos de siete especies de arbustos potencialmente facilitadores sobre la comunidad de plantas subordinadas a lo largo de un gradiente de severidad ambiental (Capítulo I).
2. Estudiar cual es el efecto de la similitud filogenética en las interacciones entre plantas y cómo varía a lo largo de un gradiente ambiental (Capítulo II).
3. Explorar como la variación fenotípica en una especie nodriza afecta a la comunidad de subordinadas y como dicha comunidad, por efectos de retroalimentación negativa, podría provocar cambios fenotípicos en la nodriza (Capítulo III).
4. Estudiar cómo afecta la similitud filogenética entre plantas a la supervivencia y crecimiento de ellas, y determinar cómo varían las interacciones a lo largo del ciclo de vida de las plantas (Capítulo IV).

CHAPTER I

Complementarity among shrub species enhances community richness and phylogenetic diversity along an environmental gradient

Nuria Pistón, Christian Schöb, Cristina Armas, Iván Prieto and Francisco I. Pugnaire



Abstract

In alpine environments, facilitator species alter microhabitats by buffering temperature extremes and wind, and maintaining soil humidity and nutrient availability higher than bare ground habitats. These modulating effects of facilitator species in severe environments are critical for the persistence of species out of their optimal range and contribute to higher community richness and diversity. We analyzed the effects of seven potential facilitator species with contrasting morphologies on subordinate plant communities along an environmental gradient, linking such effects to microhabitat conditions under their canopies. We were also interested in analyzing the complementary effects of co-occurring shrub species to community level species richness and phylogenetic diversity, and whether the effects of potential facilitator species differed along the gradient. For this, we used ecological and phylogenetic data of alpine plant communities along two altitudinal gradients on opposing aspects of the Sierra Nevada Mountains (Spain). Shrub microhabitats buffered harsh abiotic conditions by decreasing maximum temperatures and increasing relative humidity with respect to open areas along the gradient. Composition of subordinate communities was also different among shrubs and among sites and was correlated with relative humidity along the environmental gradient indicating the dependence of subordinate species on the micro-environments created by the different shrub species. Positive effects of shrubs on overall plant abundance and species richness prevailed in the most severe parts of the gradient while under relatively milder conditions shrub effects were mostly neutral or negative. In addition, we observed that such effects differed with shrub identity along the gradient. Thus, in sites where microhabitat differences were most extreme and where there was at least one shrub species showing a positive effect on richness and abundance, shrub species had complementary effects on other plant species, therefore promoting whole community species richness and

phylogenetic diversity. However, this complementary effect was absent at sites of low environmental severity where individual shrub species had non-significant or negative effects on species richness and/or overall abundance. Based on these results we can conclude that, under harsh environmental conditions, shrub effects on diversity were highest, and complementary among different species, resulting in a combined positive effect of the shrub community (i.e. all the shrub species present at a given site) that exceeded the effect of each individual shrub species alone.

Introduction

The impact of species interactions on diversity patterns has been addressed in detail in stressful environments such as the alpine belt (Reid *et al.*, 2010; Cavieres *et al.*, 2014; Liczner & Lortie, 2014). In alpine environments, facilitator species -often cushions-, alter microhabitats by buffering temperature extremes and wind, and maintaining soil humidity and nutrient availability higher than bare ground habitats (Körner, 2003; Cavieres *et al.*, 2005; Anthelme *et al.*, 2014). These modulating effects of facilitator species under stressful environments are critical for the persistence of species out of their optimal range and contribute to higher community richness (Badano & Cavieres, 2006; Schöb *et al.*, 2012). This positive effect of facilitator species changes with abiotic conditions, usually turning from positive to negative between harsh and milder environments (Callaway *et al.*, 2002). In addition, ontogeny of facilitator species (Soliveres *et al.*, 2010), soil organisms (Van der Putten, 2009) and different morphologies within and among species may also affect the outcome of facilitator-beneficiary interactions (Michalet *et al.*, 2011; Schöb *et al.*, 2012; Bråthen & Ravolainen, 2015). For example, canopy morphologies among cushion species vary widely from very compact to loose (Aubert *et al.*, 2014). Variations in canopy morphology may influence their microhabitat in different ways and facilitate different species. Thus, different facilitator species fill a larger niche space and create higher environmental heterogeneity, increasing species richness at the whole community level.

Cushion morphology is not an easy metric due to the complex variety of traits involved; alternatively, a useful approach to account for species similarity is to use phylogenetic relationships (Ackerly, 1997; Cavender-Bares *et al.*, 2009). Under the phylogenetic limiting similarity concept (Darwin, 1859; MacArthur & Levins, 1967), functional traits shaping a phenotype are considered conserved along phylogenetic lineages (Blomberg *et al.*, 2003; but see Mayfield & Levine, 2010) including those influencing species interactions

(Violle *et al.*, 2011). Given that species sharing traits within close phylogenetic lineages may have similar responses to the environment, severe environmental conditions are likely to sort species out, leading to communities with low species richness and low phylogenetic diversity (Cavender-Bares & Reich, 2012; Soliveres *et al.*, 2012b). However, and due to the effect of facilitator species in buffering extreme conditions, facilitators increase species richness and phylogenetic diversity (Butterfield *et al.*, 2013). Thus, diversity patterns would depend, on the one hand, on environmental severity and, on the other hand, on the effects of facilitator species (Valiente-Banuet & Verdú, 2013; Pistón *et al.*, 2015).

Since different species may create different microhabitats which could be colonized by different species (Díaz & Cabido, 2001), the occurrence of several facilitator species at a single site would likely increase community level diversity. However this issue has hardly been addressed (but see Zhang *et al.*, 2011; Amat *et al.*, 2015). Most reports on positive interactions concerned only one facilitator species per community, even though communities in stressful environments often include different potential facilitator species (Pugnaire *et al.*, 2004) which will differ in the microhabitats and “potential” niches they create. We could then expect a complementary effect of several facilitator species on community level species richness, abundance and phylogenetic diversity.

Our goal in this study was to analyze the effects of several potential facilitator species with contrasting morphologies on subordinate plant communities along an environmental gradient spanning the North and South aspects in a dry mountain, linking such effects to microhabitat conditions under the canopy of each shrub species. We were also interested in analyzing the contribution of co-occurring shrub species to community level species richness and phylogenetic diversity, and whether the effects of facilitator species differed along the gradient. For this purpose we selected in the Sierra Nevada Mountains, Spain, an environmental gradient characterized by

changes in temperature, water availability, and soil organic matter (Sánchez-Marañón et al. 2002; Schöb et al. 2013), and recorded plant community composition beneath seven shrub species and in adjacent open areas in 6 sites at different elevations; three sites were placed in the northern aspect and another three in the southern aspect of the Sierra Nevada range. We expected that i) subordinate species community composition would change depending on shrub identity, being related to microclimatic conditions beneath them. Although we expected species-specific effects of different shrubs on their subordinate plant community, we also expected a general trend along the gradient: individual shrubs would mostly have positive effects on plant community richness and plant abundance under relatively harsher environmental conditions while the net effect of shrubs would be neutral or competitive under milder environmental conditions as predicted by the stress gradient hypothesis (Bertness & Callaway 1994). At community level we expected ii) complementary effects of shrubs on community richness and phylogenetic diversity in stressful sites and no effects in relatively milder sites.

Methods

Field sites, species and data collection

The field sites are located in the Sierra Nevada Mountains, Southeastern Spain. We selected three sites, each ca. 1 ha, on the northern aspect of the Veleta peak at 2315 m, 2720 m and 3240 m elevation, and three on the southern aspect of the Mulhacen peak at 2040 m, 2570 m, and 2900 m elevation. Both peaks are separated by a distance of 30 km approx. The bedrock is mica-schist, and determines the rounded and gentle hillside landscape (Delgado, 2001).

Climate is dry continental Mediterranean with a hot and dry summer with means of 17 °C and 5 mm rainfall in July and -2.5 °C and 90 mm rainfall

in January (Pradollano (37°05' N 03°23' W); 2500 m elevation; <http://pendientedemigracion.ucm.es>). Mean annual temperature decreases about 0.61 °C/100 m at the North aspect and 0.59 °C/100 m at the South aspect; mean annual precipitation increases around 30 mm/100 m at the North aspect and 28 mm/100 m at the South aspect (Delgado *et al.*, 1988) resulting in a crossed climatic gradient with elevation (Schöb *et al.* 2013).

Plant communities in Sierra Nevada are characterized by a great diversity of species with a high level of endemism (Delgado, 2001). Subalpine-alpine shrubland communities (“piornales-enebrales”) are dominated by prostrate shrubs like *Cytisus galianoi* Talavera & P. E. Gibbs. (Fabaceae), *Genista versicolor* Boiss. (Fabaceae) or *Hormathophylla spinosa* (L.) P. Küpfer (Brassicaceae), and cushion-forming species like *Arenaria tetraquetra* ssp. *amabilis* (Bory) H. Lindb. fil. (Caryophyllaceae). Our analyses focused on seven species that dominated plant communities at our field sites, *A. tetraquetra* ssp. *amabilis*, *Bupleurum spinosum* Gouan (Apiaceae), *C. galianoi*, *G. versicolor*, *H. spinosa*, *Juniperus communis* ssp. *nana* Syme (Cupressaceae), and *Plantago holosteum* Scop. (Plantaginaceae). For simplicity we will refer to them as shrubs, although two of them, *A. tetraquetra* and *P. holosteum* are cushions. Regarding their morphology, and following the description of different canopy shapes by Aubert *et al.* (2014), *A. tetraquetra* shows a semi compact creeping canopy, *B. spinosum* and *H. spinosa* are thorny compact shrubs, *C. galianoi* and *G. versicolor* are radial hollow hemispherical shrubs, *J. communis* is a thorny-prostrated shrub and, *P. holosteum* is a tufted compact cushion. Shrubs and surrounding open areas were colonized by predominantly small perennial herbs and grasses (for the full list of species present at the sites see Table S4 in Supporting Information and Table S1 for a list with the number of shrub-dependent species).

In July 2010, at the peak of the growing season, we selected 30 individuals of each of the dominant shrub species present at each site and did a paired sampling beneath them and in nearby open areas; we recorded the

number of all plant species and the their number of individuals (number of shoots in the case of clonal species). For small cushions such as *A. tetraquetra* ssp. *amabilis* (mean \pm 1 SE; $209 \text{ cm}^2 \pm 12 \text{ cm}^2$) and *P. holosteam* ($190 \text{ cm}^2 \pm 19 \text{ cm}^2$) the sampling was done for the whole cushion canopy and paired with open plots of equal size. For *B. spinosum* ($4'992 \pm 506 \text{ cm}^2$), *C. galianoi* ($8'689 \pm 684 \text{ cm}^2$) and *G. versicolor* ($10'641 \pm 834 \text{ cm}^2$) we randomly placed one 625 cm^2 quadrat per canopy paired with a similar area in the open. As *G. versicolor* was larger at the low North site, we used 1250 cm^2 quadrats. For *J. communis* ssp. *nana* ($115'582 \pm 9'340 \text{ cm}^2$) we sampled five randomly placed 625 cm^2 quadrats per cushion and an equal number of open area plots. For *H. spinosa* ($1'573 \pm 101 \text{ cm}^2$) we sampled the whole canopy area and a 625 cm^2 quadrat in open areas.

Micro-environmental conditions along the gradient

We recorded air temperature and relative humidity (RH) at hourly intervals with iButtons (Maxim Integrated Products, Sunnyvale CA, USA). At each site three temperature sensors were randomly placed in the open and three into the canopy of *C. galianoi*, *G. versicolor*, *H. spinosa* and *J. communis* when the species were present. Sensors were placed 5 cm above ground and protected from direct solar radiation with a wooden cube. Using hourly values, we calculated the mean air temperature and RH for August 2010 (peak of the growing season at Sierra Nevada) and checked for differences between aspects (North and South), elevation (low, medium and high) and microhabitat (cushion vs. open areas) with a one-way factorial ANOVA. Temperature data were transformed with a power of two to meet the homogeneity of variances and normality assumptions. Post-hoc differences along the gradient were examined with Fisher's LSD tests and significant differences among shrub species at each site were tested by separate contrasts within each site.

Sign and intensity of plant interactions

To test which and whether shrub species had positive effects on the number of species and overall plant abundance (i.e., total number of individuals irrespective of the species) along the environmental gradient we measured the interaction outcome between each shrub species and the subordinate community at each site. Interaction outcome was measured as the relative change in species number and abundance beneath shrubs compared to open areas. We used the Relative Interaction Index (Armas *et al.*, 2004) as $RII = (S_{\text{shrub}} - S_{\text{open}}) / (S_{\text{shrub}} + S_{\text{open}})$, where S_{shrub} and S_{open} are the values of species richness and plant abundance of the subordinate community measured in the presence and absence of a dominant shrub. This index is positive when species richness or plant abundance is higher beneath shrubs than in open areas and negative when species richness or plant abundance is higher in open areas than below shrubs. Values of RII not differing from zero suggest that species richness and plant abundance are equal underneath shrubs and in open areas. We calculated the mean value of RII per site and shrub species and tested for differences among sites and among shrub species with general linear models. As our model was unbalanced and incomplete (not all shrub species were present at all sites), we re-parametrized the model using a single factor with 18 “Site \times Species” levels and included plot as random effect (each of the quadrats randomly distributed in each site). We performed one-sample *t*-tests to check whether RII values within each site and cushion species were different from zero (i.e., neutral interaction). We excluded *H. spinosa* from these analyses due to the differences in sampling areas between the shrub and its paired open areas.

Assessing species diversity along the environmental gradient

We tested for differences in community composition analyzing plant density (number of individuals of each subordinate species per cm²) of all understory

species associated to each shrub species and at each elevation by performing a Principal Coordinates Analysis (PCoA). For this analysis, we excluded species with a frequency below 5% in order to avoid rare species effects. We used Bray-Curtis distances and extracted 3 axes which explained a total of 43% of the variance. To test if differences in plant density among shrub species were significant we used a multivariate mean comparisons test (gDGC test) based on cluster analysis, using a diagonal covariance matrix with a single linkage and a Monte Carlo simulation with 500 permutations (Valdano & Di Rienzo, 2007). Finally, to test the relationship between microhabitats (temperature and RH along the environmental gradient) and plant density (the site scores for axis 1, 2 and 3 extracted from PCoA analysis) we calculated Spearman's rank correlation (r_s).

Community level species richness and phylogenetic diversity

We assembled a phylogenetic tree with the 86 sampled species using Phylomatic3 (<http://phylodiversity.net/phyloomatic/>). All families in our database matched family names of the angiosperm megatree (R20120829), based on the Angiosperm Phylogeny Group (Stevens 2001). Branch lengths were adjusted with the Bladj algorithm taking age estimates for the main nodes from Wikstrom *et al.* (2001) and distributing undated nodes evenly among nodes of known age as implemented in Phylocom 4.2 (<http://phylodiversity.net/phylocom/>).

To quantify the effect of all shrubs increasing all community level species richness and phylogenetic diversity we used rarefaction curves that allowed accounting for differences in sampling effort (i.e. different square sizes) among microhabitats (i.e. shrubs and open areas). With community level we refer to the whole community at each of the sites (i.e. all the species including all shrubs and species in open areas). In the case of species richness, we used the function *specaccum* with the random method (Oksanen *et al.*, 2013) to determine the number of species for a number of plots by adding the

plots in random order. For phylogenetic diversity, the total branch length of a phylogenetic tree, we used the function *phylocurve* (<http://davidnipperess.blogspot.com.es/>), which calculates expected phylogenetic diversity for every number of individual per branch and per sample, selected at random, and using sampling without replacement (Nipperess & Matsen, 2013). These two measurements provide different information, as species richness considers species as equally distinct from one another while phylogenetic diversity considers how much evolutionary history is behind the species in a community (Faith, 1992). Phylogenetic diversity represents individuals by their relative branch lengths without the need to establish absolute species identity (Nipperess & Matsen, 2013). Thus, different shrubs can increase species richness to a similar extent but may differ regarding their effect on phylogenetic diversity (i.e. if two shrub species equally increased the number of species compared to open areas but species within one of the shrub species were closer in the phylogenetic tree than species within the other shrub). To evaluate the effect of shrubs on species diversity or phylogenetic diversity we calculated for each variable two rarefaction curves per site; one including open areas only and one taking into account all shrubs along with open areas, and plotted them for each site. All statistical analyses were conducted in InfoStat v. 2014 (<http://www.infostat.com.ar/index.php>) and R 3.0.2 (<http://www.r-project.org/>).

Results

Mean temperature in summer was higher in the southern than in the northern aspect, decreasing by $30 \pm 1.8\%$ (mean \pm 1SE) and $22 \pm 1.5\%$ from low to high elevation sites in the North and South aspects, respectively. Mean temperature beneath shrubs was 1 ± 0.3 °C and 1.6 ± 0.4 °C lower than in open areas in the North and South aspects, respectively. All along the gradient, temperatures were higher in open areas than beneath each shrub

species, except in the highest sites where temperatures did not differ (14.6°C and 14.5°C for shrubs and open areas respectively in the North and 17.5°C and 18.3°C in the South). Within sites, the most contrasted shrub effects on temperature were increases between 0.02°C and 1.6°C in the lowest South site (Fig. 1a, and Tables S2 and S3 for statistical results).

Mean relative humidity in summer increased by $20.8 \pm 2.5\%$ and $5 \pm 0.9\%$ from low to high elevation in the North and South aspects, respectively. Mean relative humidity beneath the shrubs was $7.5 \pm 2.9\%$ and $8.3 \pm 2.4\%$ higher than in open areas in the North and South aspects, respectively. Moreover, relative humidity was always higher beneath shrub species than in open areas all along the gradient except for *H. spinosa* at medium sites in both aspects. Within sites, the most contrasting shrub species differed on average from 1% to 10.3% at the low southern site (Fig. 1b and Table S2 and S3 for statistical results).

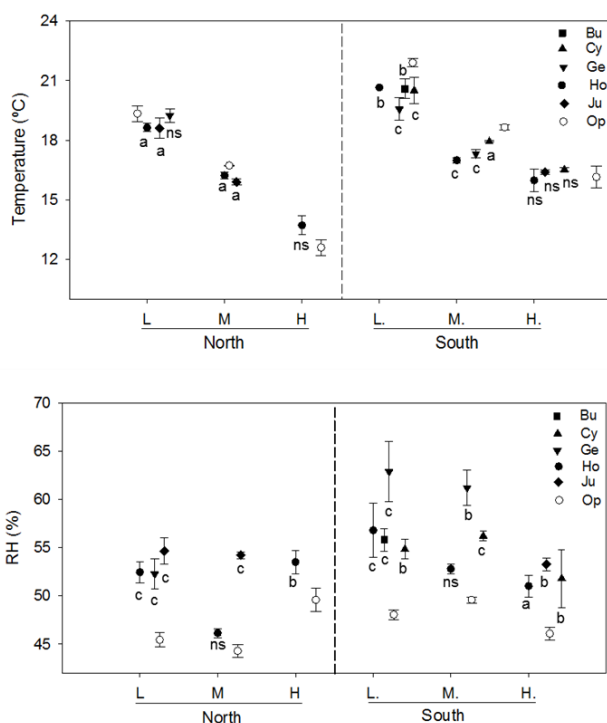


Fig. 1. Mean temperature and relative humidity (RH) of the air beneath shrub species and open areas along the environmental gradient. Values are means \pm 1SE. $n = 3$ for each species and site. L = Low elevation; M = Medium elevation; H = High elevation; Bu = *Bupleurum spinosum*; Cy = *Cytisus galianoi*; Ge = *Genista versicolor*; Ho = *Hormathophylla spinosa*; Ju = *Juniperus communis* ssp. *nana*; Op = open microhabitats. Asterisks indicate the results of the contrasts between each shrub species compared to open areas at each site: a= $p < 0.05$, b= $p < 0.01$, c= $p < 0.001$ and ^{ns} not significant results.

The net effect of shrubs on plant abundance (i.e., total number of individuals) and species richness changed in intensity and sign (RII) depending on shrub identity and environmental conditions. In general, we found positive and neutral effects at the most severe sites (i.e., highest and lowest elevations) and neutral to negative effects at mild environments (Intermediate sites; Fig. 2). In general, *A. tetraquetra* and *C. galianoi* had positive effects under harsh conditions and showed neutral or weak positive effects in mild environments. By contrast, *G. versicolor* and *P. holosteam* showed consistent neutral interactions all over the gradient while *J. communis* always had a negative effect.

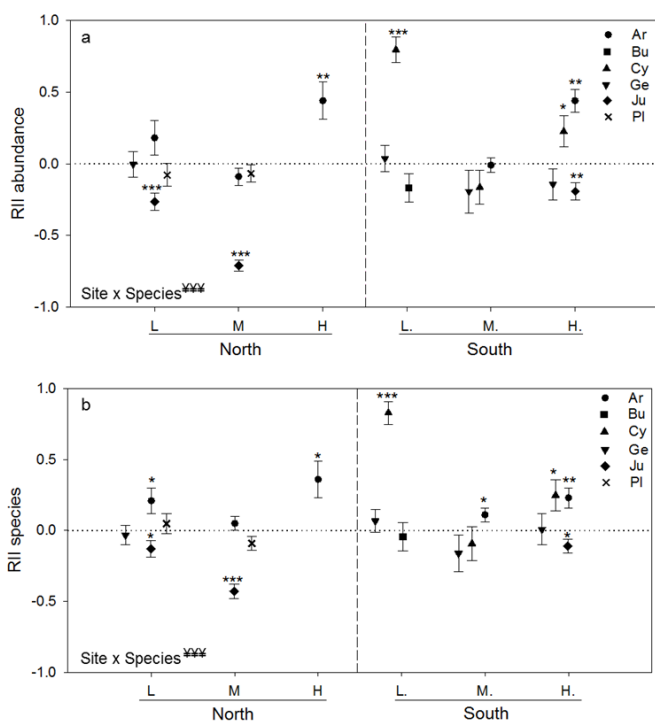


Fig. 2. Relative interaction index (RII) of every shrub species on the abundance (i.e., total number of individuals over all species) (a) and on the species richness (b) of the subordinate plant species along the elevation gradient (n = 30 per shrub and site). Data are means \pm 1SE. L = Low elevation; M = Medium elevation; H = High elevation; Ar = *Arenaria tetraquetra* ssp. *amabilis*; Bu = *Bupleurum spinosum*; Cy = *Cytisus galianoi*; Ge = *Genista versicolor*; Ho = *Hormathophylla spinosa*; Ju = *Juniperus communis* ssp. *nana*. Symbols with an asterisk represent RII values significantly different from 0; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Significant differences ($p < 0.001$) of the interaction between site and shrub species shown by ***.

Community composition of subordinate species (i.e., number of individuals per species and cm^2) differed among shrubs as shown by PCoA analysis (Fig. 3a) and post-hoc gDGC tests (Fig. 3b). Community composition was significantly different under the different shrub species in the same site, with some exceptions (e.g., *C. galianoi* and *H. spinosa* in the low site, S aspect; *C. galianoi* and *G. versicolor* at intermediate S site, and *H. spinosa* and *J. communis* at the high South site). Plant communities beneath shrub species changed among sites and between aspects, except for *J. communis* at the intermediate and low sites in the North. The number of dependent

subordinate species under each shrub species was variable, ranging 1-13 (Table S1). Subordinate communities changed with microhabitat conditions along the gradient. Axis 3 extracted from the PCoA analysis was positively correlated with RH; however, axis 1 and 2 from the PCoA did not correlate with any of the abiotic factors measured (i.e., temperature or RH, Table 1).

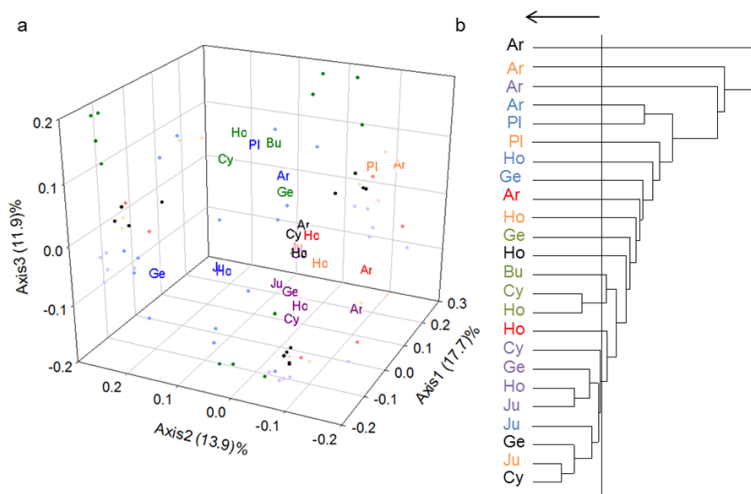


Fig. 3. Differences in subordinate species composition (individuals per species and cm^2) among sites and shrubs. Species with a frequency less than 5% were excluded (a) ($n = 59$). Multivariate mean comparisons (gDGCtest) testing differences in plant densities among shrubs along an environmental gradient based on cluster analysis. We used diagonal covariance matrix with a single linkage and Monte Carlo simulation with 500 permutations. The direction of the arrow indicates significant differences in the subordinate plant species density ($p < 0.05$) among each shrub (b). North aspect low in blue; North medium in orange; North high in red; South aspect low in green; South medium in black; South high in magenta. Ar: *Arenaria tetraquetra* ssp. *amabilis*; Bu: *Bupleurum spinosum*; Cy: *Cytisus galianoi*; Ge: *Genista versicolor*; Ho: *Hormathophylla spinosa*; Ju: *Juniperus communis* ssp. *nana*; Pl: *Plantago holosteum*.

Shrubs increased community level species richness (Fig. 4) and phylogenetic diversity (Fig. 5) in three out of the four most environmentally extreme sites of the gradient (i.e., the low site in the northern aspect and low and high sites in the southern aspect). The highest contribution was recorded in the warmest site (Fig. 4f and 5f) and with the highest differences in abiotic

conditions (temperature and RH) beneath shrubs vs. open areas (Fig. 1). In this site, *C. galianoi* showed strong effects on the subordinate plant community whereas other shrub species had neutral effects (Fig. 3). Phylogenetic diversity paralleled species richness throughout.

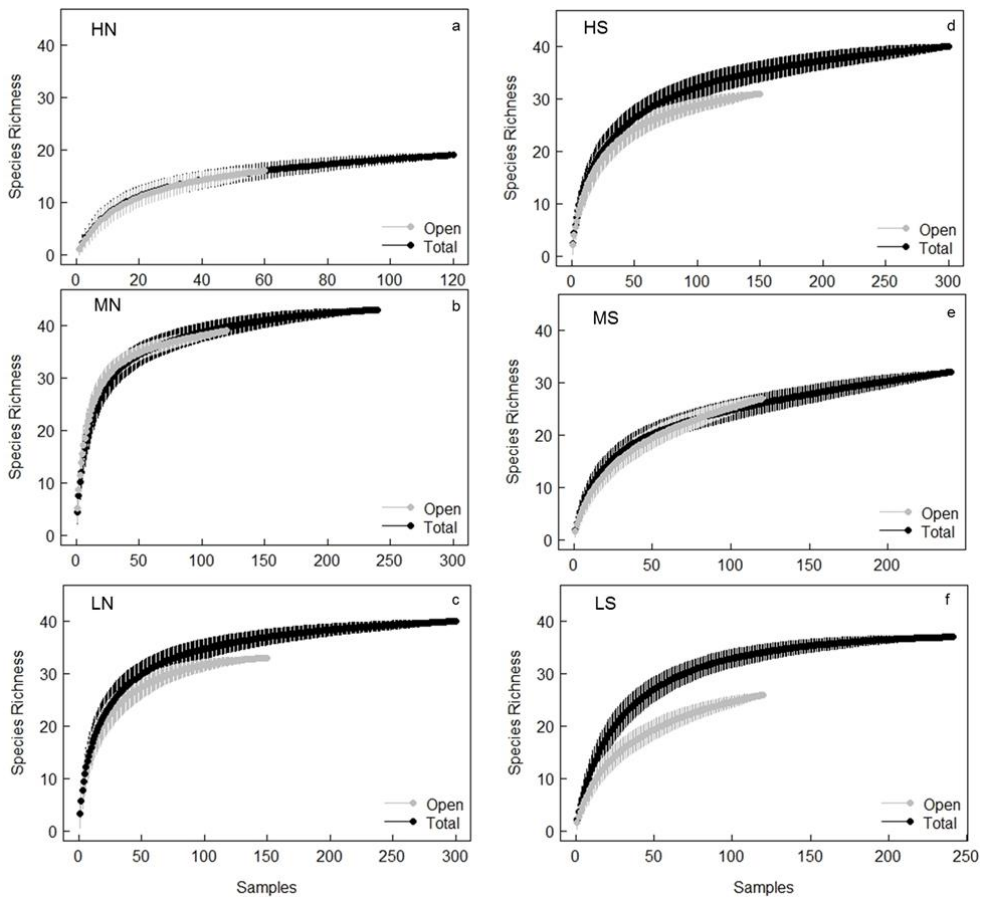


Fig. 4. Shrub contribution to community species richness at each site. (a) HN: Northern aspect high with two shrub species; (b) MN: Northern medium with four shrub species; (c) LN: Northern low with five shrub species; (d) HS: Southern aspect high with five shrub species; (e) MS: Southern medium with four shrub species; (f) LS: Southern low with four shrub species. Values are mean \pm SD. Significant differences are indicated by non-overlapping error terms of the curves at the asymptote.

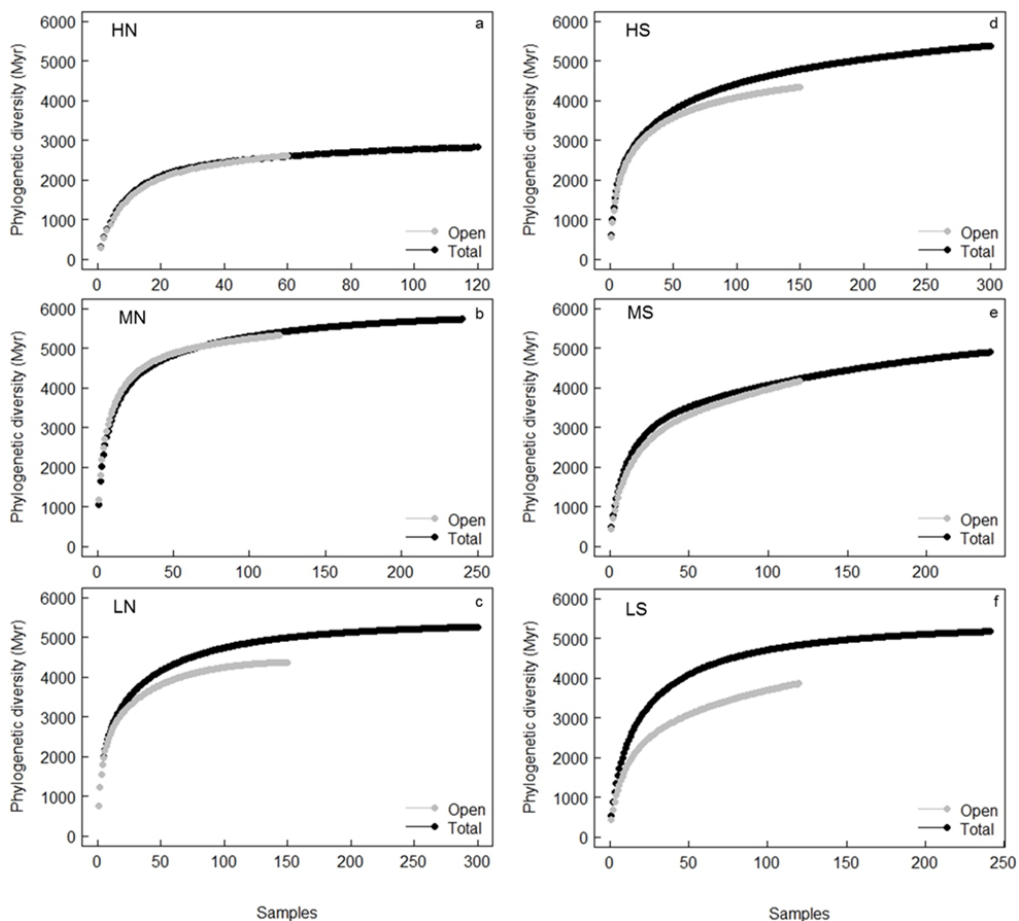


Fig. 5. Shrub contribution to community phylogenetic diversity (total branch length of the tree in Myr) for each site. (a) HN: Northern aspect high with two shrub species; (b) MN: Northern medium with four shrub species; (c) LN: Northern low with five shrub species; (d) HS: Southern aspect high with five shrub species; (e) MS: Southern medium with four shrub species; (f) LS: Southern low with four shrub species. Displayed values are mean rooted phylogenetic distance.

Discussion

Shrub microhabitats buffered harsh abiotic conditions by decreasing summer maximum temperatures and increasing RH with respect to open areas all along the gradient. Composition of subordinate communities differed among shrubs and sites, although occasionally some shrub species hosted similar

communities within a site. Differences in community composition among shrub species were related to relative humidity, suggesting a dependence of beneficiary species on micro-environments created by shrubs. Positive effects of shrubs on overall plant abundance and species richness were more evident in the most severe parts of the gradient, while under relatively milder conditions shrub effects were mostly neutral or negative. Such effects differed with shrub identity and elevation. In sites where microhabitat differences were most extreme (i.e. high North due to low temperatures or low South due to high temperatures and low relative humidity), the different shrub species had complementary effects on subordinate species richness resulting in increased whole-community species richness and phylogenetic diversity beyond the level of single-shrub effects. This complementarity was evident when at least one shrub species showed positive effects on species richness and abundance compared to open areas, and where environmental severity was greatest. However, complementarity was absent at sites of low environmental severity and where individual shrub species had non-significant or negative effects on species richness and plant abundance.

Differences in microhabitat conditions

Shrubs can modify the microhabitat beneath their canopies influencing growth and survival of beneficiary species (Brooker & Callaway, 2009; Cavieres & Badano, 2009; Anthelme *et al.*, 2014). For example, they can buffer temperature extremes under their canopies reducing summer heat shock, transpiration, and soil moisture evaporation and reducing winter frost damage (Körner, 2003). In our case not all shrub species within a site changed its microhabitat in the same way, suggesting that species with different canopy morphology created different microhabitats leading to differentiated communities of subordinate species. Molina-Montenegro *et al.* (2006) showed significant differences between microhabitat conditions of two cushion species (*Azorella monantha* and *Laretia acaulis*) in an alpine zone of Central

Chile but they did not evaluate their effects on understory plant communities; however, most studies performed in alpine systems that measured more than one cushion species did not distinguish their different effects on microhabitat conditions (Cavieres *et al.*, 2007; Wang *et al.*, 2008; Chu *et al.*, 2009).

Shrub effects on subordinate richness and overall abundance along the gradient

Plant interaction intensity and sign are expected to change depending on abiotic conditions so that positive interactions would prevail at the most severe part of the gradient while negative interactions would prevail at the milder part as predicted by the stress gradient hypothesis (SGH; Bertness & Callaway, 1994). In fact, we found that positive effects of shrubs on plant diversity were stronger under harsh conditions than under mild conditions, where shrubs had neutral and/or negative effects. This was also the case along the North aspect of the Sierra Nevada mountains for *A. tetraquetra* where Schöb *et al.* (2013) found that species richness and abundance underneath these shrubs was highest at the most stressful sites. These results support the role of facilitation as expanding distribution range of beneficiary species (Armas *et al.*, 2011; Butterfield *et al.*, 2013; Valiente-Banuet & Verdú, 2013; Pistón *et al.*, 2015).

Shrubs showing positive effects on richness and plant abundance under harsh conditions changed their effect to neutral under milder conditions, while shrubs that showed neutral or negative effects on plant diversity never had positive effects (i.e., they were always non-facilitator species). In severe environments plant interactions are dependent on plant morphology (i.e., morphological traits) and physiological traits with high degree of specificity (Armas & Pugnaire, 2011; Schöb *et al.*, 2013; Aubert *et al.*, 2014). Thus, two shrub species with similar canopies may differ in allelopathic effects, such as *J. communis* in our field sites (Castro *et al.*, 2005); others, however, may host plant symbionts (e.g., N-fixing bacteria), growth-promoting bacteria or mycorrhizas (Martínez, 2009; Rodríguez-Echeverría *et al.*, 2013). Therefore,

niches of coexisting shrub species may be similar but rarely identical, and these species-specific differences could influence the resulting subordinate community under each shrub species.

Differences in subordinate community composition among shrub species

Community composition differed among shrub species along the gradient, with few exceptions. Beneath each shrub there was a variable but rather high number of subordinate species, stressing the importance of shrub identity on shaping subordinate community composition and assembly (Wardle & Zackrisson, 2005; Schöb *et al.*, 2013; Bråthen & Ravolainen, 2015; Chen *et al.*, 2015). Although shrub species may appear functionally redundant under certain environmental conditions, the microhabitats they create could actually differ. For example, *C. galianoi* and *G. versicolor* share similar morphology but the microclimate beneath their canopies was vastly different, influencing community composition of subordinate species. Therefore, finding complementarity i.e., the process by which the effect of a diverse community of shrubs increases the performance of whole plant community above that expected from the effect of each individual shrub species (Huston, 1997), was very likely in our sites. That means that assemblages of several shrub species fill larger niche space and create more environmental heterogeneity (Harper, 1977; Jones *et al.*, 1994a) than a single species, therefore deeply influencing whole-community species richness, phylogenetic diversity and ecosystem function (Loreau *et al.*, 2001; Cadotte, 2013).

Environmental context-dependence of complementarity effects

The increase in species richness due to complementarity was parallel to an increase in phylogenetic diversity; i.e., shrubs hosting new species increased whole-community phylogenetic diversity. The highest contribution of shrubs to species richness and phylogenetic diversity was found in the low South site, the place with the most intense positive effects of shrubs.

The low species richness and phylogenetic diversity in open areas with high severity was likely consequence of non-random species loss. This process is expected if close relatives share similar adaptations (Webb *et al.*, 2002); for example, adaptation to high radiation and reduced water availability (Schöb *et al.* 2013). Shrubs could buffer species loss, resulting in richer communities with higher phylogenetic diversity. An exception of this was found at the high site of the northern aspect, where conditions were probably the most extreme and the presence of the shrubs did not ameliorate the severe microclimate.

As environmental severity decreased, community richness and phylogenetic diversity under the shrub was similar to that of open areas. In other words, under benign conditions there was no positive complementarity effect of shrubs. This result is in accordance with the stress gradient hypothesis (Bertness & Callaway, 1994), suggesting that species interactions are neutral or negative where severity is milder; in fact, this is what we observed between shrub species and their subordinate community in two of our study sites (i.e., both at mid-elevations). Another plausible explanation is that coexistence was stable at these sites following the spatial storage effect; this mechanism is the most feasible enabling coexistence in a heterogeneous environment buffering population growth rate (also called "source-sink dynamics"; Dias, 1996), thus preventing the exclusion of species inhabiting the whole community (Amarasekare, 2003; Sears & Chesson, 2007). Therefore, shrubs could act as source habitat for some species (i.e., those that show significantly higher fitness within shrubs than in opens) and as sinks for species that dominate open habitats (i.e., those that are less abundant in shrubs than in the open areas). In some places e.g., the highest plot in the South aspect, open areas could be acting as a sinks and *A. tetraquetra* and *C. galianoi* as source of species and phylogenetic diversity. However, to verify the presence of such source-sink dynamics in our study sites we would need to quantify the *per capita* growth rates of each subordinate species (Dias,

1996). Nevertheless, just by comparing species abundances beneath shrubs and in open areas in our study sites indications of such processes can be identified. As an example, for the 28 individuals of *Coincya monensis* found in shrubs at the low south site only two individuals were found in open areas (Table S4). This is a clear indication that shrubs may act as a source habitat for this species at this specific site and that spatial storage effects could be indeed be relevant in our study system.

Conclusions

Our study showed the importance of complementary effects among co-occurring shrub species on species richness and phylogenetic diversity at whole-community level. This was likely due to the positive effect of shrubs on different subsets of subordinate plant species, allowing stress-sensitive species to survive in environments too harsh without specific nurse species protection. Thus, the effect of different shrubs species on plant community richness is not redundant as they may host unique communities, particularly under harsh environmental conditions. This highlights the importance of keeping shrub species, as their complementary effects may be critical to maintain biodiversity and ecosystem functions of this fragile ecosystem.

Appendix A. Supplementary data

Aspect	Elevation	Shrubs	No of shrub dependent species
North	High	Ar	4
North	High	Ho	3
North	Medium	Ar	4
North	Medium	Ho	3
North	Medium	Ju	6
North	Medium	Pl	1
North	Low	Ar	2
North	Low	Ge	8
North	Low	Ho	5
North	Low	Ju	6
North	Low	Pl	2
South	High	Ar	5
South	High	Cy	3
South	High	Ge	7
South	High	Ho	6
South	High	Ju	9
South	Medium	Ar	2
South	Medium	Cy	2
South	Medium	Ge	1
South	Medium	Ho	4
South	Low	Bu	8
South	Low	Cy	13
South	Low	Ge	11
South	Low	Ho	11

Table A1. Number of subordinate species growing only beneath shrub species in each site. Ar: *Arenaria tetraquetra* ssp. *amabilis*; Bu: *Bupleurum spinosum*; Cy: *Cytisus galianoi*; Ge: *Genista versicolor*; Ho: *Hormathophylla spinosa*; Ju: *Juniperus communis* ssp. *nana*; Pl: *Plantago holostium*.

Variable	df	p-value
Temperature		
Aspect	1	<0.0001
Elevation	2	<0.0001
Treatment	1	<0.0001
Aspect x Elevation	2	0.061
Aspect x Treatment	1	0.029
Elevation x Treatment	2	0.003
Aspect x Elevation x Treatment	2	0.779
Relative humidity		
Aspect	1	0.0003
Elevation	2	0.888
Treatment	1	<0.0001
Aspect x Elevation	2	<0.0001
Aspect x Treatment	1	0.299
Elevation x Treatment	2	0.106
Aspect x Elevation x Treatment	2	0.546

Table A2. Statistical results of the factorial ANOVAs with temperature and relative humidity of air as response variables and mountain aspect (North vs. South), elevation (Low, Medium, High) and treatment (shrubs vs. open areas) as fixed factors. n = 3 per site and shrub species and open area. Statistically significant ($p < 0.05$) results are shown in bold.

Site	Contrast	n	p-value T (C°)	p-value RH (%)
HN	Ho vs. Op	3	0.793	0.002
MN	Ho vs. Ju	3	0.545	0.0004
MN	Ho vs. Op	3	0.046	0.343
MN	Ju vs. Op	3	0.011	<0.0001
LN	Ge vs. Ho	3	0.222	0.958
LN	Ge vs. Ju	3	0.214	0.287
LN	Ge vs. Op	3	0.275	0.0009
LN	Ho vs. Ju	3	0.978	0.311
LN	Ho vs. Op	3	0.019	0.0007
LN	Ju vs. Op	3	0.021	<0.0001
HS	Cy vs. Ho	3	0.351	0.651
HS	Cy vs. Ju	3	0.786	0.562
HS	Cy vs. Op	3	0.605	0.006
HS	Ho vs. Ju	3	0.508	0.305
HS	Ho vs. Op	3	0.151	0.018
HS	Ju vs. Op	3	0.434	0.001
MS	Cy vs. Ge	3	0.237	0.02
MS	Cy vs. Ho	3	0.078	0.091
MS	Cy vs. Op	3	0.022	0.001
MS	Ge vs. Ho	3	0.55	0.0002
MS	Ge vs. Op	3	0.0007	<0.0001
MS	Ho vs. Op	3	0.0001	0.107
LS	Bu vs. Cy	3	0.485	0.688
LS	Bu vs. Ge	3	0.007	0.0009
LS	Bu vs. Ho	3	0.71	0.609
LS	Bu vs. Op	3	0.002	0.0001
LS	Cy vs. Ge	3	0.042	0.0003
LS	Cy vs. Ho	3	0.752	0.374
LS	Cy vs. Op	3	0.0005	0.001
LS	Ge vs. Ho	3	0.02	0.004
LS	Ge vs. Op	3	<0.0001	<0.0001
LS	Ho vs. Op	3	0.001	<0.0001

Table A3. A priori contrasts analyzing differences in temperature and relative humidity (RH) among species at each site. LN: Northern aspect low; MN: Northern medium; HN: Northern high; LS: Southern aspect low; MS: Southern medium; HS:

Southern high. Bu = *Bupleurum spinosum*; Cy = *Cytisus galianoi*; Ge = *Genista versicolor*; Ho = *Hornathophylla spinosa*; Ju = *Juniperus communis* ssp. *nana*; Op = open microhabitats. Significant results ($p < 0.05$) are in bold. N is the number of sensors per shrub species and site.

	HN		MN		LN		HS		MS		LS	
	sh	op	sh	op	sh	op	sh	op	sh	op	sh	op
<i>Acinos alpinus</i>	0	0	0	0	7	1	0	0	0	0	0	0
<i>Agrostis nevadensis</i>	0	0	1	20	0	0	0	0	88	26	2	0
<i>Alyssum montanum</i>	0	0	0	0	123	124	0	0	0	0	0	0
<i>Alyssum nevadense</i>	0	0	0	0	0	0	2	1	0	1	0	2
<i>Andryala ragusina</i>	0	0	0	0	0	0	0	0	0	0	7	0
<i>Anthyllis vulneraria</i>	0	0	3	12	0	0	1	0	0	0	0	0
<i>Arenaria grandiflora</i>	0	0	0	0	0	0	71	108	8	4	2	0
<i>Arenaria pungens</i>	0	0	0	0	0	0	8	0	3	3	0	0
<i>Arenaria tetraquetra</i>	8	12	13	24	40	62	0	0	0	1	0	0
<i>Armeria filicaulis</i>	0	0	0	0	21	12	0	0	0	0	0	0
<i>Artemisia absinthium</i>	0	0	0	0	1	2	0	0	0	0	0	0
<i>Artemisia chamaemelifolia</i>	0	0	0	0	0	0	0	0	0	0	4	3
<i>Asperula aristata</i>	0	0	0	0	8	2	14	10	2	0	4	0
<i>Asterolinum sp.</i>	0	0	0	0	19	16	0	0	0	0	0	0
<i>Avena sp.</i>	0	0	1	0	0	0	0	0	0	0	0	0
<i>Avenella flexuosa</i>	3	1	53	14	133	108	75	11	3	2	2	11
<i>Avenula bromoides</i>	0	0	0	0	0	0	0	6	0	0	18	1
<i>Avenula laevis</i>	0	0	1	0	3	4	5	0	0	0	0	0
<i>Biscutella glacialis</i>	3	3	5	8	23	11	0	0	8	3	0	0
<i>Bromus tectorum</i>	0	0	0	0	11	14	0	0	0	0	11	96

	HN		MN		LN		HS		MS		LS	
	sh	op	sh	op	sh	op	sh	op	sh	op	sh	op
<i>Bupleurum spinosum</i>	0	0	0	0	0	0	0	0	0	0	22	6
<i>Campanula lusitanica</i>	0	0	0	0	0	0	29	4	0	0	5	4
<i>Carduus carlinoides</i>	6	9	0	0	0	0	0	0	0	0	6	3
<i>Centaurea sp.</i>	0	0	0	0	2	0	0	0	0	0	9	0
<i>Cerastium gibraltarium</i>	0	0	0	0	5	5	0	0	0	0	10	9
<i>Chaenorhinum glareosum</i>	8	13	0	0	0	0	0	0	0	0	0	0
<i>Coincya monensis</i>	0	0	0	0	28	2	0	0	0	0	0	0
<i>Cuscuta triumvirati</i>	0	0	0	0	0	0	7	0	2	0	5	0
<i>Cytisus galianoi</i>	0	0	0	0	12	0	96	36	19	7	20	13
<i>Dactylis glomerata</i>	0	0	4	1	0	0	0	0	0	0	0	0
<i>Dianthus brachyanthus</i>	0	0	8	23	16	15	0	5	0	0	10	4
<i>Draba hispanica</i>	0	0	4	24	5	11	0	0	0	0	0	0
<i>Erigeron frigidus</i>	1	5	0	0	0	0	0	0	0	1	0	0
<i>Eryngium bourgatii</i>	0	2	0	0	1	0	0	0	0	0	6	19
<i>Erodium cheilanthifolium</i>	0	0	0	0	0	0	1	0	13	18	0	0
<i>Erophila verna</i>	0	0	0	1	1	4	0	1	1	55	0	0
<i>Eryngium glaciale</i>	10	7	2	0	2	3	14	9	1	0	0	0
<i>Erysimum nevadense</i>	0	0	14	11	2	0	0	5	34	23	0	0
<i>Euphorbia nevadensis</i>	0	0	0	0	21	12	11	2	1	1	5	0
<i>Euphorbia nicaeensis</i>	0	0	0	0	0	0	0	0	0	0	18	0

	HN		MN		LN		HS		MS		LS	
	sh	op	sh	op	sh	op	sh	op	sh	op	sh	op
<i>Euphrasia willkommii</i>	1	1	8	204	0	0	0	0	1	0	0	0
<i>Festuca sp.</i>	0	0	0	0	0	0	0	0	0	0	1	0
<i>Festuca clementei</i>	9	2	47	5	0	0	0	0	0	0	0	0
<i>Festuca indigesta</i>	0	0	187	530	230	249	69	155	121	162	194	201
<i>Festuca pseudeskia</i>	0	0	0	0	0	0	0	0	0	0	5	1
<i>Galium nevadense</i>	0	0	2	8	0	0	0	0	0	0	0	0
<i>Galium pyrenaicum</i>	90	44	17	3	0	0	157	114	0	0	0	0
<i>Genista versicolor</i>	0	0	0	0	0	0	0	0	0	0	14	11
<i>Herniaria boissieri</i>	2	2	0	31	2	0	0	0	0	0	0	5
<i>Hormathophylla spinosa</i>	1	0	8	15	11	9	25	14	0	0	16	2
<i>Jasione amethystina</i>	56	9	132	73	0	0	107	30	10	2	0	0
<i>Jasione crispa</i>	21	34	28	31	0	0	1	21	1	0	6	1
<i>Jurinea humilis</i>	1	0	1	2	9	15	0	0	7	4	8	17
<i>Koeleria vallesiana</i>	0	0	0	0	16	0	0	0	0	0	0	0
<i>Leontodon boryi</i>	10	0	10	18	4	4	3	8	14	4	1	2
<i>Leucanthemopsis pectinata</i>	0	0	3	2	0	0	0	0	0	0	0	0
<i>Linaria aeruginea</i>	0	0	4	28	0	0	2	3	0	1	0	0
<i>Lilium sp</i>	0	0	0	0	0	0	1	0	0	0	0	0
<i>Lotus corniculatus</i>	0	0	209	58	0	0	7	0	0	0	0	0
<i>Minuartia funkii</i>	0	0	0	0	15	165	0	0	0	0	0	0

	HN		MN		LN		HS		MS		LS	
	sh	op	sh	op	sh	op	sh	op	sh	op	sh	op
<i>Nevadensia purpurea</i>	8	11	21	25	0	0	156	154	20	3	0	0
<i>Ononis sp.</i>	0	0	1	1	0	0	0	0	0	0	4	2
<i>Ononis spinosa</i>	0	0	0	0	0	0	0	5	0	0	15	0
<i>Paronychia polygonifolia</i>	0	0	0	10	0	0	0	0	0	0	0	0
<i>Pilosella sp.</i>	0	0	12	121	210	248	33	11	33	62	12	0
<i>Pimpinella procumbens</i>	0	0	28	23	0	0	0	0	0	0	0	0
<i>Plantago holosteum</i>	0	0	19	26	10	35	1	0	1	2	0	0
<i>Poa ligulata</i>	0	0	118	356	55	106	0	0	71	177	0	4
<i>Ranunculus acetosellifolius</i>	0	0	0	0	0	0	1	1	1	1	0	0
<i>Rumex angiocarpus</i>	0	0	2	37	38	110	0	0	1	3	0	0
<i>Sanguisorba verrucosa</i>	0	0	0	0	0	0	0	0	0	0	18	1
<i>Scabiosa sp.</i>	0	0	0	0	3	0	0	0	0	0	0	0
<i>Sedum amplexicaule</i>	0	0	119	795	230	346	0	0	0	0	0	3
<i>Sedum gypsicola</i>	0	0	0	0	0	2	0	0	0	0	0	0
<i>Sempervivum minutum</i>	0	0	13	35	0	0	0	0	0	0	0	0
<i>Senecio boissieri</i>	0	0	1	4	1	4	4	9	22	27	0	0
<i>Sesamoides purpurascens</i>	0	0	2	0	0	0	1	8	0	0	0	0
<i>Sideritis glacialis</i>	0	0	2	9	0	0	8	8	0	0	0	0
<i>Silene boryi</i>	0	0	5	20	98	94	10	29	0	1	0	0
<i>Silene sp.</i>	0	0	0	0	0	0	0	11	0	0	0	0

	HN		MN		LN		HS		MS		LS	
	sh	op	sh	op	sh	op	sh	op	sh	op	sh	op
<i>Tamarix sp.</i>	0	0	0	0	0	5	0	0	0	0	0	0
<i>Teucrium sp.</i>	0	0	0	0	43	13	1	2	0	0	3	2
<i>Thlaspi nevadense</i>	0	0	0	0	0	0	33	0	0	0	0	0
<i>Thymus serpylloides</i>	0	0	101	167	0	0	94	112	25	15	59	12
<i>Trisetum glaciale</i>	57	15	1	2	0	0	0	0	0	0	0	0
<i>Viola crassiuscula</i>	0	0	0	0	0	0	2	1	0	0	0	0

Table A4. Species list and the number of individuals below shrubs and in open areas for each subordinate species at each site. LN: Northern aspect low; MN: Northern medium; HN: Northern high; LS: Southern aspect low; MS: Southern medium; HS: Southern high. sh: plants growing beneath shrubs; op: plants growing in open areas.

CHAPTER II

Phylogenetic distance among beneficiary species in a cushion plant species explains interaction outcome

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Abstract

Determining which drivers lead to a specific species assemblage is a central issue in community ecology. Although many processes are involved, plant-plant interactions are among the most important. The phylogenetic limiting similarity hypothesis states that closely related species tend to compete stronger than distantly related species, although evidence is inconclusive. We used ecological and phylogenetic data on alpine plant communities along an environmental severity gradient to assess the importance of phylogenetic relatedness in affecting the interaction between cushion plants and the whole community, and how these interactions may affect community assemblage and diversity. We first measured species richness and individual biomass of species growing within and outside the nurse cushion species, *Arenaria tetraquetra*. We then assembled the phylogenetic tree of species present in both communities and calculated the phylogenetic distance between the cushion species and its beneficiary species, as well as the phylogenetic community structure. We also estimated changes in species richness at the local level due to the presence of cushions. The effects of cushions on closely related species changed from negative to positive as environmental conditions became more severe, while the interaction with distantly related species did not change along the environmental gradient. Overall, we found an environmental context-dependence in patterns of phylogenetic similarity, as the interaction outcome between nurses and their close and distantly-related species showed an opposite pattern with environmental severity.

Introduction

Finding out which mechanisms interact at different spatial scales leading to a given species assemblage is a central issue in community ecology. These mechanisms can be separated relative to the spatial scale into external and internal filters, and involve processes acting at regional scale (i.e., beyond single communities) as well as processes within local communities (Ricklefs, 2010; Violle *et al.*, 2012). External filters include factors such as climate, which sort species out from the regional pool and are important determinants of plant community composition (Gaston, 2000). Similarly, internal filters such as microscale environmental heterogeneity (Fibich *et al.*, 2013) or plant-plant interactions (Armas *et al.*, 2011) can affect community composition and structure. These filters are not independent; for instance, the outcome of plant-plant interaction varies depending on external filters such as climatic conditions (He *et al.*, 2013). Internal filters may interact with each other as well, e.g. when small-scale environmental heterogeneity affects plant-plant interactions (Choler *et al.*, 2001). Therefore, an approach unifying different drivers, including external and internal filters, is needed to disentangle their relative importance and impact on community assembly.

The integration of phylogenetic information in community analyses has proved to be a powerful tool in understanding changes in species composition. Closely related species tend to share similar trait values, hence may have similar requirements and affect their microenvironment in similar ways; as a consequence, competition is stronger between them than with distantly related species (Violle *et al.*, 2011). This idea was formalized as the phylogenetic limiting similarity hypothesis (PLSH; MacArthur & Levins, 1967) and assumes that ecological traits influencing species competition are conserved along phylogenetic lineages (Blomberg *et al.*, 2003; Wiens & Graham, 2005). However, evidence supporting this hypothesis is inconclusive (Cahill *et al.*, 2008; Mayfield & Levine, 2010).

The species pool under mild environmental conditions is larger and shows wider range of ecological niche space than under severe conditions (Grime & Pierce, 2012). Opposite, under harsh environmental conditions external filters (e.g., climate) will severely restrict the species pool, favouring only the presence of species adapted to such demanding conditions (Choler, 2005). Assuming that species strategies are phylogenetically conserved (Blomberg *et al.*, 2003; but see Mayfield & Levine, 2010), the outcome of plant-plant interactions under mild environmental conditions could render communities phylogenetically diverse, including closely related species. Hence, at the local scale closely related species can show strong competition, following PLSH predictions. By contrast, under harsh environmental conditions the species pool is reduced and it could be expected that species will be more phylogenetically related (Webb *et al.*, 2002) as the environment selects for a given suite of traits.

Under harsh environmental conditions nurse plant species allow for the presence of many other species in the community (Callaway 2007). In alpine environments, a particular case of nurses are cushion plants, which usually ameliorate environmental conditions and facilitate growth and survival of other species (beneficiaries) within them (Badano & Cavieres, 2006; Cavieres & Badano, 2009). These nurse species can create communities more phylogenetically diverse than communities in open habitats (Butterfield *et al.*, 2013). However, environmental severity restricts the species pool, selecting for species sharing similar traits and more phylogenetically related (Soliveres *et al.*, 2012a). This leads to a paradox between competition intensity among phylogenetically related species and environmental severity that needs more exploration.

To test how plant-plant interactions and phylogenetic relatedness affect each other and how they change along a severity gradient, we analysed the phylogenetic relationships between cushions on other species in three sites along an elevation gradient in the alpine belt of the Sierra Nevada Mountains,

Spain. This gradient reflects an increase in environmental severity with elevation characterized by changes in temperature, water availability, and soil nutrients (Sánchez-Marañón *et al.*, 2002; Schöb *et al.*, 2013). We used biomass data to assess interaction outcome for resources and to test the PLSH; and richness as a consequence of plant interactions on presence and survival. Specifically, we expected that a) the effects of cushion species on its closely-related beneficiary species would vary from very negative to neutral as environmental severity increased; however, the outcome of the interaction of the nurse with its distantly-related beneficiary species would change from neutral to very positive with increasing environmental severity; b) phylogenetic diversity would change along the gradient, from a community mostly characterized by distantly related species in the less severe environment to one made up by closely related species in the most severe environment; and c) the contribution of cushions to phylogenetic diversity and species richness would make communities within cushions more diverse than in open areas.

Methods

Field sites, species and data collection

The study was conducted on the north-western slope of the Sierra Nevada Mountains, Spain. We selected three field sites at 2720 m (37°05' N, 03°23' W), 3000 m (37°04' N, 03°22' W) and 3240 m elevation (37°03' N, 03°22' W) encompassing an important gradient in temperature and precipitation (Delgado *et al.*, 1988; Schöb *et al.*, 2013). In this mountain system, and in the range of altitudes where the field sites are located, environmental severity increases with elevation due to decreasing temperature and soil quality, while soil water availability may not be limiting (Schöb *et al.* 2013a).

Overall, climate is continental Mediterranean with a hot and rather dry summer. Mean annual rainfall at the closest met station (Pradollano; 2500

m elevation) is 690 mm, and mean annual temperature is 3.9 °C (Worldwide Bioclimatic Classification System 1996-2009). Above 3200 m prevail plant communities with perennial herbaceous species such as *Erigeron frigidus*, *Festuca clementei*, *Linaria glacialis*, and *Viola crassiuscula*, including a high number of endemic species. Shrublands prevail below 3000 m, with *Genista versicolor*, *Hormathophylla spinosa*, *Juniperus communis ssp. hemisphaerica*, *Sideritis glacialis*, and *Thymus serpylloides* being the dominant species (Valle, 2003). Livestock pressure within our study areas was not high due to its regulation as a National Park (Decreto 238/2011, de 12 de julio).

The dominant cushion species occurring at all three field sites was *Arenaria tetraquetra ssp. amabilis* (Bory) H. Lindb. fil. (Caryophyllaceae), a perennial shrub ranging 10-300 cm² in area that often acts as nurse for other species (Schöb *et al.*, 2012; Schöb *et al.*, 2013). To assess the contribution of *Arenaria* cushions to community structure, we sampled one hundred 50x50 cm quadrats randomly distributed in each site, identified all species and recorded the number of individuals per species growing in cushions and in the open for each plot. In general, there was at least one cushion plant (mean area of 180 cm² ± 6.17 cm²) within each quadrat except a few quadrats without any cushion, i.e. only open areas.

To evaluate the intensity of plant-plant interactions depending on their phylogenetic relatedness we collected aboveground mass of 20 mature individuals of each of the most common species (14, 12 and 9 species at the low, medium and high sites, respectively; see Appendix 1 in Supporting Information), 10 growing inside *Arenaria* cushions and 10 from open areas. Sampling was paired, collecting one individual from within the cushion and another from an adjacent open area. We selected individuals from open areas more than 35 cm away from *Arenaria* cushions in order to avoid any potential interactions with cushions. Samples were oven-dried at 70 °C for 48 h and weighed.

Effect of cushions on species biomass

We measured the interaction outcome between *Arenaria* and each of the other target species as the relative change in biomass of individuals growing within *Arenaria* compared to those growing in open areas. We used the Relative Interaction Index (Armas *et al.*, 2004) as $RII = (B_{\text{cushion}} - B_{\text{open}})/(B_{\text{cushion}} + B_{\text{open}})$, where B is the biomass of individuals of the target species growing within *Arenaria* (B_{cushion}) or in open areas (B_{open}). This index has positive values when *Arenaria* facilitates other species (i.e., the biomass of the individual growing within *Arenaria* is greater than the one growing in open areas) and is negative when the net effect of *Arenaria* is competitive. Zero RII values suggest that negative and positive effects of *Arenaria* on target species are equal. We calculated the mean value of RII per site (Fig. 1a) and tested whether RII depended on phylogenetic relatedness (Fig. 1b). For this, we assembled a phylogenetic tree for all the species recorded at our three sites (57 sp) using Phylomatic3 (<http://phylodiversity.net/phyloomatic/>). All families in our dataset matched the family names of Angiosperms megatree used in Phylomatic (R20120829), that reflects the consensus of the Group (2009). Branch lengths were adjusted with the Phylocom Bladj algorithm (<http://phylodiversity.net/phylocom/>) by computing age estimates for major nodes in our tree (Wikstrom *et al.*, 2001) and distributing undated nodes evenly between those of known ages (see Appendix 2). We then obtained phylogenetic distances (PD) among *Arenaria* and the other species using the function `cophenetic.phylo` (picante library; Kembel *et al.*, 2010) which calculates distances between pairs of tips in our phylogenetic tree using branch length. To test for changes in RII with elevation we used one-way ANOVA; we then used a second model with elevation and phylogenetic distance between *Arenaria* and each target species. As the second model was unbalanced and incomplete, we re-parametrized it in a single factor with 12 “Elevation x PD” levels. We performed one-sample *t*-tests to check whether

RII values within each site and phylogenetic distance were different from zero (i.e., neutral interaction). Post-hoc differences were examined with LSD Fisher's tests corrected by Bonferroni for multiple-comparisons.

Contribution of cushions to phylogenetic diversity

We assessed the effect of cushions on phylogenetic community structure at each site by considering all samples in the site (open + cushion) as compared to open areas within plots (open), the latter being a reflection of the intensity of environmental filtering. We calculated two metrics of phylogenetic community diversity per plot, the mean phylogenetic distance (MPD) and mean nearest phylogenetic taxon distance (MNTD) (picante library; Kembel *et al.*, 2010). Both range 0 to infinity; small values represent communities composed of species closely related and large values represent communities with species distantly related. MNTD is typically used to test PLSH as it is sensitive to co-occurrence patterns among closely related species. However, MNTD contains much less information than MPD, which reflects the phylogenetic diversity of taxa over the whole pool of species (Webb, 2000). Plots with less than two species were excluded from the analyses as they were uninformative. MPD and MNTD were calculated by weighting species abundance; abundance data were log-transformed to minimize the effect of particularly abundant species (Butterfield *et al.* 2013). We preferred observed over the expected phylogenetic distances (i.e., NRI and NTI) as we use phylogenetic distances as a factor to explain the intensity of plant-plant interactions. As such, absolute distances between species seem more appropriate than their deviations from a random pattern. To check for changes in MPD and MNTD along the severity gradient and among microhabitats, we used linear mixed models. Elevation and microhabitat (cushion+open vs. open) and their interactions were included as fixed factors. We included plot (each of the one hundred 50x50cm quadrats randomly distributed in each site) as a random effect.

Contribution of cushions to species richness

We calculated a third diversity metric, total species richness, to relate patterns of change with MPD, MNTD at each site and along the environmental gradient. Total species richness was calculated at plot level due to differences in area between *Arenaria* cushions and open areas. To test the relationship between total species richness per plot and elevation we used generalized linear models with a Poisson error structure and the log link-function. In addition, to quantify the effect of cushions on species richness at the community level we used rarefaction curves, from which we estimated community-level species richness (S_{total}) and species richness without cushions (S_{open}) per site following Cavieres *et al.* (2014) (see Appendix 3). To assess the magnitude of change in species richness at community level due to the presence of cushion species, we calculated the proportion of increase in non-cushion species richness (ISR) as:

$$\text{ISR} = (S_{\text{total}} - S_{\text{open}}) / S_{\text{total}}$$

Rarefaction analyses were performed with the software EstimateS v. 9.1 (<http://viceroy.eeb.uconn.edu/estimates/>). Statistical analyses were conducted in R 3.0.2 (<http://www.r-project.org/>) using for linear models the interface implemented in InfoStat-Statistical Software (Di Rienzo *et al.* 2013).

Results

Overall, the net effect of *Arenaria* on the biomass of other species changed in intensity and sign (RII) along the gradient, showing facilitation at the most severe site (i.e., highest elevation) and neutral effects in other points of the gradient (Fig 1a). Taking into account phylogenetic relatedness, the RII of species closely related to *Arenaria* (≤ 105.6 Myr) increased with environmental severity but in general, it did not change for medium (≥ 422.2 Myr) and distantly related species (≥ 512.5 Myr). However, there was an exception at the least severe site (i.e., lowest elevation), where species

intermediately related to *Arenaria* (475 Myr) were facilitated by the cushion (Fig 1b).

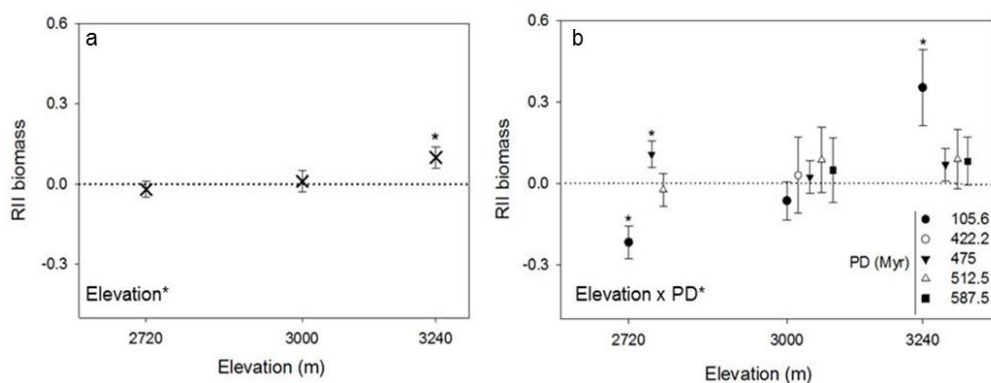


Fig. 1. Relative interaction index (RII) between *Arenaria* and selected beneficiary species along the elevation gradient (a) (n=318). RII values as a function of phylogenetic distance (b). Data are means \pm 1SE. Symbols with an asterisk represent RII values significantly different from 0. Significant differences ($p < 0.05$) along the elevation gradient and the interaction between the gradient and phylogenetic distances shown by *.

Data showed that MPD decreased and MNTD increased with environmental severity (Fig. 2a,b). Specifically, both indices increased from mid to high elevation, while total species richness remained steady (Fig. 2a,b,c). At each elevation, MPD was always higher in cushion+open than in open areas (Fig. 2a), while MNTD did not vary between cushion+open nor in the open (Fig. 2b).

Total species richness decreased as environmental severity increased, but there were no significant differences between mid and high elevations (Fig 2c). Total species richness positively correlated with MPD and negatively with MNTD ($r = 0.71$ and $r = -0.32$ respectively, $p < 0.0001$). There was a significant effect of cushions on species richness at mid and high elevations but not at low elevation (Appendix S3).

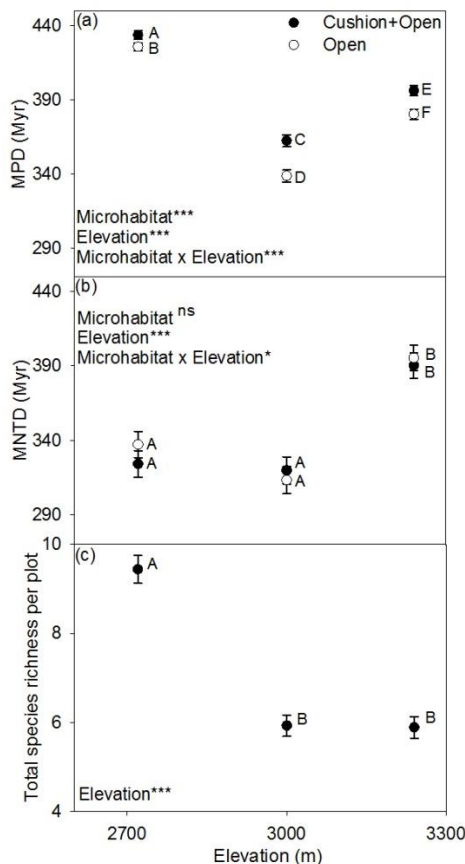


Fig. 2. Mean phylogenetic distance (a) (MPD; $n=589$), mean nearest taxon distance (b) (MNTD; $n=587$) in communities along an environmental gradient; total species richness per plot (c) at the three sites ($n=589$). Data are means \pm 1SE. Letters represent differences among factors. In each panel are included the fixed factors and their significance (linear mixed models for MPD and MNTD, and GLMM for total species richness), where * and *** indicate $p < 0.05$ and $p < 0.0001$, respectively. ^{ns} indicates non-significant differences.

Discussion

Our data suggest a relationship between phylogenetic relatedness and the outcome of plant-plant interactions along a severity gradient. Specifically, the effect of nurses on their closely related species varied from positive to negative as the environment became less severe while with more distantly-

related species it remained mostly neutral. At the site level, MPD and MNTD varied with environmental conditions, with nurses increasing mean phylogenetic distance compared to open areas. Thus, we found a remarkable context-dependent effect of phylogeny on plant-plant interactions.

Relationship between phylogenetic relatedness and plant-plant interactions

Plant-plant interaction intensity and sign are expected to change depending on abiotic conditions; following predictions of the stress gradient hypothesis (Bertness & Callaway, 1994) we expected a prevalence of positive interactions in the severe part of the gradient and negative interactions in the milder part. Indeed, we found that facilitation prevailed at the most severe site among closely related species, suggesting that cushions produced an expansion of the realized niche of stress-sensitive species, allowing them to survive in environments too harsh without nurse protection (Butterfield *et al.*, 2013; Valiente-Banuet & Verdú, 2013). Thus, the interaction effects of nurses on their closely related species varied from positive to negative as the environment became less severe while with more distantly-related species it remained, in general, neutral, with the exception of intermediately-related species that were facilitated at the less severe site. This data agree with Butterfield *et al.* (2013), who found that cushions facilitated certain lineages (in our case, those closely related to *Arenaria*) as environmental severity increased, but excluded other lineages (in our case, medium-related species) when environmental severity was highest.

Our results partially support the phylogenetic limiting similarity hypothesis (MacArthur & Levins, 1967; Violle *et al.*, 2011), as at the less severe site (i.e., low elevation) closely-related species competed with *Arenaria*. However, when environmental conditions became more severe (i.e., intermediate and high elevation sites) competition became less important in shaping plant interactions. Hence, the observed competition between *Arenaria*

and closely-related species disappeared at medium environmental severity and turned to facilitation at high environmental severity.

There is a controversy regarding phylogenetic relatedness and the outcome of plant interactions. Bennett and Cahill (2013) suggested that, should niche conservatism be common, the response of related species to environmental conditions should be similar. Although some studies support existence of the relationship between phylogenetic relatedness and interactions (Castillo *et al.*, 2010; Soliveres *et al.*, 2012b; Verdú *et al.*, 2012), other studies found no relationship (Cahill *et al.*, 2008; Fritschie *et al.*, 2014). In our alpine environment phylogenetic relatedness plays a clear role in the outcome of plant interactions but its effect is context-dependent.

The influence of interactions on community assemblage

Phylogenetic diversity was characterized by a predominance of distantly related species (high MPD) in communities at high and low severity sites and by closely related species at intermediate environmental severity. MNTD changed within species closely related from more distantly related to each other (higher MNTD) in communities at the most severe site to more closely related species in communities at the less severe site. Changes along the severity gradient could be consequence of the positive correlation between MPD with total species richness while this relationship was negative for MNTD. Thus, higher species richness increased the probability of having higher phylogenetic diversity in the community, which in turn increased the probability of higher MPD and lower MNTD.

Nevertheless, significant increases in MPD and MNTD at the high elevation site suggest that closely-related species to each other are replaced by distantly-related species. Despite the similar number of species at the most severe sites, from mid- to high-elevation sites phylogenetic distance changed significantly (33.7 Myr for MPD and 70.5 Myr for MNTD), reflecting that cushions increasingly facilitate closely-related lineages and exclude medium-

related lineages at high elevations. Changes in phylogenetic diversity, most likely due to environmental filtering caused by climatic severity, did not occur across entire lineages (Arroyo-Rodríguez *et al.*, 2012) (e.g., in our case *Arenaria serpyllifolia* and *Paronychia sp.* disappeared from 3000 m to 3200 m but not the entire clade of Caryophyllaceae); thus, at least at these two sites, environmental conditions appear very important for shaping phylogenetic community structure and may also result in different trait distribution patterns (Cavender-Bares & Reich, 2012; Purschke *et al.*, 2013).

Mean phylogenetic distance was always smaller in communities from open areas than in whole communities, while there were no differences for MNTD in any of the three sites. MPD may be more sensitive to the outcome of plant interactions as traits might be conserved within relatively older nodes (i.e., among families); MNTD, by contrast, considers a narrower phylogenetic scale and might not capture relevant trait information when analysing the effects of plant competition on phylogenetic diversity. Therefore, a smaller MPD in open-area communities suggest that harsher environmental conditions promote stronger habitat filtering, leading to a large decrease in species abundance and creating communities with species more related than in less severe sites (Webb *et al.*, 2002). In such conditions, cushion plants may provide suitable habitats for these species that are less abundant or extinct in open areas, allowing them to survive in such an extreme environment while they almost disappear in harsh open areas (Butterfield *et al.*, 2013).

Conclusion

Overall, when environmental conditions were relatively mild (i.e., at low elevation) we found partial support for the phylogenetic limiting similarity hypothesis; the interaction effects of nurses on their closely related species varied from positive to negative as the environment became less severe, while with more distantly-related species it remained, in general, neutral. Thus, under severe conditions (i.e., high elevation), facilitation became more

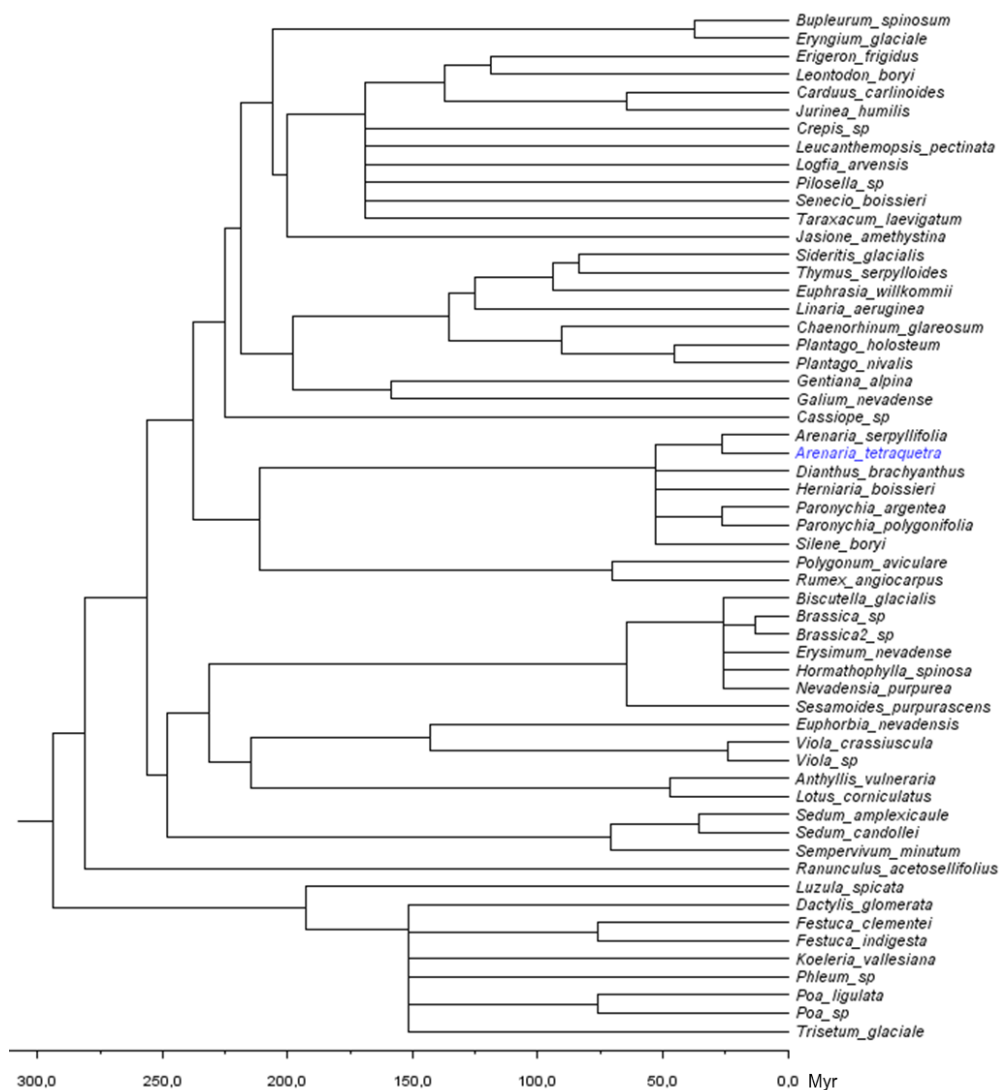
frequent and intense, favouring the growth of closely related species, which suggest that under such conditions closely-related species –i.e., sharing similar traits- cope better with environmental severity, and facilitation prevails over disadvantages of competition. Thus, there is an environmental context-dependence effect of phylogenetic relatedness which influences plant-plant interactions and shapes plant community structure.

Appendix B. Supplementary data

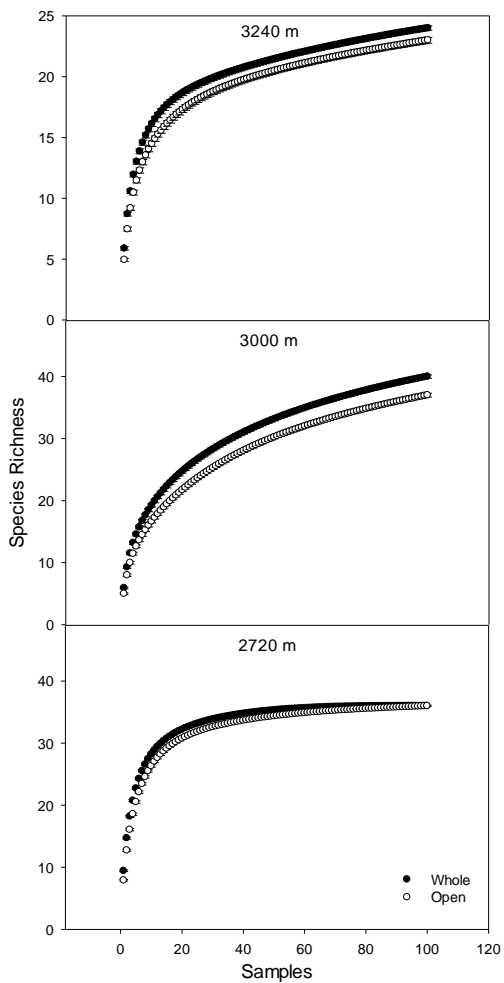
Elevation (m)	PD (Myr)	Species	n
2720	105.6	<i>Dianthus brachyanthus</i>	12
2720	475	<i>Eryngium glaciale</i>	20
2720	512.5	<i>Euphorbia nevadensis</i>	24
2720	475	<i>Euphrasia willkommii</i>	16
2720	475	<i>Galium nevadense</i>	34
2720	105.6	<i>Herniaria boissieri</i>	20
2720	475	<i>Jasione amethystina</i>	18
2720	475	<i>Linaria aeruginea</i>	13
2720	512.5	<i>Lotus corniculatus</i>	22
2720	105.6	<i>Paronychia polygonifolia</i>	24
2720	475	<i>Plantago holosteum</i>	20
2720	512.5	<i>Sedum amplexicaule</i>	31
2720	475	<i>Sideritis glacialis</i>	6
2720	105.6	<i>Silene boryi</i>	20
3000	475	<i>Eryngium glaciale</i>	18
3000	475	<i>Galium nevadense</i>	16
3000	105.6	<i>Herniaria boissieri</i>	19
3000	475	<i>Jasione amethystina</i>	24
3000	475	<i>Linaria aeruginea</i>	8
3000	475	<i>Logfia arvensis</i>	22
3000	512.5	<i>Lotus corniculatus</i>	20
3000	587.5	<i>Luzula spicata</i>	20
3000	105.6	<i>Paronychia argentea</i>	13
3000	105.6	<i>Paronychia polygonifolia</i>	27
3000	422.2	<i>Polygonum aviculare</i>	15
3000	105.6	<i>Silene boryi</i>	3
3240	512.5	<i>Biscutella glacialis</i>	6
3240	475	<i>Eryngium glaciale</i>	20
3240	475	<i>Euphrasia willkommii</i>	16
3240	475	<i>Galium nevadense</i>	20
3240	105.6	<i>Herniaria boissieri</i>	19
3240	475	<i>Jasione amethystina</i>	21
3240	587.5	<i>Luzula spicata</i>	20

Elevation (m)	PD (Myr)	Species	n
3240	512.5	<i>Nevadensia purpurea</i>	18
3240	587.5	<i>Trisetum glaciale</i>	20

Appendix B1. Species list and their phylogenetic distances (PD) to the cushion forming *Arenaria tetraquetra* ssp. *amabilis* and the elevation at which they are found. n refers to the number of biomass samples taken at each elevation.



Appendix B2. Phylogenetic tree of the regional species pool. The cushion plant *Arenaria tetraquetra* is highlighted in blue.



Appendix B3. Species accumulation curves for each site. Solid symbols correspond to species in the whole community and clear symbols to open areas. Note that the scale of the Y axis at 3240 m is from 0 to 25.

CHAPTER III

Differences in facilitation and its feedback effects between two phenotypes of a legume shrub

Nuria Pistón, Richard Michalet, Christian Schöb, Petr Macek, Francisco I. Pugnaire



Abstract

The impact of species interactions on diversity patterns has been addressed in detail in stressful environments, such as the alpine, where foundation species create new habitats for other species contributing to higher species richness and phylogenetic diversity. Foundation species with different phenotypes might modify the microhabitat differently, could differ in their subordinate community composition, and consequently receive different feedbacks from this subordinate plant community. We explored whether tight and loose canopy types of *Cytisus galianoi* are associated with differences in the microhabitat conditions and the subordinate community composition. We also wanted to experimentally test reciprocal effects between the most frequent subordinate species, *Festuca indigesta*, and the foundation species trying to evidence differences in the bi-directional interaction depending on the phenotype. For this, we performed an observational and a removal experiment in an alpine plant community in the Sierra Nevada Mountains (Spain). Both *C. galianoi* phenotypes apparently did not affect understory microhabitats differently, but hosted differentiated subordinate communities. There were differences regarding community composition and biomass between the two phenotypes, as well as differences in the physiological status of subordinate species. The tight phenotype of *C. galianoi* showed facilitation and received a negative feedback. In contrast, the loose phenotype showed higher species richness, higher plant abundance, and more phylogenetic diversity than the tight phenotype, but no negative feedback. Our results suggest that the negative feedback effects of the subordinate species on the tight phenotype might cause the phenotype change from tight to loose phenotype. Eventually, negative feedbacks may lead to the demise of the facilitator in a process which highlights the relevance of the subordinate community acting as driver of change.

Introduction

Plant-plant interactions are major processes affecting the structure of plant communities (Goldberg, 1996; Brooker *et al.*, 2008), and are dependent on environmental conditions, ontogeny, and functional strategies of interacting species (Armas & Pugnaire, 2005; Michalet, 2007; Sthultz *et al.*, 2007; Ashton *et al.*, 2010; Soliveres *et al.*, 2011; Schöb *et al.*, 2013). The impact of species interactions on diversity patterns has been addressed in detail in stressful environments, such as the alpine (Reid *et al.*, 2010; Cavieres *et al.*, 2014; Le Bagousse-Pinguet *et al.*, 2014; Liczner & Lortie, 2014). In these environments, ‘niche-constructing species’ or ‘foundation species’ create new habitats for other species (Jones *et al.*, 1994a; Odling-Smee *et al.*, 1996; Ellison *et al.*, 2005; Cavieres & Badano, 2009) contributing to increase species richness and phylogenetic diversity (Badano & Cavieres, 2006; Pistón *et al.*, 2015; Pugnaire *et al.*, 2015). Foundation species buffer temperature extremes or maintain resource levels above that of bare ground habitats (Jones *et al.*, 1994a) and their effects are species-specific (Proffitt *et al.*, 2005; Al Hayek *et al.*, 2014; Bråthen & Ravolainen, 2015). Overall, variability of foundation species represents a mechanism that explains the relationship between traits and community dynamics (Adler *et al.*, 2013).

The different microhabitats created by foundation species could be colonized by subsidiary species (Díaz & Cabido, 2001) increasing community-level diversity (Amat *et al.*, 2015; Chen *et al.*, 2015). In addition, foundation species with different phenotypes modify the microhabitat differently and differ in environmental effects. The source of variation may vary among species and environments and be caused by genetic variability, environmental plasticity, or ontogeny. For instance, niche construction by the compact cushion *Arenaria tetraquetra* increased species richness and plant abundance at high elevations but not at low elevations, in the Sierra Nevada Mountains (Spain), where loose cushions of *A. tetraquetra* could not help

with the most limiting factor, water availability (Schöb *et al.*, 2013). Similar to Michalet *et al.* (2011) who, comparing two phenotypes of the cushion species *Geum rosii* in an alpine community in Arizona, found that loose cushions had a strong facilitative effect while tight cushions had lower density of beneficiary species.

So far, most studies addressing intraspecific differences in facilitation focused on trait differences of the benefactor species (Callaway *et al.*, 1991; Crutsinger *et al.*, 2010; Michalet *et al.*, 2011; Al Hayek *et al.*, 2014; Al Hayek *et al.*, 2015). Plastic responses of foundation species to changing environmental conditions (Schöb *et al.*, 2013) or to ontogeny of woody species (Pugnaire *et al.*, 1996b; Lozano, 2014) have been documented in relation to facilitation. Therefore, drivers of phenotypic variability can influence interactions between neighbors. However, only few studies actually tried to quantify the impact of different sources of phenotypic variation on plant-plant interactions, even though such knowledge would be crucial to understand their context-dependence (Chen *et al.*, 2015).

Different phenotypes varying in facilitation intensity may also receive different feedbacks from the beneficiary community. Phenotypes showing stronger facilitation effects get stronger negative feedbacks (Michalet *et al.*, 2011; Schöb *et al.*, 2014a), most likely due to resource competition (Callaway, 2007; Schöb *et al.* 2014c). In such cases, we would expect evolutionary trait divergence. There is still little knowledge on how the interaction with beneficiaries affects facilitator species, although negative feedbacks would lead to deteriorating symptoms of facilitators when its facilitation effect increases.

In the Sierra Nevada Mountains, Spain we observed strong morphological differences in *Cytisus galianoi* shrubs, showing either loose or tight canopies. Field observations suggested that tight canopies may be younger than loose ones, and the change from tight to loose is expected to affect understory microhabitats regarding direct radiation and temperature.

We wanted to explore whether canopy type and microhabitat shifts (Jones *et al.*, 2006) influence community composition (Badano *et al.*, 2010; Jones *et al.*, 2010). The microhabitat associated to the tight canopy was expected to be most different to bare ground while loose canopies were expected to be in between. We also wanted to experimentally test reciprocal effects between the most frequent subordinate species, *Festuca indigesta*, and the facilitator species trying to evidence the link between plant traits and feedback effects. We hypothesized that i) there would be differences in *Festuca indigesta* physiological status between individuals associated to the two phenotypes of the foundation species *Cytisus galianoi*; that ii) facilitation intensity will be higher under tight canopies; and iii) the cost of facilitation would depend on facilitation intensity, thus being higher under tight than under loose canopies.

Methods

Field site and species

The field site was in the southern slope of the Sierra Nevada Mountains, SE Spain, at 2570 m elevation (36°56'N, 3°18'W). Climate is subalpine Mediterranean with hot, dry summers and cold winters. Mean annual rainfall at the closest met station (Pradollano 37°05' N 03°23' W; 2500 m elevation) is 690 mm, and mean annual temperature is 3.9 °C.

Our model species, *Cytisus galianoi* Talavera & P. E. Gibbs (Fabaceae), is found on siliceous soils between 1600 and 3000 m elevation and covers 54.3% of surface soil in the field site. It is frequently associated to *Festuca indigesta* and other perennial herbs and shrubs. Its highly branched stems reach up to 20-40 cm high and serve as main photosynthesizing organs (cladodes) because leaves are very few and tiny.

Festuca indigesta Boiss. (Poaceae) is a tussock grass common in our field site frequently found associated to *C. galianoi* canopies, where 2-3

individuals are typically found in the understory (Pistón, 2015). It grows between 1900 and 2800 m elevation and may reach a maximum size of 40 cm.

Observational study

To check for differences between “tight” and “loose” *C. galianoi* phenotypes we assessed microhabitat conditions, measured plant functional traits, and determined the subordinate plant community in each phenotype. In summer 2011 we selected 50 individuals of each phenotype and did a paired sampling with 50 nearby open areas of the same size, recording the number of individuals of each plant species (number of shoots in clonal species).

We determined the soil surface covered by each shrub as the area of an ellipse by measuring the largest diameter and its perpendicular; we also assessed canopy density (i.e. the percent surface area covered by green branches), maximum canopy height, stem density (as the number of stems in one randomly-placed 400 cm² quadrat per shrub), and terminal branch length, measured between the stem and branch tip. These functional traits were likely dependent on plant physiological status (Milla *et al.*, 2008) and were expected to influence microhabitat conditions in the understory (Crutsinger *et al.*, 2010).

We randomly selected 10 shrubs per phenotype and measured mean leaf area, specific leaf area (SLA), leaf dry matter content (LDMC), and relative water content (RWC); following Cornelissen *et al.* (2003), we selected mature and healthy leaves which were fully rehydrated before determining leaf area and saturated mass. Dry mass was measured after 72 h in the oven at 70 °C. Relative water content (RWC) measurements were performed on one branch per individual (n = 10) under cloudless conditions. Fresh branch mass was determined immediately after sampling in the field, saturated mass was measured after 30 h of rehydration in the dark, and dry mass was measured after 72 h at 70 °C.

To characterize the microhabitat in each phenotype, we measured soil convexity (semi quantitative scale with 5 levels) and soil depth under the shrub with a penetrometer at four random locations within the canopy.

To test whether community composition differed between phenotypes and with open areas (microhabitat, hereafter) we first performed a Correspondence Analysis (CA) excluding species with a frequency below 5% ($n = 12$) to avoid undesirable statistical effects; then, we identified groups of species in each microhabitat using cluster analysis and the Ward's method on the ordination axes species scores.

To test for differences in phylogenetic diversity among microhabitats, we used a phylogenetic tree (Pistón *et al.* 2015/Chapter 1) assembled Phylomatic3 (<http://phylodiversity.net/phyloomatic/>) and adjusted branch lengths with the Bladj algorithm in Phylocom 4.2. We first pruned the tree using the `drop.tip()` function (Paradis *et al.*, 2004) and then calculated phylogenetic diversity with the `pd()` function (Kembel *et al.*, 2010). Phylogenetic diversity considers how much evolutionary history is behind the species in a community (Faith, 1992) and is measured as mean branch length (in Myr) per sample unit including all species found in the community.

Canopy removal experiment

To quantify differences in the facilitation effect of both phenotypes we selected *F. indigesta*, a species naturally occurring beneath both phenotypes and in the open. In mid-July 2011 we randomly selected 20 tight and 20 loose shrubs and removed a circular area 25 cm in diameter of *C. galianoi* canopy around *F. indigesta* individuals. Another 20 *F. indigesta* individuals with intact canopies acted as control and all were compared to 20 *F. indigesta* individuals growing in open areas. In all *F. indigesta* individuals we measured maximum photochemical efficiency of photosystem II (F_v/F_m) with a saturation pulse fluorometer (Mini-PAM; Walz, Effeltrich, Germany) in 30 min dark-adapted plants. Measurements were carried out on healthy

undamaged leaves of similar appearance. Fv/Fm indicates how efficiently radiation is being processed by the plant. We also measured plant height, lateral spread (largest diameter) and number of leaves per plant. After one year, we measured survival and aboveground biomass of *F. indigesta* individuals, and repeated Fv/Fm, height and lateral spread measures. The increase in height and lateral spread during one year was calculated as (final value-initial value) / (initial value).

To test for differences in the effect of each phenotype on biomass and on leaf increment of *F. indigesta*, we used the Relative Interaction Index (RII) (Armas *et al.*, 2004) calculated as $RII = (X_{\text{with } C. \text{galianoi}} - X_{\text{in open areas}}) / (X_{\text{with } C. \text{galianoi}} + X_{\text{in open areas}})$, where $X_{\text{with } C. \text{galianoi}}$ and $X_{\text{in open areas}}$ are the values of *F. indigesta* measured in the presence and absence of *C. galianoi*, respectively. We calculated three different indices for biomass and leaf increment:

- RII_{Canopy}: the difference in performance between control and removed patches, quantifying the effect of the shrub's canopy.
- RII_{Soil}: the difference in performance between canopy-removed and open areas, quantifying the effect of the shrub's soil.
- RII_{Cytisus}: the difference in performance between control shrubs and open areas, quantifying the combined effect of canopy and soil on *F. indigesta* performance.

RII is positive when biomass or the relative leaf increment is higher in the shrub than in open areas and negative in the opposite case. Zero RII values suggest that biomass or relative leaf increment is equal in both microsites.

Beneficiary removal experiment

We assessed facilitation costs for *C. galianoi* by selecting 20 shrubs of each phenotype and removing one (out of 2-3) *F. indigesta* individuals growing within each shrub and selected another 20 shrubs of each phenotype as a control. On each *C. galianoi* individual we measured Fv/Fm as above, but on green branches instead of leaves, since branches are the main photosynthetic

tissue for this species. We recorded the number of flowers in 20 cm x 20 cm quadrats placed in the *C. galianoi* canopy with *F. indigesta* in the center. Terminal branch dry mass was measured as the dry mass of the segment between the main stem and branch tip for five randomly selected branches per shrub.

Statistical analysis

To test for differences in cushion traits and microhabitat conditions between phenotypes we used GLM; in case of differences in convexity we used a contingency table analysis, with “Microhabitat” and “Convexity” as class criteria.

To test for differences in scores on CA axis 1 and 2 we used one-way ANOVA with “microhabitat” as independent variable. In order to assess microhabitat requirements of subordinate species, we calculated the cumulative abundance of species of each cluster group and then conducted a one-way ANOVA with microhabitat as independent variable (Fig. S1a); in case of species richness we used one-way ANOVA with microhabitat as independent variable (Fig. S1b).

To check for differences in phylogenetic diversity we used a GLM to test for differences among microhabitats as independent variable (tight, loose and open areas) (Fig. S2).

To check for differences in Fv/Fm and growth rates we used GLM. Fixed factors were microhabitat type and canopy removal (with or without canopy removed). The model was incomplete because open areas has only one “level” of *F. indigesta* plants that also acted as control for those in both phenotypes. Thus, we re-parametrized all fixed factors into a single one with five “Microhabitat x Canopy removal” levels which we called “Patch”. In case of survival we used a generalized linear model with a Binomial distribution and “Patch” as independent variable (R package stats). Post-hoc differences among patches were examined with Fisher’s LSD tests.

To analyze RII indices we used a GLM with “Phenotype” (tight and loose) and “Mechanism” (canopy, soil and *Cytisus*) as independent variables. Additionally, we used one-sample *t*-tests to assess whether RII values were significantly different from zero.

For the cost of facilitation, we compared data using “Phenotype” (tight and loose) and “Removal of *Festuca*” (removal and control) as independent variables. Fv/Fm was analyzed with a GLM using the function `lm()` (R package stats); for number of flowers we used a GLM with a Poisson distribution with the function `glm()` (R package stats), and for terminal branch mass we used a GLMM with square-root transformed branch mass data as dependent variable and shrub identity as a random blocking factor using the function `lme()` (R package nlme). We used the `glht()` function (R package multcomp) for multiple comparisons of factor levels for each model. All analyses were conducted in InfoStat v. 2013 (Di Rienzo *et al.*, 2013) and R v 3.0.2 (R-CoreTeam, 2013).

Results

We found highly significant differences in functional traits between the two phenotypes of *C. galianoi*; specifically, the tight phenotype had a canopy ca. 38% denser, 43% taller, 17% greater stem density, 25% longer terminal branches, and 39% larger leaf area than the loose phenotype (Table 1). There were no significant differences regarding SLA, LDMC or RWC. Microhabitat conditions (soil depth and convexity) did not differ between phenotypes (Table 1).

Shrub traits	Phenotype		Phenotype effect
	Tight	Loose	<i>p</i> -value
Surface (cm ²)	100.2 ± 19.5	100.7 ± 13.5	0.982
Cover (%)	90.0 ± 0.7	55.5 ± 1.8	<0.0001
Height (cm)	16.0 ± 0.45	9.0 ± 0.4	<0.0001
Stem density (per 400 cm ²)	140.2 ± 4.9	115.4 ± 3.8	<0.0001
Terminal branch length (cm)	4.8 ± 0.25	3.6 ± 0.1	<0.0001
Leaf area (mm ²)	10.6 ± 1.1	6.5 ± 1.1	0.017
SLA (m ² kg ⁻¹)	17.3 ± 0.9	16.9 ± 0.9	0.753
LDMC (g kg ⁻¹)	212.1 ± 18.6	210.5 ± 17.6	0.951
RWC	0.68 ± 0.02	0.67 ± 0.03	0.662
Microhabitat			
Convexity index	class 3 with a 30%	class 2 with a 35%	0.069
Penetration depth (cm)	7.6 ± 0.3	8.3 ± 0.2	0.067

Table 1. Functional traits and microhabitat measurements (mean ± 1SE, n = 10) of tight and loose phenotypes of the shrub *Cytisus galianoi* in the Sierra Nevada mountains, Spain. For convexity we used ranks (1 = very convex, 2 = convex, 3 = flat, 4 = concave, 5 = very concave) and estimated the highest frequency (shown as percentage). Significant differences of GLM (*p*-values) between the two phenotypes are shown in bold.

The CA ordination revealed significant differences in species composition among microhabitats (Fig. 1a). The first axis was related to differences in community composition in the tight phenotype versus the other microhabitats. The second axis was related to differences between phenotypes. Three groups of subordinate species, characteristic for each of the three microhabitats, were identified with CA conducted on species scores (Fig. 1b). Group A included species of the tight phenotype with two herbs and the shrub *G. versicolor*. Group B included species of the loose phenotype, which were most abundant (Fig. S1a) and with the highest species richness (Fig. S1b), being therefore the most phylogenetically diverse group (Fig. S2). It included a mix of tall (e.g., *Festuca indigesta* and *Armeria filicaulis*) and short (e.g., *Jurinea humilis* and

Senecio boissieri) herb species. Group C included species characteristic of open areas with only two short herb species.

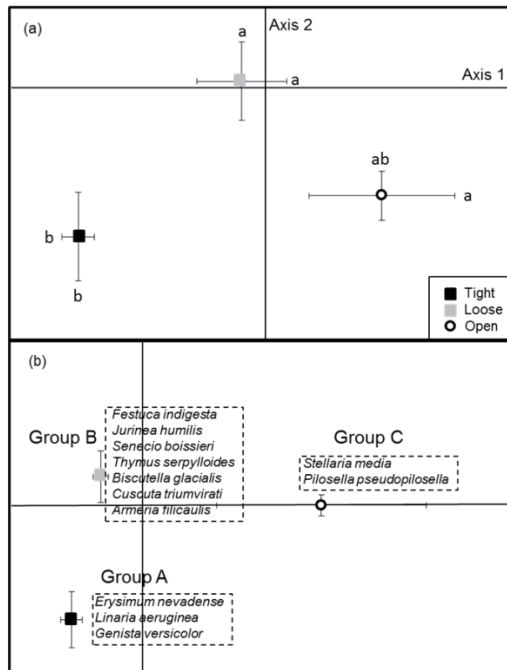


Figure 1. CA diagrams of microhabitats (for loose and tight phenotypes and open areas $n = 50$). Panel (a) shows mean scores (\pm SE) of species composition for each microhabitat and panel (b) mean scores (\pm SE) for three species groups identified by the cluster analysis (see text for details). Microhabitat scores differed significantly along the first ($p < 0.001$) and two ($p < 0.05$) ordination axes. Different letters above indicate significant difference between microhabitats.

Festuca indigesta showed 21% higher Fv/Fm values in the intact canopy of the tight phenotype than in the other patches, resulting in the only significant difference among sites (Fig. 2a). There was ca. 60% decrease in *F. indigesta* height in the removal treatments compared with intact canopies and open areas (Fig. S3a). There were no significant differences among patches in lateral spread (Fig. S3b). *Festuca indigesta* survival was 100% in control canopies and 75% in altered canopies of the tight phenotype, but there were

no differences in the loose phenotype or between phenotypes with canopies removed and open areas (Fig. 2b).

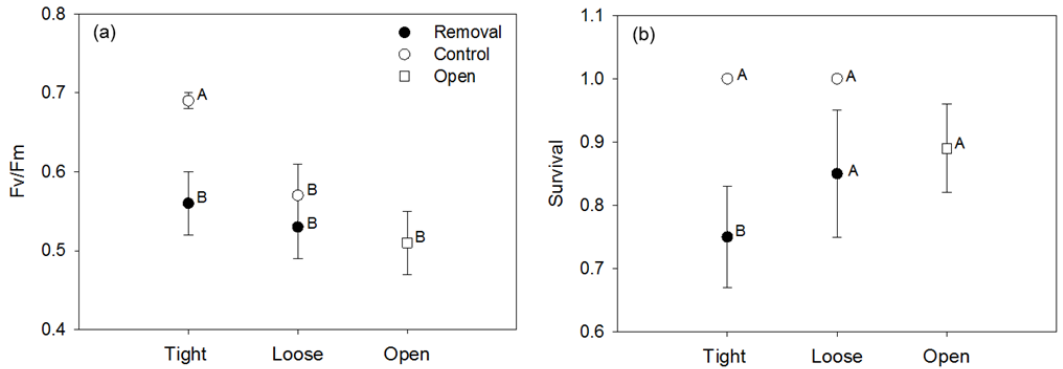


Figure 2. Photosynthetic efficiency of photosystem II (Fv/Fm) of *Festuca indigesta* leaves (a), and survival (b), in five different patches, tight and loose canopies intact, tight and loose canopies removed, and open areas. Different letters indicate significant differences in Fv/Fm ($p < 0.001$) and survival ($p < 0.05$). Values are mean \pm 1SE; $n=20$.

Regarding biomass (Fig. 3a) and leaf growth (Fig. 3b) the canopy effect was positive in both phenotypes whereas the soil effect was neutral and negative, respectively. There were significant differences for both biomass and leaf growth among mechanisms ($p = 0.01$ and $p < 0.0001$, respectively) but not between phenotypes ($p = 0.93$ and $p = 0.55$, respectively) and their interaction ($p = 0.17$ and $p = 0.70$, respectively).

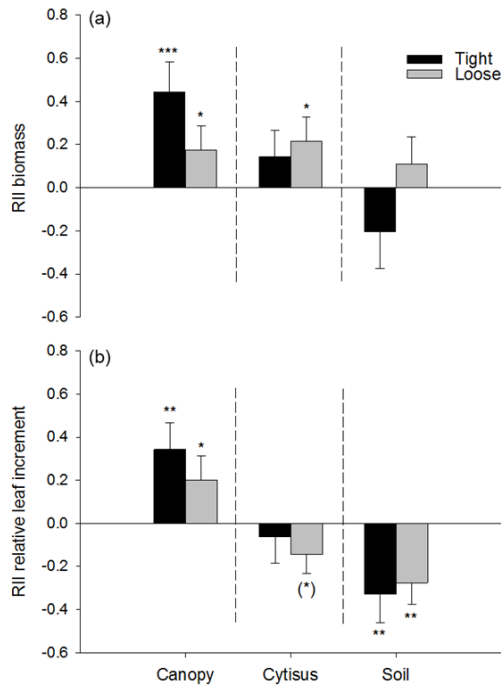


Figure 3. Relative differences (RII) in biomass (a) and leaf growth (b) of *Festuca indigesta* individuals growing within tight and loose phenotypes of *Cytisus galianoi*. “Canopy” quantifies the effect of the *C. galianoi* canopy on the grass; “Cytisus” quantifies the net effect (canopy + soil) of the shrub on the grass; “Soil” quantifies the effect of the soil beneath the shrub on the grass. There were significant differences for both, biomass and leaf growth among mechanism ($p = 0.01$ and $p < 0.0001$, respectively) but not between phenotypes ($p = 0.93$ and $p = 0.55$, respectively) or their interaction ($p = 0.17$ and $p = 0.70$, respectively). Differences of RII from 0 (one sample t -tests) are indicated by (*) < 0.1 , * < 0.05 , ** < 0.01 , *** < 0.001 ; positive bar values indicate facilitation and negative values indicate competition. Values are means + SE; $n=20$.

Cytisus galianoi tended to have lower Fv/Fm when *Festuca* was removed than control plants, and the loose phenotype tended to have lower Fv/Fm than the tight phenotype (Fig. 4a). There was a significant interaction “Phenotype x Removal of *Festuca*” regarding flower number, showing that *Festuca* removal increased the number of flowers in the tight phenotype but not in the loose (Fig. 4b). Finally, mean terminal branch of *C. galianoi* was

significantly heavier in the tight than in the loose phenotype and tended to increase after *F. indigesta* removal (Fig. 4c).

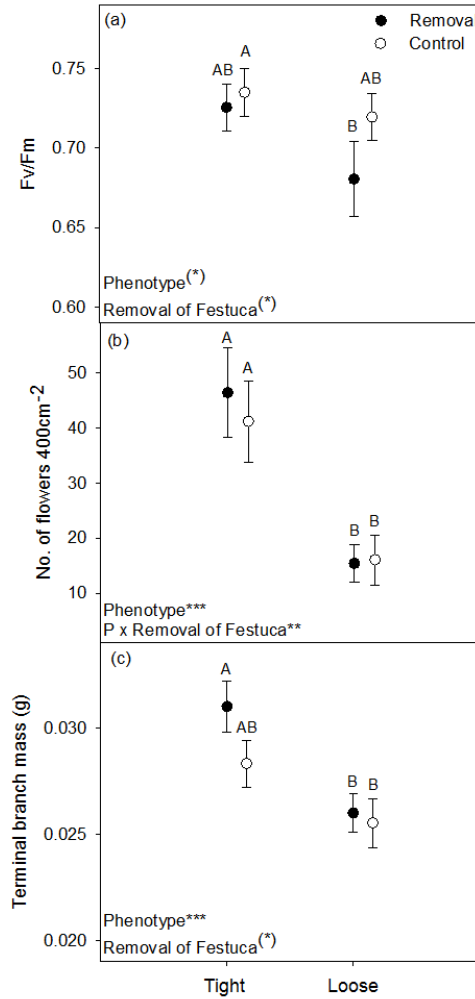


Figure 4. *Cytisus galianoi* traits in control plants and in plants where one *Festuca indigesta* individual was removed. Measurements are photosynthetic efficiency of photosystem II (Fv/Fm; a), flower density (b), and terminal branch mass (c). Significant effects of phenotype (loose or tight), Removal of *Festuca* and their interaction are shown on the lower left part of panels when significant indicated by (*) $p < 0.1$, ** $p < 0.01$, and *** $p < 0.001$. Different letters indicate significant differences between treatments. Values are mean $\pm 1SE$; $n=20$.

Discussion

The foundation species, *Cytisus galianoi*, has tight and loose canopy phenotypes which apparently do not affect understory microhabitats differently, but host clearly differentiated subordinate plant communities. There were differences regarding community composition and biomass between the two phenotypes, as well as physiological effects on subordinate species. The tight phenotype showed facilitation; however, this positive effect also had a negative feedback on *C. galianoi*. This negative feedback may actually be behind *C. galianoi* canopy change from tight to loose, as it means reducing facilitation costs. The plant community hosted by the loose phenotype showed higher species richness, higher plant abundance, and more phylogenetic diversity than the tight phenotype. Eventually, negative feedbacks may lead to the demise of the facilitator individual in a process which highlights the relevance of the subordinate community acting as driver of change.

Functional traits, environment and community composition

Facilitator species modify microhabitats beneath their canopies influencing growth and survival of beneficiary species (Brooker & Callaway 2009; Cavieres & Badano 2009; Anthelme *et al.* 2014). For example, they can buffer temperature extremes, reducing summer heat shock, transpiration, and soil moisture evaporation, as well as preventing frost damage (Körner, 2003). Canopy functional traits may be proxies for microhabitat changes (Schöb *et al.*, 2012) and serve as indicators of plant interaction outcome. In our case, both *C. galianoi* phenotypes showed differences in functional traits such as height, stem density, or terminal branch length, and we assume that such variation led to differences in subordinate community composition. Similar to Michalet *et al.* (2011), our results showed that beneath loose phenotypes thrived a dense community with higher species richness than under the tight

phenotype. Such microhabitat differences, and parallel changes in subordinate communities could be due to ontogeny (Pugnaire *et al.*, 1996c; Lozano, 2014), genotypic variability (Crutsinger *et al.*, 2010; Al Hayek *et al.*, 2014) or plasticity (Schöb *et al.*, 2013), which increase community diversity through niche complementarity (Jones *et al.*, 1994a).

Reciprocal effects

Many mechanisms with which shrubs promote the establishment of other plants have been linked to plant age and canopy size (Pugnaire *et al.*, 1996c; Callaway, 2007) which affect soil chemical and physical properties underneath, increasing fertility compared to open areas (Pugnaire *et al.*, 2004). We found that the strongest facilitation effect of *C. galianoi* on *F. indigesta* was linked to canopy type (the tight phenotype). However, understory soil had a negative effect on leaf growth and a neutral effect on biomass, whereas the net balance of both, soil and canopy (i.e., the overall *Cytisus* effect) had only a positive effect in the loose phenotype. In addition, *F. indigesta* individuals growing within tight phenotypes showed higher Fv/Fm, which can indirectly be linked to higher photosynthetic rates and daily carbon gain (Monje & Bugbee, 1998; but see Baker, 2008). Canopies buffered temperature and increased relative humidity compared to open areas (7.83 ± 0.47% decrease in temperature and 16.04 ± 0.76% increase in relative humidity; Pistón *et al.* Chapter 1) which resulted in higher *F. indigesta* growth. The lower abundance and species richness under the tight phenotype could be related to the shading effect of the canopy.

Cost of facilitation

Reciprocal interactions have been rather overlooked in facilitation studies so far (but see e.g., Pugnaire *et al.*, 1996c; Schöb *et al.*, 2014a; Schöb *et al.*, 2014b). The facilitation concept has an unidirectional origin (Bertness & Callaway, 1994) but plant-plant interactions are bi-directional. Where the

beneficiary species neither harm or benefit the facilitator, the interaction would be neutral (Rodríguez-Echeverría *et al.*, 2012; Martínez-García *et al.*, 2013); however, if the cost of hosting other species outweighs facilitator benefits, we can expect either niche divergence or, ultimately, the demise of the facilitator (Fig. 5) (Aguiar & Sala, 1999; Bronstein, 2009). In our case, we found weak feedback effects between facilitator and facilitated species, although there was a general trend to negative effects of *F. indigesta* on *C. galianoi* due to competition and a benefit on Fv/Fm in the loose phenotype. Decreased terminal branch mass was also likely a consequence of competition for resources (Pugnaire *et al.*, 2011), whereas increased Fv/Fm might be a consequence of shading and improved soil water balance (Monje & Bugbee, 1998). Schöb *et al.* (2014c), analyzing reciprocal effects between *Arenaria tetraquetra* and subordinate species found a facilitator cost for hosting beneficiaries, which increased with increasing beneficiary cover. These data suggest that costs and benefits of facilitator species are not only species-specific, but depend on traits of both facilitator and facilitated species.

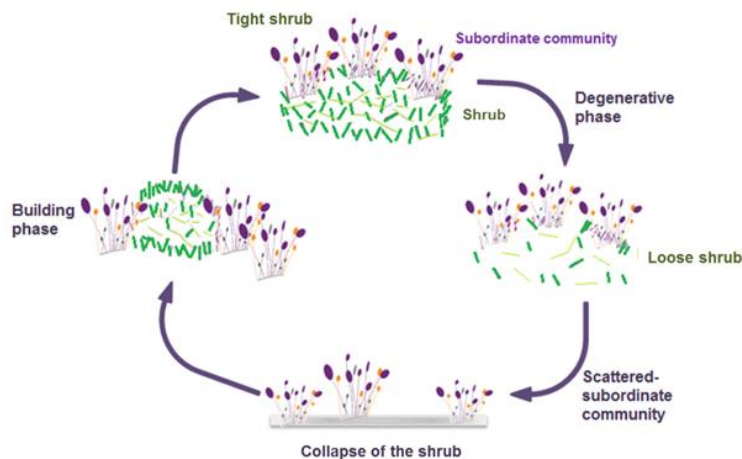


Figure 5. Diagram showing the possible dynamics between the two *Cytisus galianoi* phenotypes (modified from Aguiar & Sala, 1999). As the shrub grows (building phase) promotes both seed accumulation and seedling establishment. As grasses are established, competition between the grasses overshadows facilitation by the shrub. The shrub begins to collapse (degenerative phase) and canopy protection disappears. When the shrub dies, the remnant grass individuals form the scattered-subordinate community.

Conclusion

Our data highlight the importance of plant interactions for trait selection in facilitator-facilitated species, and stress the relevance of feedback effects of the subordinate community as driver of change in cushion morphology and, ultimately, leading to complementarity effects at the whole community level.

Appendix C. Supplementary material.

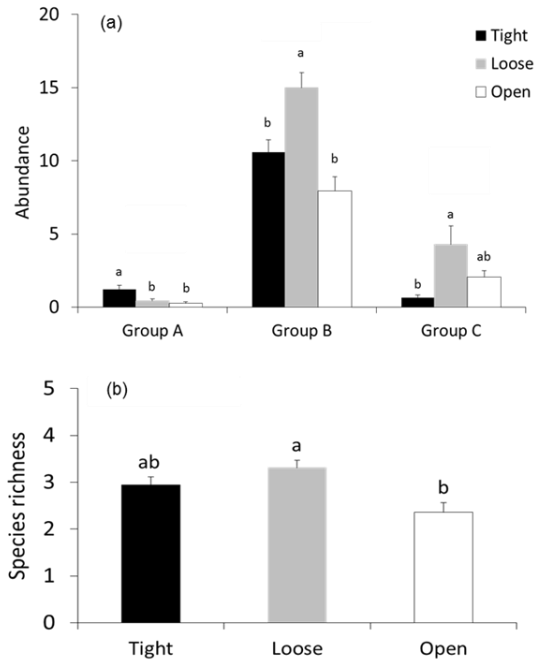


Figure C1. Plant abundance (a) and species richness (b) in subordinate communities growing within the tight and loose phenotypes of *Cytisus galianoi* shrubs and in open areas. In panel (a) abundance is split into three groups identified in the cluster analysis on species scores (Fig. 1). Different letters show differences among microhabitats (tight, loose, open) within each group (a) or variable (b). Values are means \pm SE; n=50.

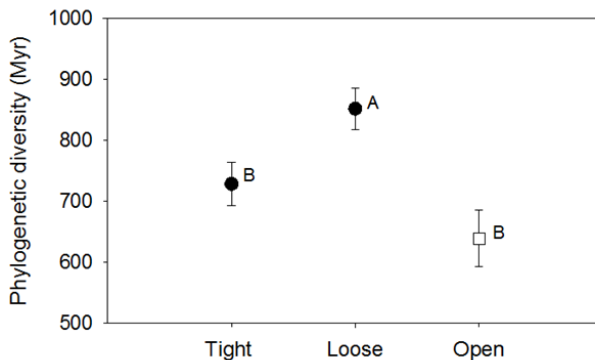


Figure C2. Phylogenetic diversity (Myr) within tight and loose (solid dots) phenotypes of *Cytisus galianoi* and open areas (clear dot). Different letters indicate significant differences among microhabitats ($p < 0.001$). Values are mean \pm 1SE; n=50.

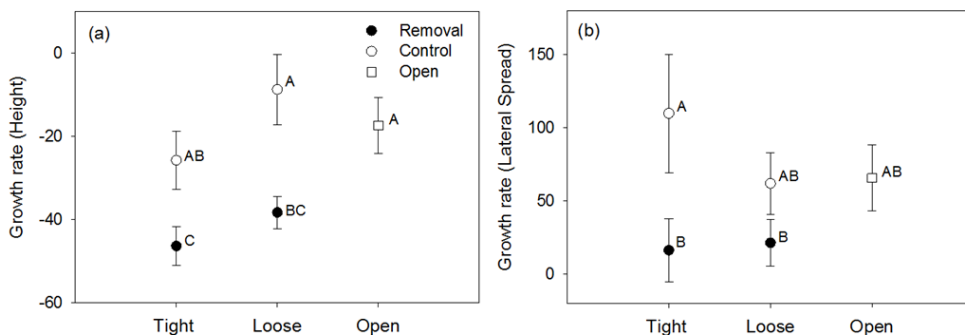
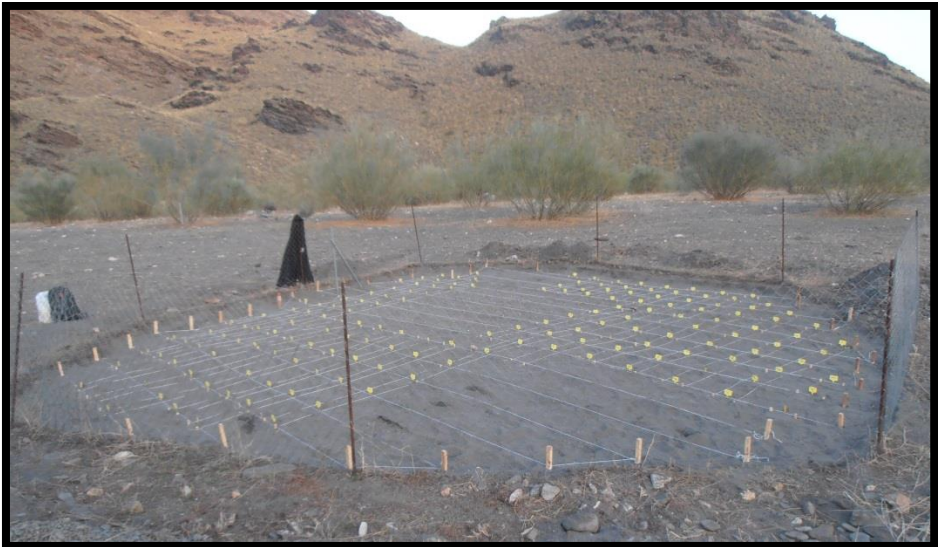


Figure C3. Growth rate (final value - initial value) / (initial value) of *Festuca indigesta* based on height (a) and lateral spread (b) in five patches. Different letters indicate significant differences among patches ($p < 0.05$). Values are mean \pm 1SE; n=20.

CHAPTER IV

Ontogenetic shifts and phylogenetic relatedness in annual plants interactions in a semi-arid community

Nuria Pistón, Cristina Armas, Christian Schöb, Carme Estruch, Fernando Casanoves, Francisco I. Pugnaire.



Abstract

Patterns of relatedness among species have been used to infer processes governing species assemblages. Although abiotic processes alter the trait distribution of both community and species, empirical tests of the effects of biotic interactions on plant trait variation remain uncommon. Ultimately, the sign and intensity of species interactions may shift over the lifetime of a plant and these ontogenetic shifts appear to be context-dependent. Thus, we wanted to evidence how interaction intensity changes with phylogenetic relatedness, from early stages to survival and reproduction. For this purpose we created an environmentally uniform outdoor field site in southeastern Spain in order to avoid confounding effects and analyzed the outcome of plant interactions between annual species growing with conspecifics, close-, medium- and distantly-related species. We found that competition was less intense between distant relatives than between species with increasing degree of relatedness. As a consequence, there was higher survival and number of flowers in far- than close- related species or the intraspecific treatment. We found a high variance difference in intraspecific interaction and the other treatments regarding no. of leaves, showing this trait responded plastically to competition. Plastic responses were apparent across life stages but for the first and last stages, which highlights the need to incorporate multiple life stages when assessing the factors contributing to individual survival and species coexistence.

Introduction

A primary goal of ecology is to understand processes such as interactions governing species coexistence. Interactions are dependent on trait differences between species (Albert *et al.*, 2010a; Albert *et al.*, 2010b; Jung *et al.*, 2010), which have been shown to covary with phylogenetic similarity (Cavender-Bares *et al.*, 2009; Pellissier *et al.*, 2013; Yang *et al.*, 2014). Patterns of relatedness among species thus have been used to infer processes governing species assemblages (Webb *et al.*, 2002; Silvertown *et al.*, 2006; Prinzing *et al.*, 2008). Two opposing forces affect coexistence: abiotic conditions, which cause trait clustering due to habitat affinities of closely related species (Soliveres *et al.*, 2012a; Pistón *et al.*, 2015) and biotic interactions such as competition, which is strongest at small spatial scales preventing close relatives from co-occurring and lead to trait overdispersion (Webb *et al.*, 2002). However, some studies pointed out the possibility that when competition is an important assembly process it may differentially exclude distantly related species (Chesson, 2000; Mayfield & Levine, 2010; Godoy *et al.*, 2014). Thus, although abiotic filtering alters both community- and species-level trait distribution, empirical tests of the effects of biotic interactions on plant trait variation remain uncommon (Burns & Strauss, 2012).

Ecological traits are conserved along phylogenetic lineages leading to niche conservatism (Blomberg *et al.*, 2003; Wiens & Graham, 2005). But in the analysis of community assembly, either important traits could be overlooked or uninformative traits be included in the study, thus reducing our ability to explain patterns of species coexistence. Limitations of trait-based measures were the reason for using phylogenetic approaches; it is assumed that the phylogeny may represent unmeasured traits better than a limited number of measured traits (Helmus *et al.*, 2007; Mouquet *et al.*, 2012). Moreover, different traits may show differing amounts of conservatism and

convergence/divergence along phylogenies (Blomberg *et al.*, 2003; Cavender-Bares *et al.*, 2006). Thus, phylogenies and measured traits may represent different aspects of a species' ecology and it is advisable to use both approximations to address species coexistence (Cadotte *et al.*, 2013).

Using phylogenetic distance among competitors may be a good predictor of plant success under competition (Webb *et al.*, 2006; Castillo *et al.*, 2010). Ultimately, sign and intensity of species interactions may shift over the lifetime of a plant (Gonzalez *et al.*, 2010; Uriarte *et al.*, 2010; Lebrija-Trejos *et al.*, 2013; Zhu *et al.*, 2015), these ontogenetic shifts being context-dependent (Schiffers & Tielbörger, 2006; le Roux *et al.*, 2013). Therefore, attempts to explain species coexistence must integrate species interactions across all life stages (Comita & Hubbell, 2009; Trinder *et al.*, 2012; Trinder *et al.*, 2013). This changing scenario may explain why different studies report contradictory shifts in a phylogenetic context, because the outcome of studies may depend on the life stage of target species (Webb *et al.*, 2006; Swenson *et al.*, 2007; Buckley *et al.*, 2010; Zhu *et al.*, 2015).

Here we wanted to address how ontogenetic shifts in species interactions vary with relatedness of the interacting species. In other words, we wanted to quantify changes in the intensity of species interactions with phylogenetic relatedness from early stages to survival and reproduction. For this purpose we created an environmentally uniform outdoor field site in southeastern Spain in order to avoid confounding effects and analyzed the outcome of plant interactions between annual species growing with conspecifics and closely, mid- and distantly-related species. We hypothesized that under the same environmental conditions, ontogenetic shifts towards negative interactions would occur among closely related species, in contrast to distantly-related species which shift towards positive interactions.

Methods

Field site, species and experimental setup

The experiment was conducted between December 2011 and April 2012 in the Rambla del Saltador valley, on the southern slope of the Sierra de Filabres range in Almería, Spain (37°08'N 2°22'W, 630 m altitude). The Rambla del Saltador valley has a semiarid climate with a pronounced dry season from May to September with almost no rain. Mean annual temperature is 17.9 °C and mean annual precipitation is 235 mm, mild winter temperatures (mean minimum temperature of 4.1°C) and hot summers (average maximum temperature of 34.7°C) (Lázaro *et al.*, 2001). Extreme air temperatures above 45°C and below freezing temperatures are not uncommon in the hottest and coldest months, respectively (Spanish National Meteorological Institute 2012). The valley bottom is filled with alluvial deposits on mica schist bedrock with low organic matter and low nutrient concentrations (Pugnaire *et al.*, 1996c). This area is dominated by *Retama sphaerocarpa* (L.) Boiss (Fabaceae) shrubs, with a density of 500 plants/ha and a perennial cover of ca. 40%, with open areas between shrubs almost bare except in winter-spring, when they are covered by annuals (Pugnaire *et al.*, 1996b). For the experiment we fenced a homogeneous 5x5 m plot without perennial species. To reduce environmental heterogeneity we homogenized the topsoil by removing soil from the first 20 cm. Soil was thoroughly mixed, sieved to remove stones, and replaced in the plot.

We selected nine annual plant species from the local species pool and collected seeds from many mother plants at the field site the year prior to the experiment. Seeds were cleaned and mixed, and finally stored in a cold, dry place until use. Before sowing, seeds were counted in the laboratory and disinfected with 75% ethanol for two minutes. Seeds were sown in the field and watered with 100 ml of distilled water per plot every 2 weeks over 6

weeks. Plants other than our target species appearing in the plot were removed frequently.

We established 170 15x15 cm plots which were randomly allocated to each of 4 interaction treatments and 81 5x5 cm plots for controls. In each plot, each species either grew alone (i.e., control with only 1 individual), with the same species (intraspecific interaction), and with other species selected by phylogenetic relatedness, i.e. ‘close’ for individuals of other species in the same genus (e.g., *M. minima* + *M. truncatula*), ‘medium’ for individuals of species in the same family (e.g., *B. rubens* + *L. rigidum*) and ‘far’ for individuals of species from different families (e.g., *M. minima* + *B. rubens*). We replicated each interaction treatment 10 times except the control that was replicated 9 times.

In each plot we sowed 50 seeds, 25 seeds of each species in plots with two species and 50 in single-species plots. For ‘control’ plots we sowed several seeds in a 5x5 cm plot and weeded to leave 1 individual per plot.

Plots were regularly distributed within the experimental area and randomly assigned to treatments (Fig. 1). Plots were covered with a fine mesh until emergence to avoid dispersal and contamination with foreign seeds (Ariza & Tielbörger, 2011).



Fig. 1. Experimental site of 25 m² after mixing the soil and removing all stones and reallocating the soil. Plots were distributed regularly and we randomly assigned them treatments. Plots were covered with organza until cotyledon germination to avoid dispersal and contamination with foreign seeds.

Seeds of five of the nine species did not germinate, and the four remaining species were *Bromus rubens* L. (Poaceae), *Lolium rigidum* Gaudin (Poaceae), *Medicago minima* (L.) L. (Fabaceae) and *Medicago truncatula* Gaertn. (Fabaceae).

To evaluate interaction outcome through time, we recorded the number of individuals per plot and per species and as well as number of leaves, length of mature leaves (starting on census 3), plant height, number of flowers, number of fruits (although there were few and we did not have enough replicates for statistical tests) on individuals in every plot fortnightly (i.e, 5 censuses over 10 weeks). At the end of the experiment we harvested plants by species and averaged specific leaf area (SLA) and aboveground biomass; following Cornelissen *et al.* (2003), we selected mature and healthy leaves which were fully rehydrated before leaf area determinations. All samples were oven-dried at 70 °C for 72 h and weighed.

We recorded these traits because the production and maintenance of leaves (i.e., total number of leaves through time) is one of the mechanisms by which plants compete and has also a direct link to resource capture (Trinder *et al.*, 2013); leaf length has an important influence on the energy balance of the plant and is associated with reproductive morphology (Parkhurst & Loucks, 1972; Ackerly, 1998; Ackerly, 2009); height is the dominant factor influencing access to light, which is the major driving force affecting not only photosynthetic activity, but also leaf temperature, water status, and other physiological processes (Falster & Westoby, 2003; Valladares & Niinemets, 2007). SLA is generally associated with photosynthetic capacity, relative growth rate and leaf longevity (Cornelissen *et al.*, 2003) and increases in response to increasing temperatures and decreasing light availability (Poorter *et al.*, 2009); it is positively correlated with soil N (Ordoñez *et al.*, 2009) and negatively with leaf longevity and resource-use efficiency (Wright *et al.*, 2004); flowers and fruits indicated the reproductive output and, finally, we also measured herbivory as an estimate of disturbance (Weiblen *et al.*, 2006; Berendse *et al.*, 2007).

Net interaction outcome and phylogenetic relatedness

We measured net interaction outcome (using data from the last census) in treatments as trait differences of individuals growing in competition and growing alone. We used the Relative Interaction Index (Armas *et al.*, 2004) as $RII = (B_{\text{interaction}} - B_{\text{alone}}) / (B_{\text{interaction}} + B_{\text{alone}})$, where B is the trait of individuals growing in competition ($B_{\text{interaction}}$) or alone (B_{alone}). This index has positive values indicating facilitation among individuals growing together and negative values indicating competition when individuals grow better alone. Zero RII values indicate neutral interaction among individuals.

Assessing trait variation through time

To characterize interaction dynamics and trait values and how they might contribute to survival, we analysed trait changes through time (i.e., no. of leaves and height from censuses 1 to 5, and leaf length from census 3 to 5).

Statistical analysis

We used one-way ANOVA to test whether phylogenetic grouping was more important than random differences between species. We specifically compared the magnitude of RII values within groups of phylogenetic relatedness. We checked if RII for the two grass species, *L. rigidum* and *B. rubens*, was significantly different from RII of the two legume species, *M. minima* and *M. truncatula*. If differences were non-significant we assumed that differences between clades were as important as differences between species, and therefore we could assume that the effects of phylogenetic relatedness were not driven by species-specific interactions.

We then determined whether phylogenetic relatedness predicts survival and the net interaction outcome in mature individuals. We calculated survival as percentage of individuals at the last census divided by maximum number of germinated seeds. We used phylogenetic relatedness as fixed factor and number of individuals in each plot as co-variable. We treated species as random effects. Appropriate checks and corrections were made for the requirements of homogeneity of variances and normality of residuals to be met. Post-hoc differences were examined with LSD Fisher test corrected by Bonferroni for multiple-comparisons.

Additionally, we analysed trait changes across ontogeny (in census 3-5). We performed a general mixed model using phylogenetic relatedness as fixed factor and number of individuals in each plot as co-variable; we included the compound symmetry as the temporal correlation model and covariance structure (Zuur *et al.*, 2009) including species within plot within

phylogenetic relatedness as the unit repeatedly measured in time. We also checked homogeneity of variances and normality of residuals requirements. Data were analyzed using lme (Pinheiro *et al.*, 2015) in R (<http://www.r-project.org/>) interfaced by InfoStat statistical software version 2014 (<http://www.infostat.com.ar/>).

Results

We found that, comparing clades, differences in RII between species within a clade were not significant for individual biomass ($n = 17$; p -value = 0.62), height ($n = 16$; p -value = 0.33), leaf length ($n = 16$; p -value = 0.24), no. of leaves ($n = 16$; p -value = 0.46) nor no. of flowers ($n = 16$; p -value = 0.23) and thus RII differences between clades were more (or equally) important than species-specific differences.

Plants growing with far-related species survived at higher percentages than those in the ‘intraspecific’ treatment; survival in the ‘close’ and ‘medium’ relatedness treatments were in between. Overall, survival of plants growing with far relatives reached up to 55.8% compared to 52.64 and 48.49% in the ‘medium’ and ‘close’ relatedness treatments, and 41.5% in the intraspecific treatment (Fig. 2).

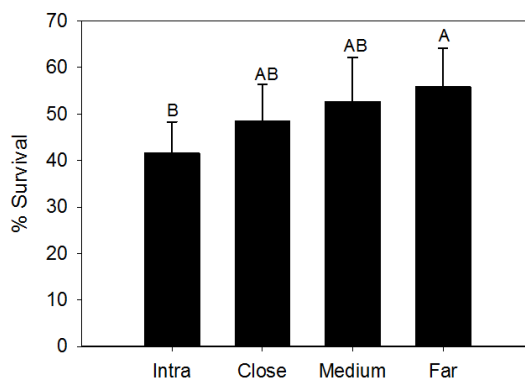


Fig. 2. Survival percentage (mean + SE) for the four interaction treatments: intraspecific ($n = 33$), close ($n = 16$), medium ($n = 8$) and far ($n = 15$). The effects of

the treatment was non-significant ($p = 0.12$). Different letters indicate post-hoc differences among treatments.

The results of net interaction outcome for individual mass and no. of flowers indicated that net interactions were always competitive and that there were significant differences among treatments. We found higher competition intensity regarding biomass between far relatives compared to ‘medium’ and ‘intraspecific’ treatments with close relatives displaying intermediate values (Fig. 3a). On the other hand, competition intensity for no. of flowers was higher between close relatives than in medium and far relatives, and plants in intraspecific interaction displayed intermediate values (Fig. 3b). We did not find significant differences of interaction intensity on SLA a (see Fig. S1 in Supporting Information).

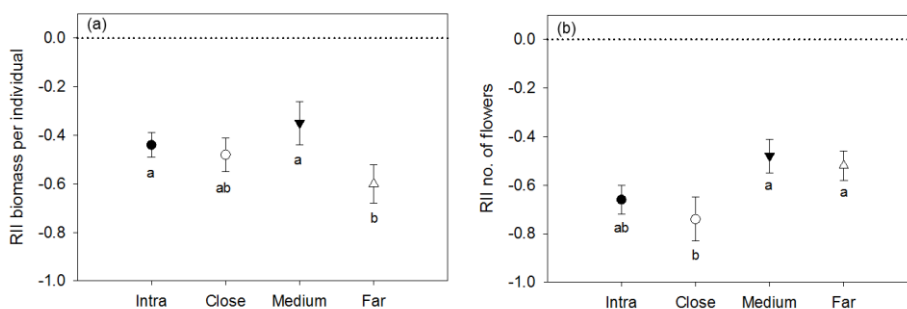


Fig. 3. RII biomass per individual (a) and RII no. of flowers (b) (mean \pm SE) for the four interaction treatments: intraspecific ($n = 33$), close ($n = 16$), medium ($n = 8$) and far ($n = 15$). The effects of the treatment were marginally significant for RII biomass per individual ($p = 0.07$) and significant for RII no. of flowers ($p = 0.05$). Different letters indicate post-hoc differences among treatments. Results of one sample t -tests on RII differences from 0 are indicated above errors, ** < 0.01 , *** < 0.001 ; positive values indicate facilitation and negative values indicate competition.

Finally, studying the dynamics of plant interactions across life stages, we found that one trait, no. of leaves (Fig. 4), but not leaf length (Fig. S2) neither height (although marginally significant; Fig. S3), showed a significant Time \times Phylogenetic relatedness interaction. One phylogenetic relatedness treatment was responsible of such significant interaction; the medium

relatives. All treatments tended to reduce RII leaf length trough time while increase RII no. of leaves. No. of leaves competition intensity changed from strongly negative RII values at the first census to weakly negative RII values at the last census. Moreover, intraspecific competition was the harsher for the no. of leaves at third and fourth census showing significant differences compared with the rest of treatments.

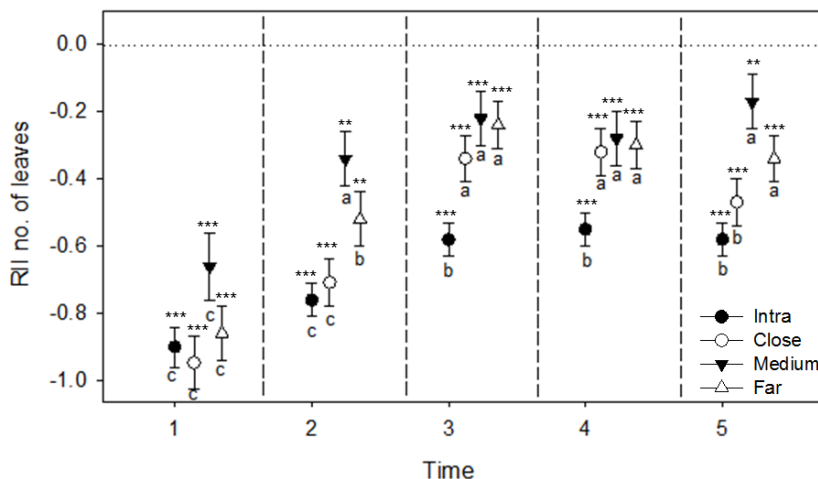


Fig. 4. RII no. of leaves (mean \pm SE) for the four competition treatments: intraspecific, close, medium and far ($n = 9-32$). The effects of the time ($p < 0.0001$), treatment ($p < 0.0001$) and the interaction of both factors ($p = 0.05$) were significant. Different letters indicate post-hoc differences among treatments. Results of one sample t -tests on RII differences from 0 are indicated above errors, ** < 0.01 , *** < 0.001 ; positive values indicate facilitation and negative values indicate competition.

Discussion

Our data showed that competition was, overall, less intense between distantly related species than in other treatments with closer relatives. As a consequence, there was higher survival and more flowers in far- than close relatives or in the intraspecific treatment. We also found a high variance in the interaction between intraspecifics and other treatments concerning no. of leaves, suggesting that this trait responded plastically to competition. Plastic changes were apparent across plants life stages except for the first and last

stages, which highlight the need to incorporate multiple life stages when assessing factors contributing to individual survival and species coexistence.

Plant performance and survival

Plant survival and growth were expected to vary depending on phylogenetic relatedness of species in a community. Some studies have shown that seedling establishment increases in presence of distantly related species compared to closely related species (Webb *et al.*, 2006; Castillo *et al.*, 2010) as they might reduce niche overlap and reduce potential competition (Valiente-Banuet & Verdú, 2008). It is not always the case and, for example, Lebrija-Trejos *et al.* (2013) showed the opposite with seedlings in a moist tropical forests in Panama, where survival tended to increase in the presence of closely-related neighbours while distantly-related neighbours did not affect survival.

In terms of plant performance and survival, most reports support the idea of higher probability of positive interactions among distantly-related species than in closely-related species (e.g., Valiente-Banuet *et al.*, 2006; Valiente-Banuet & Verdú, 2007; Verdú *et al.*, 2012) although recent reports showed the opposite (Cahill *et al.*, 2008; Godoy *et al.*, 2014). Our results showed that competition was less intense between distantly related species, overall showing higher survival and less negative effects on no. of flowers than pairs of close relatives. This supports the idea that distant relatives are more likely to coexist than closely-related species, probably due to higher niche differences (Valiente-Banuet & Verdú, 2007; Violle *et al.*, 2011; Verdú *et al.*, 2012) and/or through indirect interactions by avoiding shared pests and host-specific belowground enemies (Van der Putten, 2009; Castagneyrol *et al.*, 2014; Parker *et al.*, 2015).

Trait plasticity

Recent studies claim the importance of testing multiple life stages when assessing species coexistence and survival (e.g., Trinder *et al.*, 2012; Lebrija-

Trejos *et al.*, 2013; Trinder *et al.*, 2013; Zhu *et al.*, 2015). Plants change along ontogenetic development and these changes may be permanent or transient (Baldwin, 1999; Pearcy, 1999). The production and maintenance of leaves is one mechanism by which plants compete and capture resources (Trinder, Brooker & Robinson 2013); we found that competition effects on no. of leaves varied among treatments through time in a way that intraspecific competition was harsher at third and fourth census compared with other treatments; however, the significant interaction Time \times Phylogenetic relatedness was mainly a consequence of changes in the ‘medium’ treatment. Medium relatives increased competition intensity in no. of leaves at the fourth stage and decreased at the last; both species forming this treatment belonged to the family Poaceae, which are known to have trait conservatism to soil resources (e.g. adventitious root growth and high root allocation) and regrowth following grazing (e.g. basal meristem and high root allocation) (Coughenour, 1985; Chase, 2004). This suggests that these species could be able to respond better than the other selected species to aboveground and belowground processes (Bennett & Cahill, 2013)

Conclusion

Few studies have tested empirically how phylogenetic relatedness affects species interactions through trait similarity (Pearse & Hipp, 2009; Violle *et al.*, 2011). Some of them pointed out, however, that plant ontogenetic shifts can be dependent on environmental conditions (e.g., Schiffers & Tielbörger, 2006; le Roux *et al.*, 2013). Our approach included critical issues that have been overlooked in previous studies: multiple direct measurements of the process of competition as opposed to indirect mechanisms of competitive outcomes, and a consideration of temporal dynamics. Overall our results show that ontogenetic changes in species interactions depend on their phylogenetic relatedness and strongly affect interaction outcome.

Appendix D. Supplementary material.

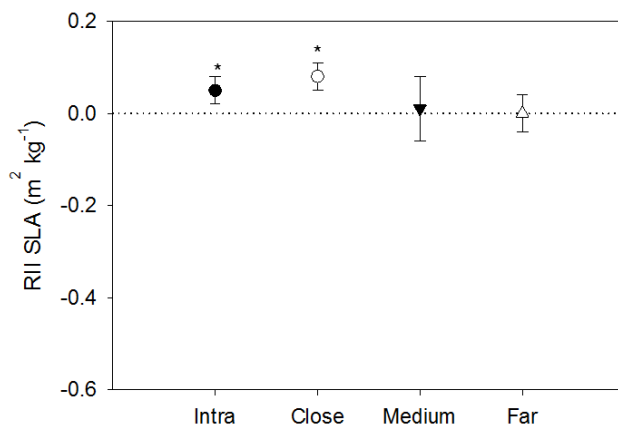


Figure D1. RII SLA (mean ± SE) for the four interaction treatments: intraspecific ($n = 25$), close ($n = 12$), medium ($n = 5$) and far ($n = 13$). The effect of the treatment was non-significant ($p = 0.23$). Results of one sample t -tests on RII differences from 0 are indicated above errors, * < 0.05 ; positive values indicate facilitation and negative values indicate competition.

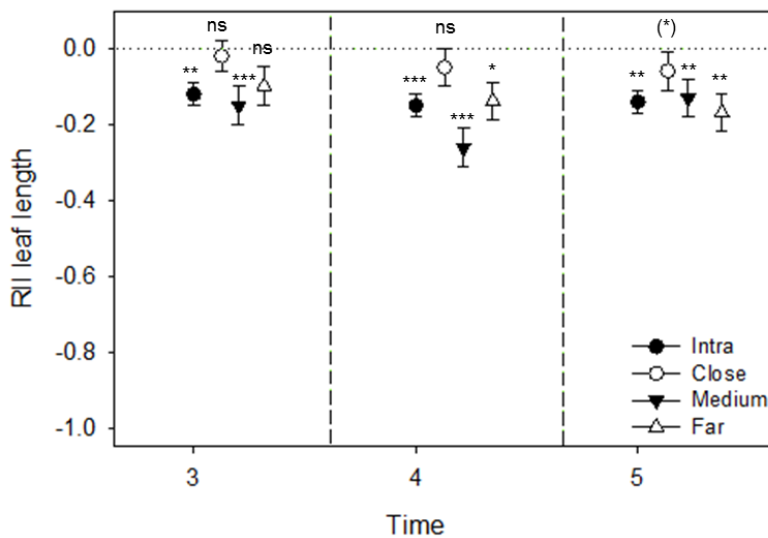


Figure D2. RII leaf length (mean ± SE) for the four competition treatments: intraspecific, close, medium and far ($n = 11-31$). The effects of time ($p = 0.05$) was

significant while treatment ($p = 0.10$) and their interaction ($p = 0.15$) were non-significant. Different letters indicate post-hoc differences among treatments. Results of one sample t -tests on RII differences from 0 are indicated above errors, ns > 0.1 , (*) < 0.1 , * < 0.05 , ** < 0.01 , *** < 0.001 ; positive values indicate a positive effect and negative values indicate a negative effect on leaf length.

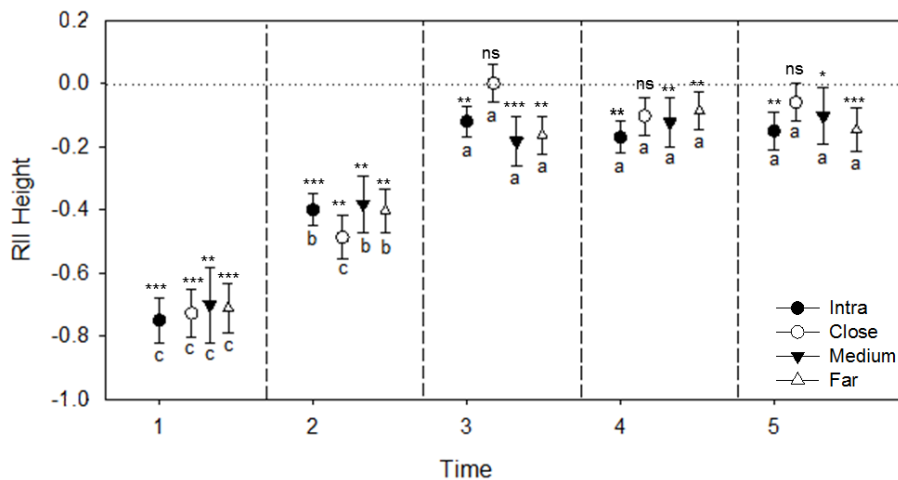


Figure D3. RII height (mean \pm SE) for the four interaction treatments: intraspecific, close, medium and far ($n = 9-32$). The effects of time were significant ($p < 0.0001$), whereas the effects of treatment ($p = 0.58$) was not significant and their interaction ($p = 0.09$) was marginally significant. Different letters indicate post-hoc differences among treatments. Results of one sample t -tests on RII differences from 0 are indicated above errors, ns > 0.1 , * < 0.05 , ** < 0.01 , *** < 0.001 ; positive values indicate facilitation and negative values indicate competition.

GENERAL CONCLUSIONS

1. Composition of subordinate communities differed among shrubs and among sites, and correlated with relative humidity, showing the dependence of subordinate species on the micro-environments created by the different shrub species.
2. There was a positive effect of shrubs on overall plant abundance, and species richness prevailed in the most severe parts of the gradient, while under relatively milder conditions shrub effects were mostly neutral or negative. Such effects differ with shrub identity.
3. Where microhabitat differences were more extreme, shrub species had complementary effects, promoting whole-community species richness and phylogenetic diversity. Such complementary effect is absent at sites of low environmental severity where individual shrub species has non-significant or negative effects on species richness and/or abundance.
4. There is environmental context-dependence in patterns of phylogenetic similarity, as interaction outcome depends on species relatedness.
5. Both phenotypes of *Cytisus galianoi* showed facilitation effects on the species *Festuca indigesta* but the tight phenotype received a negative feedback. By contrast, the loose phenotype showed higher species richness, plant abundance, and more phylogenetic diversity than the tight phenotype, but no negative feedback. This suggests that negative feedback effects of the subordinate species may cause a phenotype change from tight to loose.
6. Competition was, overall, less intense between distantly related species compared with the rest of treatments differing in degree of relatedness under homogeneous environmental conditions.
7. Ontogenetic changes in species interactions depend on their phylogenetic relatedness. Therefore, there is a need to incorporate multiple life stages when assessing factors contributing to individual survival and species coexistence.

1. La composición de la comunidad de subordinadas fue distinta entre arbustos y sitios y estaba relacionada con la humedad relativa del micro-hábitat a lo largo del gradiente mostrando así la dependencia de estas especies a las condiciones creadas por los arbustos.
2. Los efectos positivos de los arbustos sobre la abundancia y riqueza de especies subordinadas prevalecieron en las partes más severas del gradiente, mientras que bajo condiciones relativamente suaves, los efectos fueron neutros o negativos. Estos efectos fueron además dependientes de la identidad del arbusto.
3. En las comunidades donde las diferencias en micro-hábitat fueron más extremas y donde al menos un arbusto presentaba efectos positivos sobre la comunidad de subordinadas, las especies de arbustos tenían efectos complementarios sobre la riqueza y diversidad filogenética a nivel de comunidad. Sin embargo, estos efectos desaparecían en sitios de baja severidad ambiental donde los arbustos tenían un efecto neutro o negativo sobre la abundancia y riqueza de subordinadas.
4. Los patrones filogenéticos fueron dependientes del ambiente ya que el resultado de la interacción entre especies cercanas y la nodriza variaba a lo largo del gradiente.
5. Ambos fenotipos de *Cytisus galianoi* mostraron facilitación sobre la especie *Festuca indigesta* pero el fenotipo compacto sufría un coste por dicha facilitación. Por el contrario, el fenotipo abierto mostró mayor riqueza y abundancia de especies y mayor diversidad filogenética y no sufría ningún coste. Esto sugiere que los costes de facilitación pueden ser la causa del cambio de fenotipo compacto a abierto.
6. La competencia fue más intensa entre especies lejanas en la filogenia comparada con el resto de tratamientos dependiendo de la similitud filogenética entre plantas en condiciones ambientales homogéneas.

7. Los cambios en las interacciones entre plantas a lo largo de la ontogenia dependen de su relación filogenética entre ellas. Por tanto, existe una necesidad de incorporar múltiples etapas del ciclo de vida para evaluar los factores que contribuyen a la supervivencia y coexistencia entre especies.

REFERENCIAS

- Ackerly DD. 1997.** Plant life histories: A meeting of phylogeny and ecology. *Trends in Ecology & Evolution* **12**(1): 7-9.
- Ackerly DD. 1998.** Leaf size, sapling allometry, and Corner's rules: phylogeny and correlated evolution in maples (*Acer*). *The American Naturalist* **152**(6): 767.
- Ackerly DD. 2009.** Conservatism and diversification of plant functional traits: Evolutionary rates versus phylogenetic signal. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 19699-19706.
- Adler PB, Fajardo A, Kleinhesselink A, Kraft NJB, Scherber C. 2013.** Trait-based tests of coexistence mechanisms. *Ecology Letters* **16**(10): 1294-1306.
- Aguiar MR, Sala O. 1999.** Patch structure, dynamics and implications for the functioning of arid ecosystems. *Trends in Ecology & Evolution* **14**(7): 273-277.
- Al Hayek P, Maalouf J-P, Baumel A, Bou Dagher-Kharrat M, Médail F, Touzard B, Michalet R. 2015.** Differential effects of contrasting phenotypes of a foundation legume shrub drive plant–plant interactions in a Mediterranean mountain. *Journal of Vegetation Science* **26**(2): 373-384.
- Al Hayek P, Touzard B, Le Bagousse-Pinguet Y, Michalet R. 2014.** Phenotypic differentiation within a foundation grass species correlates with species richness in a subalpine community. *Oecologia* **176**(2): 533-544.
- Albert CH, Thuiller W, Yoccoz NG, Douzet R, Aubert S, Lavorel S. 2010a.** A multi-trait approach reveals the structure and the relative importance of intra- vs. interspecific variability in plant traits. *Functional Ecology* **24**(6): 1192-1201.
- Albert CH, Thuiller W, Yoccoz NG, Soudant A, Boucher F, Saccone P, Lavorel S. 2010b.** Intraspecific functional variability: extent, structure and sources of variation. *Journal of Ecology* **98**(3): 604-613.
- Amarasekare P. 2003.** Competitive coexistence in spatially structured environments: a synthesis. *Ecology Letters* **6**(12): 1109-1122.
- Amat B, Cortina J, Zubcoff JJ. 2015.** Community attributes determine facilitation potential in a semi-arid steppe. *Perspectives in Plant Ecology, Evolution and Systematics* **17**(1): 24-33.
- Anthelme F, Cavieres LA, Dangles O. 2014.** Facilitation among plants in alpine environments in the face of climate change. *Frontiers in Plant Science* **5**(387).
- Ariza C, Tielbörger K. 2011.** An evolutionary approach to studying the relative importance of plant–plant interactions along environmental gradients. *Functional Ecology* **25**(4): 932-942.
- Armas C, Kikvidze Z, Pugnaire FI. 2009.** Abiotic conditions, neighbour interactions, and the distribution of *Stipa tenacissima* in a semiarid mountain range. *Journal of Arid Environments* **73**(12): 1084-1089.
- Armas C, Ordiales R, Pugnaire FI. 2004.** Measuring plant interactions: A new comparative index. *Ecology* **85**(10): 2682-2686.
- Armas C, Pugnaire FI. 2005.** Plant interactions govern population dynamics in a semi-arid plant community. *Journal of Ecology* **93**(5): 978-989.

- Armas C, Pugnaire FI. 2009.** Ontogenetic shifts in interactions of two dominant shrub species in a semi-arid coastal sand dune system. *Journal of Vegetation Science* **20**(3): 535-546.
- Armas C, Pugnaire FI. 2011.** Plant neighbour identity matters to belowground interactions under controlled conditions. *Plos One* **6**(11).
- Armas C, Rodríguez-Echeverría S, Pugnaire FI. 2011.** A field test of the stress-gradient hypothesis along an aridity gradient. *Journal of Vegetation Science* **22**(5): 818-827.
- Arroyo-Rodríguez V, Cavender-Bares J, Escobar F, Melo FPL, Tabarelli M, Santos BA. 2012.** Maintenance of tree phylogenetic diversity in a highly fragmented rain forest. *Journal of Ecology* **100**(3): 702-711.
- Ashton IW, Miller AE, Bowman WD, Suding KN. 2010.** Niche complementarity due to plasticity in resource use: plant partitioning of chemical N forms. *Ecology* **91**(11): 3252-3260.
- Aubert S, Boucher F, Lavergne S, Renaud J, Choler P. 2014.** 1914–2014: A revised worldwide catalogue of cushion plants 100 years after Hauri and Schröter. *Alpine Botany* **124**(1): 59-70.
- Badano EI, Cavieres LA. 2006.** Impacts of ecosystem engineers on community attributes: effects of cushion plants at different elevations of the Chilean Andes. *Diversity and Distributions* **12**: 388-396.
- Badano EI, Jones CG, Cavieres LA, Wright JP. 2006.** Assessing impacts of ecosystem engineers on community organization: a general approach illustrated by effects of a high-Andean cushion plant. *Oikos* **115**(2): 369-385.
- Badano EI, Marquet PA, Cavieres LA. 2010.** Predicting effects of ecosystem engineering on species richness along primary productivity gradients. *Acta Oecologica* **36**: 46-54.
- Baker NR. 2008.** Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annual Review of Plant Biology* **59**(1): 89-113.
- Baldwin I. 1999.** Inducible nicotine production in native nicotiana as an example of adaptive phenotypic plasticity. *Journal of Chemical Ecology* **25**(1): 3-30.
- Bennett JA, Cahill JF. 2013.** Conservatism of responses to environmental change is rare under natural conditions in a native grassland. *Perspectives in Plant Ecology, Evolution and Systematics* **15**(6): 328-337.
- Bennett JA, Lamb EG, Hall JC, Cardinal-McTeague WM, Cahill JF. 2013.** Increased competition does not lead to increased phylogenetic overdispersion in a native grassland. *Ecology Letters* **16**(9): 1168-1176.
- Berendse F, de Kroon H, Braakhekke WG 2007.** Chapter 8 - Acquisition, use and loss of nutrients. In: Pugnaire FI, Valladares F eds. *Functional plant ecology*. Boca Ratón, FL: Boca Ratón, FL : CRC Press.
- Bertness MD, Callaway RM. 1994.** Positive interactions in communities. *Trends in Ecology & Evolution* **9**(5): 191-193.
- Blomberg SP, Garland TJ, Ives AR. 2003.** Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**(4): 717-745.
- Bråthen KA, Ravolainen VT. 2015.** Niche construction by growth forms is as strong a predictor of species diversity as environmental gradients. *Journal of Ecology*: doi: 10.1111/1365-2745.12380.
- Bronstein JL. 2009.** The evolution of facilitation and mutualism. *Journal of Ecology* **97**(6): 1160-1170.

- Brooker RW. 2006.** Plant-plant interactions and environmental change. *New Phytologist* **171**(2): 271-284.
- Brooker RW, Callaway RM. 2009.** Facilitation in the conceptual melting pot. *Journal of Ecology* **97**(6): 1117-1120.
- Brooker RW, Maestre FT, Callaway RM, Lortie CL, Cavieres LA, Kunstler G, Liancourt P, Tielborger K, Travis JMJ, Anthelme F, et al. 2008.** Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology* **96**(1): 18-34.
- Buckley Y, Buckley S, Ramula S, Blomberg J, Burns E, Crone J, Ehrlén T, Knight J-B, Pichancourt H, Quested G, et al. 2010.** Causes and consequences of variation in plant population growth rate: a synthesis of matrix population models in a phylogenetic context. *Ecology Letters* **13**(9): 1182-1197.
- Burns JH, Strauss SY. 2011.** More closely related species are more ecologically similar in an experimental test. *Proceedings of the National Academy of Sciences* **108**(13): 5302-5307.
- Burns JH, Strauss SY. 2012.** Effects of competition on phylogenetic signal and phenotypic plasticity in plant functional traits. *Ecology* **93**(8s): S126-S137.
- Butterfield BJ, Cavieres LA, Callaway RM, Cook BJ, Kikvidze Z, Lortie CJ, Michalet R, Pugnaire FI, Schöb C, Xiao S, et al. 2013.** Alpine cushion plants inhibit the loss of phylogenetic diversity in severe environments. *Ecology Letters* **16**(4): 478-486.
- Cadotte MW. 2013.** Experimental evidence that evolutionarily diverse assemblages result in higher productivity. *Proceedings of the National Academy of Sciences of the United States of America* **110**(22): 8996-9000.
- Cadotte MW, Albert CH, Walker SC. 2013.** The ecology of differences: assessing community assembly with trait and evolutionary distances. *Ecology Letters* **16**(10): 1234-1244.
- Cahill JF, Kembel SW, Lamb EG, Keddy PA. 2008.** Does phylogenetic relatedness influence the strength of competition among vascular plants? *Perspectives in Plant Ecology, Evolution and Systematics* **10**(1): 41-50.
- Callaway RM. 1995.** Positive interactions among plants. *Botanical Review* **61**(4): 306-349.
- Callaway RM. 1998.** Are positive interaction species-specific? *Oikos* **82**: 202-207.
- Callaway RM. 2007.** Positive interactions and interdependence in plant communities *Springer, Dordrecht, The Netherlands*.
- Callaway RM, Brooker RW, Choler P, Kikvidze Z, Lortie CJ, Michalet R, Paolini L, Pugnaire FI, Newingham B, Aschehoug ET, et al. 2002.** Positive interactions among alpine plants increase with stress. *Nature* **417**(6891): 844-848.
- Callaway RM, Howard TG. 2007.** Competitive networks, indirect interactions, and allelopathy: a microbial view point on plant communities. *Progress in Botany* **68**(317-335).
- Callaway RM, Nadkarni NM, Mahall BE. 1991.** Facilitation and interference of *Quercus douglasii* on understory productivity in Central California. *Ecology* **72**(4): 1484-1499.
- Callaway RM, Walker LR. 1997.** Competition and facilitation: A synthetic approach to interactions in plant communities. *Ecology* **78**(7): 1958-1965.

- Castagneyrol B, Jactel H, Vacher C, Brockerhoff EG, Koricheva J. 2014.** Effects of plant phylogenetic diversity on herbivory depend on herbivore specialization. *Journal of Applied Ecology* **51**(1): 134-141.
- Castillo JP, Verdú M, Valiente-Banuet A. 2010.** Neighborhood phylodiversity affects plant performance. *Ecology* **91**(12): 3656-3663.
- Castro J, Zamora R, Hodar JA, Gómez JM. 2005.** Ecology of seed germination of *Pinus sylvestris* L. at its southern, Mediterranean distribution range. *Forest Systems* **14**(2).
- Cavender-Bares J, Keen A, Miles B. 2006.** Phylogenetic structure of Floridian plant communities depends on taxonomic and spatial scale. *Ecology* **87**(7 Suppl): S109-122.
- Cavender-Bares J, Kozak KH, Fine PVA, Kembel SW. 2009.** The merging of community ecology and phylogenetic biology. *Ecology Letters* **12**(7): 693-715.
- Cavender-Bares J, Reich PB. 2012.** Shocks to the system: community assembly of the oak savanna in a 40-year fire frequency experiment. *Ecology* **93**(8): S52-S69.
- Cavieres LA, Badano EI. 2009.** Do facilitative interactions increase species richness at the entire community level? *Journal of Ecology* **97**: 1181-1191.
- Cavieres LA, Badano EI, Sierra-Almeida A, Gómez-González S, Molina-Montenegro MA. 2005.** Positive interactions between alpine plant species and the nurse cushion plant *Laretia acaulis* do not increase with elevation in the Andes of central Chile. *New Phytologist* **169**: 59-69.
- Cavieres LA, Badano EI, Sierra-Almeida A, Molina-Montenegro MA. 2007.** Microclimatic modifications of cushion plants and their consequences for seedling survival of native and non-native herbaceous species in the High Andes of Central Chile. *Arctic, antarctic, and alpine research* **39**(2): 229-236.
- Cavieres LA, Brooker RW, Butterfield BJ, Cook BJ, Kikvidze Z, Lortie CJ, Michalet R, Pugnaire FI, Schöb C, Xiao S, et al. 2014.** Facilitative plant interactions and climate simultaneously drive alpine plant diversity. *Ecology Letters* **17**(2): 193-202.
- Comita LS, Hubbell SP. 2009.** Local neighborhood and species' shade tolerance influence survival in a diverse seedling bank. *Ecology* **90**(2): 328-334.
- Cornelissen JHC, Lavorel S, Garnier E, Díaz S, Buchmann N, Gurvich DE, Reich PB, Ter Steege H, Morgan HD, Van Der Heijden MGA, et al. 2003.** A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany* **51**(4): 335-380.
- Coughenour MB. 1985.** Graminoid responses to grazing by large herbivores: adaptations, exaptations, and interacting processes. *Annals of the Missouri Botanical Garden* **72**(4): 852-863.
- Crutsinger GM, Strauss SY, Rudgers JA. 2010.** Genetic variation within a dominant shrub species determines plant species colonization in a coastal dune ecosystem. *Ecology* **91**(4): 1237-1243.
- Chase MW. 2004.** Monocot relationships: an overview. *American Journal of Botany* **91**(10): 1645-1655.
- Chen J, Schöb C, Zhou Z, Gong Q, Li X, Yang Y, Li Z, Sun H. 2015.** Cushion plants can have a positive effect on diversity at high elevations in the

- Himalayan Hengduan Mountains. *Journal of Vegetation Science* doi: 10.1111/jvs.12275.
- Chesson P. 2000.** Mechanisms of Maintenance of Species Diversity. *Annual Review of Ecology and Systematics* **31**(ArticleType: research-article / Full publication date: 2000 / Copyright © 2000 Annual Reviews): 343-366.
- Choler P. 2005.** Consistent shifts in alpine plant traits along a mesotopographical gradient. *Arctic, Antarctic and Alpine Research* **37**(4): 444-453.
- Choler P, Michalet R, Callaway RM. 2001.** Facilitation and competition on gradients in alpine plant communities. *Ecology* **82**(12): 3295-3308.
- Chu C, Wang Y, Li Q, Zhao L, Ren Z, Xiao S, Yuan J, Wang G. 2009.** Effects of traits, species identity and local environmental conditions on the assessment of interactions: insights from an alpine meadow community. *Journal of Plant Ecology* **2**(3): 135-141.
- Darwin C. 1859.** *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life.* London: John Murray.
- Decreto 238/2011 de 12 de julio.** por el que se establece la ordenación y gestión de Sierra Nevada. España: Boletín Oficial de la Junta de Andalucía. 114-314.
- Delgado R, Castillo, A., Valle, F., Lorite, J., Blanca, G., Tinaz, A., Pascual, F., Pleguezuelos, JM., Rivas, JM., Pérez, J., Moleon, M., Titos, M., Castellón, F. and Muñoz, A. 2001.** *Parque Nacional de Sierra Nevada.* Talavera de la Reina, España.: Canseco editores.
- Delgado R, Delgado G, Párraga J, Gámiz E, Sánchez M, Tenorio MA. 1988.** *Proyecto LUCDEME Mapa de Suelos, Güejar-Sierra 1027.* Spain: Universidad de Granada.
- Di Rienzo J, Casanoves F, Balzarini M, Gonzalez L, Tablada M, Robledo C. 2013.** InfoStat v. 2013. *InfoStat Group, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina.*
- Dias PC. 1996.** Sources and sinks in population biology. *Trends in Ecology & Evolution* **11**(8): 326-330.
- Díaz S, Cabido M. 2001.** Vive la différence: plant functional diversity matters to ecosystem processes. *Trends in Ecology & Evolution* **16**(11): 646-655.
- Ellison AM, Bank MS, Clinton BD, Colburn EA, Elliott K, Ford CR, Foster DR, Kloeppe BD, Knoepp JD, Lovett GM, et al. 2005.** Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* **3**(9): 479-486.
- Engler R, Randin CF, Thuiller W, Dullinger S, Zimmermann NE, Araujo MB, Pearman PB, Le Lay G, Piedallu C, Albert CH, et al. 2011.** 21st century climate change threatens mountain flora unequally across Europe. *Global Change Biology* **17**(7): 2330-2341.
- Faith DP. 1992.** Conservation evaluation and phylogenetic diversity. *Biological Conservation* **61**(1): 1-10.
- Falster DS, Westoby M. 2003.** Plant height and evolutionary games. *Trends in Ecology & Evolution* **18**(7): 337-343.
- Fibich P, Vítová A, Macek P, Lepš J. 2013.** Establishment and spatial associations of recruits in meadow gaps. *Journal of Vegetation Science* **24**(3): 496-505.
- Fritschie KJ, Cardinale BJ, Alexandrou MA, Oakley TH. 2014.** Evolutionary history and the strength of species interactions: testing the phylogenetic limiting similarity hypothesis. *Ecology* **95**: 1407-1417.
- Gaston KJ. 2000.** Global patterns in biodiversity. *Nature* **405**(6783): 220-227.

- Godoy O, Kraft NJB, Levine JM. 2014.** Phylogenetic relatedness and the determinants of competitive outcomes. *Ecology Letters* **17**(7): 836-844.
- Goldberg DE. 1996.** Competitive ability: Definitions, contingency and correlated traits. *Philosophical Transactions of the Royal Society B: Biological Sciences* **351**(1345): 1377-1385.
- Gómez-Aparicio L. 2009.** The role of plant interactions in the restoration of degraded ecosystems: a meta-analysis across life-forms and ecosystems. *Journal of Ecology* **97**(6): 1202-1214.
- Gonzalez MA, Roger A, Courtois EA, Jabot F, Norden N, Paine CET, Baraloto C, Thébaud C, Chave J. 2010.** Shifts in species and phylogenetic diversity between sapling and tree communities indicate negative density dependence in a lowland rain forest. *Journal of Ecology* **98**(1): 137-146.
- Grime JP. 1977.** Evidence for existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist* **111**(982): 1169-1194.
- Grime JP, Pierce S. 2012.** *The evolutionary strategies that shape ecosystems*: John Wiley & Sons.
- Gross N, Kunstler G, Liancourt P, de Bello F, Suding KN, Lavorel S. 2009.** Linking individual response to biotic interactions with community structure: a trait-based framework. *Functional Ecology* **23**(6): 1167-1178.
- Group TAP. 2009.** An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161**(2): 105-121.
- Guisan A, Theurillat JP. 2000.** Assessing alpine plant vulnerability to climate change: a modeling perspective. *Integrated Assessment* **1**: 307-320.
- Harper JL. 1977.** *The Population Biology of Plants*. New York Academic Press.
- He Q, Bertness MD, Altieri AH. 2013.** Global shifts towards positive species interactions with increasing environmental stress. *Ecology Letters* **16**(5): 695-706.
- Helmus MR, Bland TJ, Williams CK, Ives AR. 2007.** Phylogenetic measures of biodiversity. *The American Naturalist* **169**(3): E68-E83.
- HilleRisLambers J, Adler PB, Harpole WS, Levine JM, Mayfield MM. 2012.** Rethinking community assembly through the lens of coexistence theory. *Annual Review of Ecology, Evolution, and Systematics* **43**(1): 227-248.
- Huston MA. 1997.** Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia* **110**(4): 449-460.
- Jones CG, Gutierrez JL, Byers JE, Crooks JA, Lambrinos JG, Talley TS. 2010.** A framework for understanding physical ecosystem engineering by organisms. *Oikos* **119**(12): 1862-1869.
- Jones CG, Gutierrez JL, Groffman PM, Shachak M. 2006.** Linking ecosystem engineers to soil processes: a framework using the Jenny State Factor Equation. *European Journal of Soil Biology* **42**: S39-S53.
- Jones CG, Lawton J, Shachak M. 1994a.** Organisms as ecosystem engineers. *Oikos* **69**(3): 373-386.
- Jones CG, Lawton JH, Shachak M. 1994b.** Organisms as ecosystems engineers. *Oikos* **69**(3): 373-386.
- Jung V, Violle C, Mondy C, Hoffmann L, Muller S. 2010.** Intraspecific variability and trait-based community assembly. *Journal of Ecology* **98**(5): 1134-1140.

- Kawai T. 2007.** Testing the facilitation-competition paradigm under the stress-gradient hypothesis: Decoupling multiple stress factors. *Proceedings of the Royal Society of London B Biological Sciences* **274**(1624): 2503-2508.
- Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO. 2010.** Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* **26**(11): 1463-1464.
- Kikvidze Z, Michalet R, Brooker RW, Cavieres LA, Lortie CJ, Pugnaire FI, Callaway RM. 2011.** Climatic drivers of plant-plant interactions and diversity in alpine communities. *Alpine Botany* **121**(1): 63-70.
- Kikvidze Z, Pugnaire FI, Brooker RW, Choler P, Lortie CJ, Michalet R, Callaway RM. 2005.** Linking patterns and processes in alpine plant communities: A global study. *Ecology* **86**(6): 1395-1400.
- Körner C. 2003.** *Alpine plant life: functional plant ecology of high mountain ecosystems*. Berlin: Springer.
- Lázaro R, Rodrigo FS, Gutiérrez L, Domingo F, Puigdefábreas J. 2001.** Analysis of a 30-year rainfall record (1967–1997) in semi-arid SE Spain for implications on vegetation. *Journal of Arid Environments* **48**(3): 373-395.
- Le Bagousse-Pinguet Y, Maalouf J-P, Touzard B, Michalet R. 2014.** Importance, but not intensity of plant interactions relates to species diversity under the interplay of stress and disturbance. *Oikos* **123**(7): 777-785.
- le Roux PC, Shaw J, Chown S. 2013.** Ontogenetic shifts in plant interactions vary with environmental severity and affect population structure. *New Phytologist* **200**(1): 241-250.
- Lebrija-Trejos E, Wright SJ, Hernández A, Reich PB. 2013.** Does relatedness matter? Phylogenetic density-dependent survival of seedlings in a tropical forest. *Ecology* **95**(4): 940-951.
- Liczner AR, Lortie CJ. 2014.** A global meta-analytic contrast of cushion-plant effects on plants and on arthropods. *PeerJ* **2**: e265.
- Loreau M, Hector A. 2001.** Partitioning selection and complementarity in biodiversity experiments. *Nature* **412**(6842): 72-76.
- Loreau M, Naeem S, Inchausti P, Bengtsson J, Grime JP, Hector A, Hooper DU, Huston MA, Raffaelli D, Schmid B, et al. 2001.** Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science* **294**(5543): 804-808.
- Lortie CJ, Brooker RW, Choler P, Kikvidze Z, Michalet R, Pugnaire FI, Callaway RM. 2004.** Rethinking plant community theory. *Oikos* **107**(2): 433-438.
- Losos JB. 2008.** Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology Letters* **11**(10): 995-1003.
- Lozano YM. 2014.** *Interacciones entre plantas y microorganismos del suelo: consecuencias para la dinámica de comunidades vegetales*. PhD, University of Almería Almería.
- MacArthur R, Levins R. 1967.** The limiting similarity, convergence, and divergence of coexisting species. *The American Naturalist* **101**(921): 377-385.
- Maestre FT, Callaway RM, Valladares F, Lortie CJ. 2009.** Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology* **97**(2): 199-205.

- Maestre FT, Valladares F, Reynolds JF. 2005.** Is the change of plant-plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. *Journal of Ecology* **93**(4): 748-757.
- Martínez-García LB, García K, Hammer EC, Vayssières A. 2013.** Mycorrhiza for all: an under-earth revolution. *New Phytologist* **198**(3): 652-655.
- Martínez LB, Pugnaire, F.I. 2009.** Interacciones entre las comunidades de hongos formadores de micorrizas arbusculares y de plantas. Algunos ejemplos en los ecosistemas semiáridos. *Ecosistemas* **18**(2): 44-54.
- Mayfield MM, Levine JM. 2010.** Opposing effects of competitive exclusion on the phylogenetic structure of communities. *Ecology Letters* **13**(9): 1085-1093.
- Michalet R. 2006.** Do biotic interactions shape both sides of the humped-back model of species richness in plant communities? *Ecology Letters* **9**(7): 767-773.
- Michalet R. 2007.** Highlighting the multiple drivers of change in interactions along stress gradients. *New Phytologist* **173**(1): 3-6.
- Michalet R, Xiao S, Touzard B, Smith DS, Cavieres LA, Callaway RM, Whitham TG. 2011.** Phenotypic variation in nurse traits and community feedbacks define an alpine community. *Ecology Letters* **14**(5): 433-443.
- Milla R, Gimenez-Benavides L, Montserrat-Marti G. 2008.** Replacement of species along altitude gradients: the role of branch architecture. *Annals of Botany* **102**(6): 953-966.
- Miriti MN. 2006.** Ontogenetic shift from facilitation to competition in a desert shrub. *Journal of Ecology* **94**(5): 973-979.
- Molina-Montenegro MA, Badano EI, Cavieres LA. 2006.** Cushion plants as microclimatic shelters for two ladybird beetles species in alpine zone of Central Chile. *Arctic, antarctic, and alpine research* **38**(2): 224-227.
- Monje O, Bugbee B. 1998.** Adaptation to high CO₂ concentration in an optimal environment: radiation capture, canopy quantum yield and carbon use efficiency. *Plant, Cell & Environment* **21**(3): 315-324.
- Mouquet N, Devictor V, Meynard CN, Munoz F, Bersier L-F, Chave J, Coueron P, Dalecky A, Fontaine C, Gravel D, et al. 2012.** Ecophylogenetics: advances and perspectives. *Biological Reviews* **87**(4): 769-785.
- Nipperess DA, Matsen FA. 2013.** The mean and variance of phylogenetic diversity under rarefaction. *Methods in Ecology and Evolution* **4**(6): 566-572.
- OdlingSmee FJ, Laland KN, Feldman MW. 1996.** Niche construction. *The American Naturalist* **147**(4): 641-648.
- Oksanen J, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Simpson G, Solymos P, Henry M, Stevens H, et al. 2013.** vegan: Community Ecology Package. R package version 2.0-10.
- Ordoñez JC, van Bodegom PM, Witte J-PM, Wright IJ, Reich PB, Aerts R. 2009.** A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Global Ecology and Biogeography* **18**(2): 137-149.
- Paradis E, Claude J, Strimmer K. 2004.** APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**: 289-290.
- Parker IM, Saunders M, Bontrager M, Weitz AP, Hendricks R, Magarey R, Suiter K, Gilbert GS. 2015.** Phylogenetic structure and host abundance drive disease pressure in communities. *Nature* **520**(7548): 542-544.
- Parkhurst DF, Loucks OL. 1972.** Optimal Leaf Size in Relation to Environment. *Journal of Ecology* **60**(2): 505-537.

- Pausas J, Verdu M. 2010.** The jungle of methods for evaluating phenotypic and phylogenetic structure of communities. *Bioscience* **60**(8): 614-625.
- Pearcy RW 1999.** Responses of plants to heterogeneous light environments. In: F. Valladares and F. I . Pugnaire e ed. *Functional Plant Ecology, Second Edition*. Marcel Dekker, New York, New York, USA.: CRC Press, 269-314.
- Pearse IS, Hipp AL. 2009.** Phylogenetic and trait similarity to a native species predict herbivory on non-native oaks. *Proceedings of the National Academy of Sciences* **106**(43): 18097-18102.
- Pellissier L, Pradervand J-N, Williams PH, Litsios G, Cherix D, Guisan A. 2013.** Phylogenetic relatedness and proboscis length contribute to structuring bumblebee communities in the extremes of abiotic and biotic gradients. *Global Ecology and Biogeography* **22**(5): 577-585.
- Pinheiro J, Bates D, DebRoy S, Sarkar D, Team RDC. 2015.** *nlme: Linear and Nonlinear Mixed Effects Models*. <http://CRAN.R-project.org/package=nlme>: R package version 3.1-120.
- Pistón N, Armas C, Schöb C, Macek P, Pugnaire FI. 2015.** Phylogenetic distance among beneficiary species in a cushion plant species explain interaction outcome. *Oikos*: doi: 10.1111/oik.01979.
- Poorter H, Niinemets U, Poorter L, Wright IJ, Villar R. 2009.** Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist* **182**(3): 565-588.
- Prieto I, Martínez-Tilleria K, Martínez-Manchego L, Montecinos S, Pugnaire FI, Squeo FA. 2010.** Hydraulic lift through transpiration suppression in shrubs from two arid ecosystems: patterns and control mechanisms. *Oecologia* **163**(4): 855-865.
- Prinzing A, Reiffers R, Braakhekke WG, Hennekens SM, Tackenberg O, Ozinga WA, Schaminée JHJ, Van Groenendael JM. 2008.** Less lineages – more trait variation: phylogenetically clustered plant communities are functionally more diverse. *Ecology Letters* **11**(8): 809-819.
- Proffitt CE, Chiasson RL, Owens AB, Edwards KR, Travis SE. 2005.** *Spartina alterniflora* genotype influences facilitation and suppression of high marsh species colonizing an early successional salt marsh. *Journal of Ecology* **93**(2): 404-416.
- Pugnaire FI, Armas C, Maestre FT. 2011.** Positive plant interactions in the Iberian Southeast: Mechanisms, environmental gradients, and ecosystem function. *Journal of Arid Environments* **75**(12): 1310-1320.
- Pugnaire FI, Armas C, Valladares F. 2004.** Soil as a mediator in plant-plant interactions in a semi-arid community. *Journal of Vegetation Science* **15**(1): 85-92.
- Pugnaire FI, Haase P, Incoll LD, Clark SC. 1996a.** Response of the tussock grass *Stipa tenacissima* to watering in a semi-arid environment. *Functional Ecology* **10**(2): 265-274.
- Pugnaire FI, Haase P, Puigdefábregas J. 1996b.** Facilitation between higher plant species in a semiarid environment. *Ecology* **77**(5): 1420-1426.
- Pugnaire FI, Haase P, Puigdefábregas J, Cueto M, Clark SC, Incoll LD. 1996c.** Facilitation and succession under the canopy of a leguminous shrub, *Retama sphaerocarpa*, in a semi-arid environment in south-east Spain. *Oikos* **76**(3): 455-464.

- Pugnaire FI, Zhang L, Li R, Luo T. 2015.** No evidence of facilitation collapse in the Tibetan plateau. *Journal of Vegetation Science* **26**(2): 233-242.
- Purschke O, Purschke B, Schmid M, Sykes P, Poschlod S, Michalski W, Durka I, Kühn M, Winter H, Prentice J, et al. 2013.** Contrasting changes in taxonomic, phylogenetic and functional diversity during a long-term succession: insights into assembly processes. *Journal of Ecology* **101**(4): 857-866.
- R-CoreTeam 2013.** R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Reid AM, Lamarque LJ, Lortie CJ. 2010.** A systematic review of the recent ecological literature on cushion plants: the new heavy weight champions of plant facilitation. *Web Ecology* **10**: 44.
- Ricklefs RE. 2010.** Evolutionary diversification, coevolution between populations and their antagonists, and the filling of niche space. *Proceedings of the National Academy of Sciences of the United States of America* **107**(4): 1265-1272.
- Rodríguez-Echeverría S, Armas C, Pistón N, Hortal S, Pugnaire FI. 2013.** A role for below-ground biota in plant-plant facilitation. *Journal of Ecology* **101**(6): 1420-1428.
- Rodríguez-Echeverría S, Fajardo S, Ruiz-Díez B, Fernández-Pascual M. 2012.** Differential effectiveness of novel and old legume–rhizobia mutualisms: implications for invasion by exotic legumes. *Oecologia* **170**(1): 253-261.
- Rodríguez-Echeverría S, Pérez-Fernández MA. 2003.** Soil fertility and herb facilitation mediated by *Retama sphaerocarpa*. *Journal of Vegetation Science* **14**(6): 807-814.
- Sánchez-Marañón M, Soriano M, Delgado G, Delgado R. 2002.** Soil quality in Mediterranean mountain environments: Effects of land use change. *Soil Science Society of America journal* **66**(3): 948-958.
- Schiffers K, Tielbörger K. 2006.** Ontogenetic shifts in interactions among annual plants. *Journal of Ecology* **94**(2): 336-341.
- Schöb C, Armas C, Guler M, Prieto I, Pugnaire FI. 2013.** Variability in functional traits mediates plant interactions along stress gradients. *Journal of Ecology* **101**(3): 753-762.
- Schöb C, Butterfield BJ, Pugnaire FI. 2012.** Foundation species influence trait-based community assembly. *New Phytologist* **196**(3): 824-834.
- Schöb C, Callaway RM, Anthelme F, Brooker RW, Cavieres LA, Kikvidze Z, Lortie CJ, Michalet R, Pugnaire FI, Xiao S, et al. 2014a.** The context dependence of beneficiary feedback effects on benefactors in plant facilitation. *New Phytologist* **204**(2): 386-396.
- Schöb C, Michalet R, Cavieres LA, Pugnaire FI, Brooker RW, Butterfield BJ, Cook BJ, Kikvidze Z, Lortie CJ, Xiao S, et al. 2014b.** A global analysis of bidirectional interactions in alpine plant communities shows facilitators experiencing strong reciprocal fitness costs. *New Phytologist* **202**(1): 95-105.
- Schöb C, Prieto I, Armas C, Pugnaire FI. 2014c.** Consequences of facilitation: one plant's benefit is another plant's cost. *Functional Ecology* **28**(2): 500-508.
- Schupp EW. 1995.** Seed seedling conflicts, habitat choice, and patterns of plant recruitment. *American Journal of Botany* **82**(3): 399-409.

- Sears ALW, Chesson P. 2007.** New methods for quantifying the spatial storage effect: an illustration with desert annuals. *Ecology* **88**(9): 2240-2247.
- Silvertown J, Dodd M, Gowing D, Lawson C, McConway K. 2006.** Phylogeny and the hierarchical organization of plant diversity. *Ecology* **87**(7): S39-S49.
- Soliveres S, DeSoto L, Maestre FT, Olano JM. 2010.** Spatio-temporal heterogeneity in abiotic factors modulate multiple ontogenetic shifts between competition and facilitation. *Perspectives in Plant Ecology, Evolution and Systematics* **12**(3): 227-234.
- Soliveres S, Eldridge DJ, Maestre FT, Bowker MA, Tighe M, Escudero A. 2011.** Microhabitat amelioration and reduced competition among understorey plants as drivers of facilitation across environmental gradients: Towards a unifying framework. *Perspectives in Plant Ecology, Evolution and Systematics* **13**(4): 247-258.
- Soliveres S, Smit C, Maestre FT. 2014.** Moving forward on facilitation research: response to changing environments and effects on the diversity, functioning and evolution of plant communities. *Biological Reviews* **90**(1): 297-313.
- Soliveres S, Torices R, Maestre FT. 2012a.** Environmental conditions and biotic interactions acting together promote phylogenetic randomness in semi-arid plant communities: new methods help to avoid misleading conclusions. *Journal of Vegetation Science* **23**(5): 822-836.
- Soliveres S, Torices R, Maestre FT. 2012b.** Evolutionary relationships can be more important than abiotic conditions in predicting the outcome of plant-plant interactions. *Oikos* **121**(10): 1638-1648.
- Sthultz CM, Gehring CA, Whitham TG. 2007.** Shifts from competition to facilitation between a foundation tree and a pioneer shrub across spatial and temporal scales in a semiarid woodland. *New Phytologist* **173**(1): 135-145.
- Swenson NG, Enquist BJ, Thompson J, Zimmerman JK. 2007.** The influence of spatial and size scale on phylogenetic relatedness in tropical forest communities. *Ecology* **88**(7): 1770-1780.
- Tirado R, Pugnaire FI. 2005.** Community structure and positive interactions in constraining environments. *Oikos* **111**(3): 437-444.
- Trinder CJ, Brooker RW, Davidson H, Robinson D. 2012.** Dynamic trajectories of growth and nitrogen capture by competing plants. *New Phytologist* **193**(4): 948-958.
- Trinder CJ, Brooker RW, Robinson D. 2013.** Plant ecology's guilty little secret: understanding the dynamics of plant competition. *Functional Ecology* **27**(4): 918-929.
- Uriarte M, Swenson NG, Chazdon RL, Comita LS, John Kress W, Erickson D, Forero-Montaña J, Zimmerman JK, Thompson J. 2010.** Trait similarity, shared ancestry and the structure of neighbourhood interactions in a subtropical wet forest: implications for community assembly. *Ecology Letters* **13**(12): 1503-1514.
- Valdano SG, Di Rienzo JA. 2007.** Discovering meaningful groups in hierarchical cluster analysis. An extension to the multivariate case of a multiple comparison method based on cluster analysis. <http://interstat.statjournals.net/YEAR/2007/abstracts/0704002.php>.
- Valiente-Banuet A, Rumebe AV, Verdú M, Callaway RM. 2006.** Modern quaternary plant lineages promote diversity through facilitation of ancient

- tertiary lineages. *Proceedings of the National Academy of Sciences of the United States of America* **103**(45): 16812-16817.
- Valiente-Banuet A, Verdú M. 2007.** Facilitation can increase the phylogenetic diversity of plant communities. *Ecology Letters* **10**: 1029-1036.
- Valiente-Banuet A, Verdú M. 2008.** Temporal shifts from facilitation to competition occur between closely related taxa. *Journal of Ecology* **96**(3): 489-494.
- Valiente-Banuet A, Verdú M. 2013.** Plant Facilitation and Phylogenetics. *Annual Review of Ecology, Evolution, and Systematics* **44**(1): 347-366.
- Valladares F, Niinemets U 2007.** Chapter 4 - The architecture of plant crowns: from design to light capture and performance. In: Pugnaire FI, Valladares F eds. *Functional plant ecology*. Boca Ratón, FL: Boca Ratón, FL : CRC Press.
- Valle F. 2003.** *Mapa de Series de Vegetación de Andalucía. Escala 1:400.000.*: Consejería de Medio Ambiente. Junta de Andalucía, Sevilla.
- Van der Putten WH. 2009.** A multitrophic perspective on functioning and evolution of facilitation in plant communities. *Journal of Ecology* **97**(6): 1131-1138.
- Verdú M, Gómez-Aparicio L, Valiente-Banuet A. 2012.** Phylogenetic relatedness as a tool in restoration ecology: a meta-analysis. *Proceedings of the Royal Society of London B Biological Sciences* **279**(1734): 1761-1767.
- Verdú M, Pausas JG. 2007.** Fire drives phylogenetic clustering in Mediterranean Basin woody plant communities. *Journal of Ecology* **95**(6): 1316-1323.
- Violle C, Enquist BJ, McGill BJ, Jiang L, Albert CH, Hulshof C, Jung V, Messier J. 2012.** The return of the variance: intraspecific variability in community ecology. *Trends in Ecology & Evolution* **27**(4): 244-252.
- Violle C, Nemergut D, Pu Z, Jiang L. 2011.** Phylogenetic limiting similarity and competitive exclusion. *Ecology Letters* **14**(8): 782-787.
- Wang YS, Chua CJ, Maestre FT, Wang G. 2008.** On the relevance of facilitation in alpine meadow communities: An experimental assessment with multiple species differing in their ecological optimum. *Acta Oecologica-International Journal of Ecology* **33**(1): 108-113.
- Wardle DA, Zackrisson O. 2005.** Effects of species and functional group loss on island ecosystem properties. *Nature* **435**(7043): 806-810.
- Webb CO. 2000.** Exploring the phylogenetic structure of ecological communities: an example for rain forest trees. *The American Naturalist* **156**(2): 145-155.
- Webb CO, Ackerly DD, McPeck MA, Donoghue MJ. 2002.** Phylogenies and community ecology. *Annual Review of Ecology, Evolution, and Systematics* **33**: 475-505.
- Webb CO, Gilbert GS, Donoghue MJ. 2006.** Phylodiversity-dependent seedling mortality, size structure, and disease in a Bornean rain forest. *Ecology* **87**(sp7): S123-S131.
- Weiblen GD, Webb CO, Novotny V, Basset Y, Miller SE. 2006.** Phylogenetic dispersion of host use in a tropical insect herbivore community *Ecology* **87**(sp7): S62-S75.
- Wiens JJ, Graham C. 2005.** Niche conservatism: Integrating evolution, ecology, and conservation biology. *Annual Review of Ecology, Evolution, and Systematics* **36**: 519-539.
- Wikstrom N, Savolainen V, Chase MW. 2001.** Evolution of the angiosperms: calibrating the family tree. *Proceedings of the Royal Society of London B Biological Sciences* **268**(1482): 2211-2220.

- Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, et al. 2004.** The worldwide leaf economics spectrum. *Nature* **428**(6985): 821-827.
- Yang J, Ci X, Lu M, Zhang G, Cao M, Li J, Lin L. 2014.** Functional traits of tree species with phylogenetic signal co-vary with environmental niches in two large forest dynamics plots. *Journal of Plant Ecology* **7**(2): 115-125.
- Zhang M-J, Liu M, Li Y, Xu C, An S. 2011.** The combined positive effects of two dominant species in an arid shrub-herbaceous community: implications from the performance of two associate species. *Plant Ecology* **212**(9): 1419-1428.
- Zhu Y, Comita LS, Hubbell SP, Ma K. 2015.** Conspecific and phylogenetic density-dependent survival differs across life stages in a tropical forest. *Journal of Ecology*: doi: 10.1111/1365-2745.12414.
- Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM. 2009.** *Mixed effects models and extensions in ecology with R*. Dordrecht, The Netherlands.: Springer.

Otras aportaciones científicas derivadas de la Tesis Doctoral

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Book chapters

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Conferences contributions

Pistón N., C. Armas, C. Schöb, P. Macek and F.I. Pugnaire. Multiple-cushion contribution along a severity gradient in the Sierra Nevada mountains. Modern Phylogenetic Comparative Methods. Seville, 11-15 November 2014.

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Rodríguez-Echeverría, S., Armas, C., Pistón, N., Hortal, S., & Pugnaire, F.I. La microbiota edáfica modula las interacciones positivas entre plantas. XI Congreso Nacional de la AEET. Pamplona, Spain, 6-10 May 2013.

Pistón, N., Schöb, C., Armas C., & Pugnaire F.I. The effects of alpine cushion shrubs on plant biodiversity in a dry mountain range, Sierra Nevada, Spain. XII European Ecological Congress. Ávila, Spain, September 2011.

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Índice de figuras y tablas

INTRODUCCIÓN

<u>Fig. I1. Imagen de la planta nodriza</u>	7
<u>Figura I2. Gradiente opuesto</u>	8
<u>Figura I3. Esquema mostrando las relaciones entre los distintos factores que afectan al ensamblaje de la comunidad</u>	10

CHAPTER I: Complementarity among shrub species enhances community richness and phylogenetic diversity along an environmental gradient.

<u>Fig. 1. Mean temperature and relative humidity</u>	32
<u>Fig. 2. Relative interaction index (RII)</u>	33
<u>Fig. 3. Differences in subordinate species composition</u>	34
<u>Fig. 4. Shrub contribution to community species richness at each site</u>	35
<u>Fig. 5. Shrub contribution to community phylogenetic diversity</u>	36
<u>Table A1. Number of subordinate species growing only beneath shrub species in each site</u>	42
<u>Table A2. Statistical results of the factorial ANOVAs</u>	43
<u>Table A3. A priori contrasts analyzing differences in temperature and relative humidity</u>	44
<u>Table A4. Species list</u>	50

CHAPTER II: Phylogenetic distance among beneficiary species in a cushion plant species explains interaction outcome.

<u>Fig. 1. Relative interaction index (RII)</u>	62
<u>Fig. 2. Mean phylogenetic distance</u>	63
<u>Appendix B1. Species list and their phylogenetic distances (PD)</u>	69
<u>Appendix B2. Phylogenetic tree of the regional species pool. The cushion plant <i>Arenaria tetraquetra</i> is highlighted in blue</u>	70
<u>Appendix B3. Species accumulation curves for each site</u>	71

CHAPTER III: Differences in facilitation and its feedback effects between

<u>Table 1. Functional traits and microhabitat measurements</u>	85
<u>Figure 1. CA diagrams of microhabitats</u>	86

<u>Figure 2. Photosynthetic efficiency of photosystem II</u>	87
<u>Figure 3. Relative differences (RII) in biomass</u>	88
<u>Figure 4. Cytisus galianoi traits in control plants and in plants where one Festuca indigesta individual was removed</u>	89
<u>Figure 5. Diagram showing the possible dynamics between the two Cytisus galianoi phenotypes</u>	92
<u>Figure C1. Plant abundance</u>	94
<u>Figure C2. Phylogenetic diversity</u>	95
<u>Figure C3. Growth rate</u>	95

CHAPTER IV: Ontogenetic shifts and phylogenetic relatedness in annual plants interactions in a semi-arid community

<u>Fig. 1. Experimental site of 25 m²</u>	105
<u>Fig. 2. Survival percentage</u>	108
<u>Fig. 3. RII biomass per individual</u>	109
<u>Fig. 4. RII no. of leaves</u>	110
<u>Figure D1. RII SLA</u>	113
<u>Figure D2. RII leaf length</u>	113
<u>Figure D3. RII height</u>	114