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TESIS DOCTORAL

Atributos de las comunidades bióticas y funcionamiento del
ecosistema en zonas áridas: Efectos del cambio climático e
implicaciones para la restauración

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CERTIFICAN

Que los trabajos de investigación desarrollados en la memoria de tesis doctoral **“Atributos de las comunidades bióticas y funcionamiento del ecosistema en zonas áridas: Efectos del cambio climático e implicaciones para la restauración”**, son aptos para ser presentados por el Licenciado Enrique Valencia Gómez ante el tribunal que en su día se consigne, para aspirar al Grado de Doctor en el Programa de Doctorado de Conservación de Recursos Naturales por la Universidad Rey Juan Carlos de Madrid.

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A mi abuela

*A veces sentimos que lo que hacemos es
tan sólo una gota en el mar, pero el mar
sería menos si le faltara una gota.*

Madre Teresa de Calcuta

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RESUMEN

SÍNTESIS

El cambio global afecta directamente la biodiversidad, provocando cambios fisiológicos, fenológicos y/o de crecimiento a nivel de individuo, y cambios en la composición, distribución y/o abundancia a nivel de comunidad, que afectan a la estructura y funcionamiento del ecosistema. Dada la elevada sensibilidad de los ecosistemas áridos a los principales motores del cambio global, es decir, cambios en usos de suelo y cambio climático, resulta fundamental estudiar en mayor profundidad los efectos actuales y futuros de dichos cambios en estos ecosistemas. En la presente tesis, se han llevado a cabo una serie de estudios observacionales, a escala regional, y experimentales, a escala local, para evaluar el impacto del cambio climático y los cambios en el uso del suelo sobre la estructura de la comunidad. Asimismo, se han evaluado los efectos de estos cambios sobre los procesos ecosistémicos en zonas Mediterráneas semiáridas. Para la consecución de estos objetivos se ha utilizado una aproximación basada en rasgos funcionales.

Los capítulos 1 y 2 corresponden a los estudios observacionales a escala regional. En el capítulo 1, se evaluó cómo la aridez y las interacciones bióticas determinan la estructura funcional de comunidades de matorral de zonas semiáridas Mediterráneas, muestreadas a lo largo de un gradiente de aridez en España. Para dicho fin, se utilizó una aproximación basada en la evaluación de rasgos funcionales y diferentes escalas dentro de la comunidad. A escala regional, las distintas especies se distribuyeron alrededor de cuatro ejes de especialización, en base a rasgos relacionados con la arquitectura de la planta y morfología de la hoja. A escala de comunidad, se evaluó el impacto relativo de la hipótesis de filtrado de hábitat y diferenciación de nicho en la estructura de la comunidad. Ambos mecanismos actúan de manera independiente en diferentes rasgos para determinar la estructura de la comunidad a lo largo del

gradiente de aridez. Por último, a escala de vecindad de las plantas, se analizó el impacto de las interacciones bióticas en la estructura de la comunidad, destacando la importancia del impacto de los procesos de competencia y facilitación en los cambios observados en la diversidad funcional de la comunidad objeto de estudio.

En el capítulo 2, se utilizó una aproximación basada en rasgos funcionales para analizar cómo la aridez y la alteración en los usos del suelo, provocada por la matorralización (i.e., incremento en la abundancia o cobertura de matorrales), impactaban en la estructura funcional de las comunidades de zonas semiáridas Mediterráneas y determinar cómo afectan todos estos cambios a los procesos ecosistémicos. Esta estructura funcional a nivel de comunidad puede ser explicada a través de la identidad de las especies dominantes (“community-weighted mean trait values” [CWM]) o de la diversidad funcional. Nuestro estudio muestra que ambos mecanismos son igual de importantes para explicar las respuestas de los procesos ecosistémicos a la aridez y a la matorralización. Los rasgos funcionales relacionados con el tamaño de la planta (altura vegetativa y propagación lateral) y con las hojas (área específica foliar y porcentaje de materia seca) resumen los efectos de la matorralización en los procesos ecosistémicos de manera contundente ($r^2=0.63$). Cabe destacar la importancia de la diversidad funcional, ya que mantiene el funcionamiento del ecosistema frente a incrementos de aridez.

Ya a escala local en el capítulo 3, se evaluó el efecto de un incremento de temperatura de $\sim 3^\circ\text{C}$ sobre la capacidad fotosintética de diez especies de ecosistemas áridos con rasgos funcionales contrastados, y se comprobó si dichos rasgos modulaban la respuesta fotosintética al calentamiento. Con este propósito, se realizó un experimento manipulativo en condiciones de vivero durante dos años con condiciones climáticas muy diferentes (primer año más seco que el segundo). En el año más seco, el

calentamiento provocó un incremento de la tasa fotosintética al comienzo de la estación de crecimiento, lo que sugirió un adelanto de la primavera bajo condiciones de calentamiento. En el año más húmedo, los rasgos funcionales modularon la respuesta de la fotosíntesis, especialmente al final de la estación de crecimiento. Las especies más grandes, pero con hojas más pequeñas, tuvieron mayores tasas fotosintéticas con el incremento de temperatura, situación contraria a la dada en las especies más pequeñas, pero con hojas más grandes.

En el capítulo 4, se empleó la misma aproximación experimental que en el capítulo anterior para conocer las respuestas fenológicas de catorce especies herbáceas de zonas semiáridas Mediterráneas, durante dos estaciones de crecimiento a un tratamiento de calentamiento pasivo (incremento de $\sim 3^{\circ}\text{C}$ de temperatura). Estas especies pertenecen a tres grupos funcionales (gramíneas, leguminosas fijadoras de N y herbáceas no fijadoras de N), y se evaluó su respuesta fenológica y los efectos del calentamiento sobre dicha respuesta. Asimismo, se comprobó si los ejes de especialización, promovidos por los rasgos funcionales de estas especies, influían en la respuesta fenológica. Los resultados mostraron que el calentamiento provoca un adelanto de la fecha de inicio de la floración, fecha de inicio y pico de fructificación, aumento de la duración de la floración y reducción de la producción de flores y frutos. En el caso de los grupos funcionales, las herbáceas no fijadoras de N florecieron y fructificaron antes que los otros dos grupos funcionales. Sin embargo, el calentamiento no reveló cambios en la respuesta fenológica entre los grupos funcionales. Por último, los resultados mostraron el papel de los rasgos funcionales, siendo las especies más grandes, pero con hojas más pequeñas y con menor área específica foliar, las que florecieron más temprano y las que tuvieron períodos de floración más largos.

Por último en el capítulo 5 se evaluó, mediante un experimento manipulativo factorial completo en condiciones de vivero, los efectos del calentamiento, reducción de precipitación y riqueza de especies sobre la estructura funcional de la comunidad vegetal y de la comunidad de microorganismos (protistas y bacterias), y también se cuantificaron los efectos sobre los procesos ecosistémicos. Dicho experimento fue llevado a cabo con especies presentes en ecosistemas de zonas semiáridas Mediterráneas. En cuanto a los tratamientos, los niveles de riqueza en la comunidad fueron de 1, 3 y 6 especies, el calentamiento incrementó la temperatura en $\sim 3^{\circ}\text{C}$ y la reducción de la precipitación disminuyó la cantidad de lluvia en un 35%. Estos tratamientos promovieron cambios poco importantes en los ciclos de C y P, y productividad (biomasa superficial), y ninguno en la estructura funcional de la comunidad vegetal, en la abundancia de microorganismos o en el conjunto de los procesos ecosistémicos. La riqueza de especies afectó a los procesos ecosistémicos, pero fundamentalmente a través de efectos indirectos mediados por la estructura funcional de las plantas, promoviendo incrementos en los tamaños de la comunidad, y por la abundancia de microorganismos.

SUMMARY (ENGLISH VERSION)

Global change impacts biodiversity, promoting changes in physiology, phenology and/or growth of individual species, and will inevitably alter composition, abundances and/or distribution of the community, affecting structure and functioning of worldwide ecosystems. These impacts are especially important in dryland ecosystems, since they are among the more sensitive to global change drivers, such as climate and land use changes. Although some first attempts to evaluate these impacts have been made, there are too many uncertainties about the effect of biodiversity and climate change drivers on community functional structure and/or ecosystem functioning in drylands. Therefore, in this PhD thesis, we used observational and experimental studies at regional and local scale, respectively, to evaluate the responses of community functional structure to climate land use changes, and to determine how functional attributes ultimately affect ecosystem functioning in semi-arid Mediterranean ecosystems. To do so, we used a functional trait-based approach.

At regional scale in chapter 1, we assess how aridity and biotic interactions determine the functional community structure of semi-arid Mediterranean shrublands sampled along a large aridity gradient in Spain. We used functional trait-based and multi-scale approaches to evaluate their impact on community structure. Surveyed species can be separated along four axes of specialization, based on their above-ground architecture and leaf morphology. At the community scale, we assessed the relative impact of habitat filtering and niche differentiation on community structure. Both mechanisms acted independently on different traits to determine community structure along the aridity gradient. At the plant neighbourhood scale, we evaluated the impact of biotic interactions on community structure. The results suggested that competition and

facilitation impacted on the observed changes in the functional diversity of the studied communities.

In Chapter 2, we used a functional trait-based approach to assess how aridity and a major land use change (shrub encroachment) impact in functional structure of Mediterranean dryland communities (community-weighted mean trait values [CWM] and functional diversity) at regional scale. Then, we determine whether aridity and shrub encroachment affect ecosystem functioning directly or indirectly via changes in functional structure. We found that functional diversity and CWM were equally important as drivers of multifunctionality (i.e. the provision of multiple ecosystem functions and services simultaneously) responses to both aridity and shrub encroachment. Plant size traits (e.g., vegetative height or lateral spread) and leaf traits (e.g., specific leaf area and leaf dry matter content) grasped the effect of shrub encroachment on multifunctionality with a relative high accuracy ($r^2=0.63$). Functional diversity also improved the resistance of multifunctionality to increases in aridity.

In Chapter 3, we evaluate how a $\sim 3^\circ\text{C}$ increase in temperature affected the photosynthetic capacity of ten dryland species with contrasting functional attributes, and to test whether their functional traits modulated warming effects on their photosynthetic performance. For this purposes we used a common garden experiment over two years with contrasting environmental conditions (drier first year vs. wetter second year). In the drier year, warming increased photosynthetic rates at the beginning of the growing season, suggesting a modification in the growing period (earlier spring). In the wetter year, functional traits modulated photosynthetic responses to warming, especially at the end of the growing period. Larger species with small leaves had higher photosynthetic rates under warming compared to smaller species with larger leaves.

In chapter 4, we used a manipulative experiment conducted over two growing seasons to evaluate the responses in reproductive phenology of fourteen Mediterranean semiarid species to a $\sim 3^{\circ}\text{C}$ increase in temperature. Warming advanced the onset of flowering and fruit production, extended the duration of flowering and reduced the production of flowers and fruits. Also, we evaluated the responses of three functional groups (grasses, nitrogen-fixing legumes and forbs) to warming, and how leaf and size traits influenced phenological responses. Functional groups differed in their timing of flowering; for instance, forbs had an earlier reproductive phenology than legumes and grasses. However, they did not show an advanced of phenological response to warming among functional groups. We found that larger species with small leaves and lower specific leaf area had an earlier flowering phenology and longer flowering period.

Finally in chapter 5, we report results from a common garden experiment conducted over two years with Mediterranean semiarid species. We evaluate how changes in plant functional structure and microorganism communities (bacteria and protest) promoted by climate change drivers (a 3°C temperature increase and a 35% rainfall reduction) and species richness, impact on multifunctionality. Species richness effects on multifunctionality were largely mediated by ‘indirect effects’, via changes in plant community structure (functional identity) and increases in microorganism abundance. However, our climate change treatments promoted slight changes on variables related to C and P cycling and no changes in community structure and microorganism abundance.

INTRODUCCIÓN

ANTECEDENTES

Se denomina cambio global (Vitousek, 1992) al impacto de la actividad humana sobre los procesos y el funcionamiento del sistema terrestre (Duarte *et al.*, 2006). Este fenómeno es, posiblemente, uno de los problemas más importantes para la humanidad debido a sus profundos efectos en los diversos ecosistemas (Frenette-Dussault *et al.*, 2012). La alteración del hábitat, el cambio climático, introducción de especies invasoras, la sobreexplotación y la contaminación (nitrógeno, fósforo) son los motores del cambio global. Entre ellos, los cambios en el uso del suelo y el cambio climático son los principales motores que inciden en la biodiversidad global (Sala *et al.*, 2000). Los cambios del uso del suelo engloban diferentes factores de cambio, como pueden ser la expansión de las zonas de cultivo y pastoreo o la matorralización, entre otros (Lambin & Meyfroidt, 2011; Naito & Cairns, 2011). Por otra parte, el cambio climático engloba incrementos del dióxido de carbono, cambios en las precipitaciones e incrementos de temperatura que influyen directa e indirectamente en los ecosistemas terrestres. Los registros climáticos indican un aumento de las temperaturas globales anuales de 0,85°C en los últimos 130 años, destacando este incremento a partir de la década de los ochenta (IPCC, 2013). Asimismo, como consecuencia de este calentamiento se están produciendo modificaciones en los patrones de precipitación (IPCC, 2007). La importancia de estos cambios ha generado un gran interés en la comunidad científica, que intenta evaluar no sólo los efectos actuales sino predecir los futuros sobre el cambio climático en la estructura y funcionamiento de los ecosistemas (Root *et al.*, 2003; De Castro *et al.*, 2005; Holmgren *et al.*, 2006; Maestre & Reynolds, 2007; Woodward, 2007; González-Megías & Menéndez, 2012; Matías *et al.*, 2012; Peñuelas *et al.*, 2013).

A pesar de los estudios sobre los efectos del cambio climático, es necesaria una mayor investigación para poder evitar las consecuencias generadas por el cambio.

El cambio climático afecta directamente la biodiversidad (Thomas *et al.*, 2004; Ohlemüller *et al.*, 2006; Montoya & Raffaelli, 2010), alterando los ecosistemas naturales en todo el mundo (Maestre & Reynolds, 2007; Maestre *et al.*, 2012a). Los diferentes efectos sobre la biodiversidad van desde cambios en la fisiología y crecimiento de los organismos a nivel individual, modificaciones en la composición, distribución o abundancia a nivel de comunidad, hasta cambios en la estructura y funcionamiento del ecosistema en su conjunto (Hughes, 2000; Peñuelas & Filella, 2001; Ohlemüller *et al.*, 2006; Parmesan, 2006; González-Megías, 2012). Existen numerosos estudios sobre el efecto entre la diversidad vegetal y los procesos ecosistémicos (Tilman & Downing, 1994; Naeem *et al.*, 1994; Tilman *et al.*, 1996; Héctor *et al.*, 1999; Tilman *et al.*, 2012; Lundholm, 2015). En ellos, se afirma que la conservación de la diversidad vegetal es crucial para el mantenimiento de la productividad (Díaz & Cabido, 2001; Cardinale *et al.*, 2011; Hooper *et al.*, 2012), así como para preservar las distintas funciones relacionadas con los ciclos de nutrientes (Vitousek *et al.*, 1997; Maestre *et al.*, 2012a). Sin embargo, los cambios en la biodiversidad producidos por el cambio climático actual y futuro y sus efectos en los ecosistemas, aún deben ser abordados con mayor profundidad.

El cambio climático puede afectar de forma directa a los procesos ecosistémicos, modificando la producción primaria, la descomposición de la hojarasca o el reciclado de nutrientes (Aguiar *et al.*, 1996; Castro *et al.*, 2010; Delgado-Baquerizo *et al.*, 2013a, 2013b), o indirectamente a través de cambios en la estructura de las comunidades (Suding *et al.*, 2008). La comprensión de cómo el cambio climático provoca modificaciones en las comunidades de plantas, y cómo influye en el funcionamiento del

ecosistema requiere la evaluación de los rasgos funcionales de las especies involucradas (Chapin III & Walker, 1997; Chapin III *et al.*, 2000).

Utilización de la aproximación funcional para determinar la respuesta de la estructura de la comunidad al cambio climático y sus efectos en los procesos ecosistémicos

Rasgos funcionales

Los rasgos funcionales de las plantas nos proporcionan información acerca del estado de la planta y su respuesta ante variaciones ambientales (Cornelissen *et al.*, 2003). Se definen como características fisiológicas, morfológicas y/o fenotípicas, determinadas de manera individual en cada especie, relacionado con el rendimiento de la planta que le permiten sobrevivir en su medio ambiente (Violle *et al.*, 2007). Los rasgos funcionales pueden ser variables continuas o discretas, que permiten la comparación de diferentes ecosistemas y la evaluación de los cambios en la comunidad a lo largo de gradientes ambientales o a través del tiempo (Fig. 1; Díaz & Cabido 2001; Violle *et al.*, 2007). Ya que los rasgos funcionales son obtenidos a nivel individual, para ser aplicados a nivel de comunidad, se debe promediar el valor a nivel de ésta, pero ponderándose con la abundancia de cada especie, y así quedará caracterizada la estructura funcional de la comunidad estudiada (“community-weighted trait” [CWT], Violle *et al.*, 2007). La alteración de estos rasgos funcionales a lo largo de variaciones ambientales pone de manifiesto la importancia de los mecanismos de adaptación de las plantas (Liu *et al.*, 2010). Por ejemplo, Cornwell y Ackerly (2009) estudiaron el cambio en los caracteres funcionales en 44 comunidades de plantas de la Costa de California, para evaluar los procesos de adaptación y evolución de estos rasgos. En este estudio, los rasgos se diferencian entre ambientes húmedos y secos, teniendo en cuenta que en los primeros

las especies se segregan dependiendo de la altura y en los segundos se segregan en función del nitrógeno foliar. En los últimos años, se han incrementado los estudios que utilizan rasgos funcionales, tanto en la evaluación de los cambios de plantas a nivel individual, en respuesta a cambios ambientales, tales como temperatura, radiación incidente o estrés bioclimático (Woodward & Diament, 1991; Ackerly *et al.*, 2002; McGill *et al.*, 2006), como a nivel de comunidad (Cornwell & Ackerly, 2009). Posteriormente, estos resultados se han empleado para predecir la respuesta de la vegetación ante diferentes escenarios climáticos (Woodward & Diament, 1991). La utilización de los rasgos funcionales es, por tanto, una manera de integrar la complejidad de los ecosistemas, y la posibilidad de poder comparar entre comunidades diferentes y generalizar los resultados, convirtiéndolos en un eje fundamental en el estudio de la ecología de comunidades (Cornwell & Ackerly, 2009).

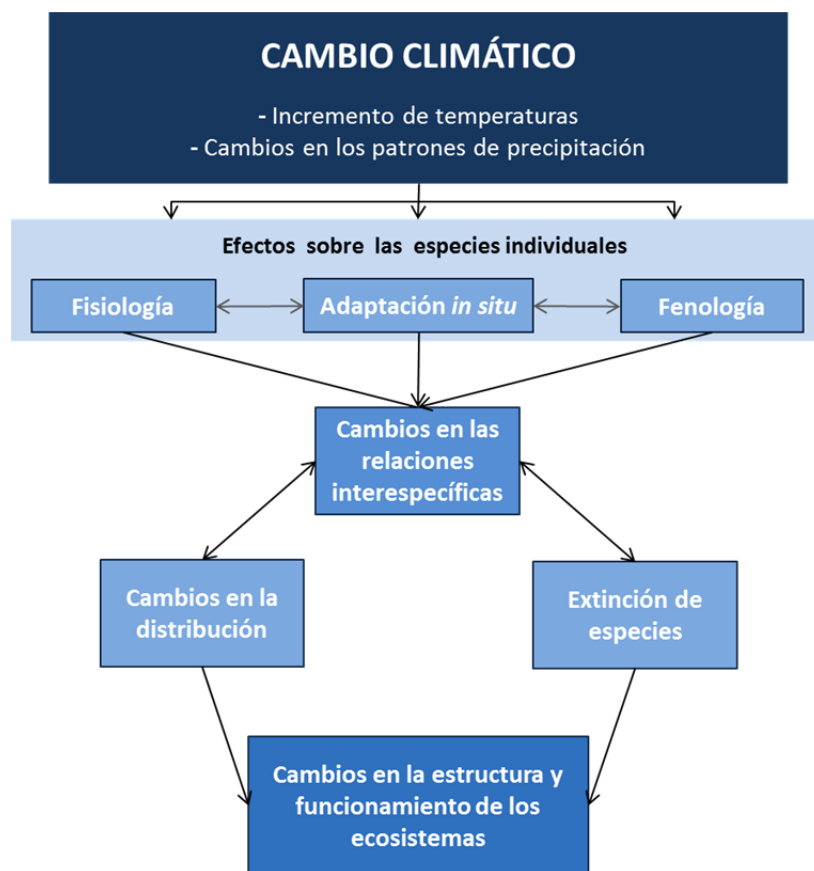


Fig. 1. Rutas de cambio y adaptación de comunidades de plantas al cambio climático (Modificada de Hughes, 2000).

Los rasgos funcionales proporcionan información acerca del papel que desempeñan las especies en la comunidad y cómo responden ante los cambios, así como también permiten valorar el impacto que provocan los cambios en la comunidad sobre los procesos ecosistémicos (Garnier *et al.*, 2004; Violle *et al.*, 2007). Para evaluar los impactos del cambio climático se ha propuesto un marco conceptual que evalúa la respuesta de la comunidad a los cambios y el efecto de éstos sobre los procesos ecosistémicos (Fig. 2; Esquema respuesta-efecto; Lavorel & Garnier, 2002; Suding *et al.*, 2008), basado en los rasgos funcionales de las plantas. La aplicación de esta metodología de trabajo proporciona una herramienta muy útil para entender los cambios en los ecosistemas y poder predecir comportamientos futuros.

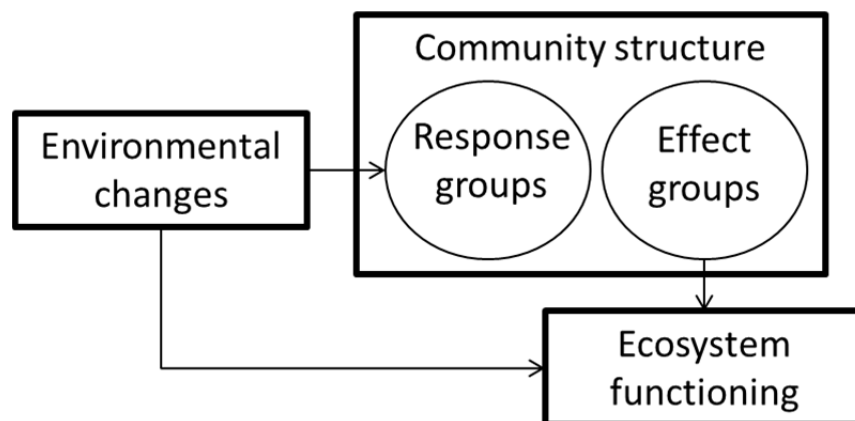


Fig. 2. Marco conceptual que relaciona la respuesta de los rasgos a los cambios ambientales y los efectos de estos cambios en el funcionamiento de los ecosistemas. Tomado de Suding *et al.*, (2008).

Esquema respuesta-efecto de los rasgos funcionales

La hipótesis central del esquema respuesta-efecto de los rasgos funcionales proviene de Lavorel & Garnier (2002) y la denominaron “Santo Grial”, por ser aplicable en una gran variedad de situaciones. Esta hipótesis se basa en que los mismos rasgos funcionales pueden captar la respuesta de la comunidad a los cambios, y a su vez los efectos sobre los procesos ecosistémicos (Fig. 2), lo que simplificaría el estudio de estas relaciones

complejas. Esta hipótesis se hace realidad mediante el uso de variables continuas y comparables entre comunidades diferentes, que agrupan los rasgos en ejes de especialización (Westoby *et al.*, 2002; Wright *et al.*, 2004), superando la identidad taxonómica de cada especie (McGill *et al.*, 2006). Estos ejes de especialización representan los distintos nichos de las especies (Gross *et al.*, 2007; Devictor *et al.*, 2010). El marco conceptual previamente comentado, se ha aplicado fundamentalmente sobre las especies vegetales de la comunidad, y se ha podido observar la relación entre la disponibilidad de recursos y rasgos de respuesta, como área específica foliar, concentración de nitrógeno en hojas o altura vegetativa, que a su vez afectan a los procesos ecosistémicos (Díaz *et al.*, 2007; Laliberté & Tylianakis 2012). Sin embargo, se necesitan más estudios relacionados de la aplicación del esquema respuesta-efecto para poder conocer los cambios en los procesos ecosistémicos producidos por el cambio climático, y de esta forma mejorar las predicciones de los efectos del cambio climático.

Actualmente, existe una imperiosa necesidad de ampliar los estudios sobre los vínculos entre la biodiversidad y el funcionamiento de los ecosistemas teniendo en cuenta distintas comunidades y las interacciones entre ellas, con la finalidad de tener una visión más integradora de la complejidad de los ecosistemas (Cardinale *et al.*, 2006, 2012; Hillebrand & Matthiessen, 2009; Reiss *et al.*, 2009; Lavorel *et al.*, 2013). Por ejemplo, cambios en la biodiversidad vegetal pueden afectar a la productividad biológica, la cual a su vez afecta a la abundancia y composición de las comunidades microbianas del suelo, alterando procesos ecosistémicos clave que dependen de las mismas, como la descomposición de la materia orgánica y el reciclado de nutrientes (Angers & Caron, 1998; Hooper *et al.*, 2000; Carney & Matson, 2005). Aunque estas relaciones han sido poco estudiadas hasta ahora, se han demostrado relaciones entre los rasgos de respuesta de las plantas a los factores abióticos y su efecto sobre la estructura

de otras comunidades con las que las plantas interactúan, afectando también a los procesos ecosistémicos. El estudio de Grigulis *et al.* (2013), basado en el análisis de las interacciones entre los rasgos de las plantas y los microorganismos del suelo, demostró que los cambios en la respuesta de la planta a la disponibilidad de recursos también puede influir en la actividad de los microorganismos del suelo, afectando a la producción de biomasa y la retención de carbono y nitrógeno en el suelo. La literatura en este ámbito es todavía muy escasa, por lo que se requieren más estudios sobre estos aspectos que ayuden a mejorar las predicciones bajo la amenaza del cambio climático.

Respuesta de la estructura funcional de la comunidad a los motores del cambio global, y efectos sobre los procesos ecosistémicos

La estructura funcional de la comunidad puede responder al cambio climático de diferentes formas, destacando dos hipótesis (Fig. 3): el filtrado ambiental del hábitat (Keddy 1992; Weiher *et al.*, 1998) y la diferenciación de nicho (Maire *et al.*, 2012). Se denomina filtrado ambiental al mecanismo por el que las condiciones ambientales imponen un proceso de selección de rasgos viables en estas condiciones, afectando a las especies que no dispongan de estos rasgos (Díaz *et al.*, 1998; Maire *et al.*, 2012). A nivel de comunidad, este proceso conduce a un cambio del CWT y puede reducir la diversidad funcional, ya que las especies convergen en el rasgo que es más viable, eliminando las especies que no poseen esta característica. Por otra parte, la diferenciación de nicho está relacionada con procesos que seleccionan especies con rasgos funcionales contrastados, permitiendo la coexistencia de especies con rasgos diferenciados (Maire *et al.*, 2012). Este mecanismo puede ser consecuencia directa de los cambios ambientales, debido a que especies funcionalmente muy diferentes conviven al tener una respuesta diferente, pero igual de óptima, frente a las mismas

condiciones ambientales. Por ejemplo, grupos que tienen rasgos totalmente diferenciados, como las especies malacófilas que evitan el estrés frente a especies esclerófilas que lo toleran (Freschet *et al.*, 2011). Este mecanismo también puede estar mediado por el efecto de las interacciones bióticas, como son los procesos de competencia o facilitación (Grime, 1973; Callaway, 1995). A nivel de comunidad, una alta diferenciación de nicho conlleva una alta diversidad funcional. *A priori*, ambas hipótesis pueden parecer opuestas, sin embargo pueden actuar simultáneamente y determinar los cambios en la estructura funcional de la comunidad.

Los cambios producidos en la estructura funcional de la comunidad promueven efectos en los procesos ecosistémicos y también pueden ser explicados mediante dos hipótesis (Fig. 3; Díaz *et al.*, 2007; de Bello *et al.*, 2010), denominadas “mass-ratio” (Grime, 1998) y complementariedad de recursos (Loreau *et al.*, 2001). La hipótesis “mass-ratio” afirma que la respuesta del ecosistema se explica principalmente por los rasgos de las especies dominantes, pudiendo ser evaluado mediante el CWT (Garnier *et al.*, 2004; Violle *et al.*, 2007). La hipótesis de complementariedad de recursos sugiere que las especies con rasgos funcionales contrastados pueden optimizar la utilización de los recursos del ecosistema, ya que utilizan diferentes o iguales recursos, pero en diferente momento o lugar (Loreau *et al.*, 2001; Hooper *et al.*, 2005; Gross *et al.*, 2007). El efecto de la complementariedad de recursos en los procesos ecosistémicos se puede captar mediante los cambios en la diversidad funcional (Loreau *et al.*, 2001; Díaz *et al.*, 2007).

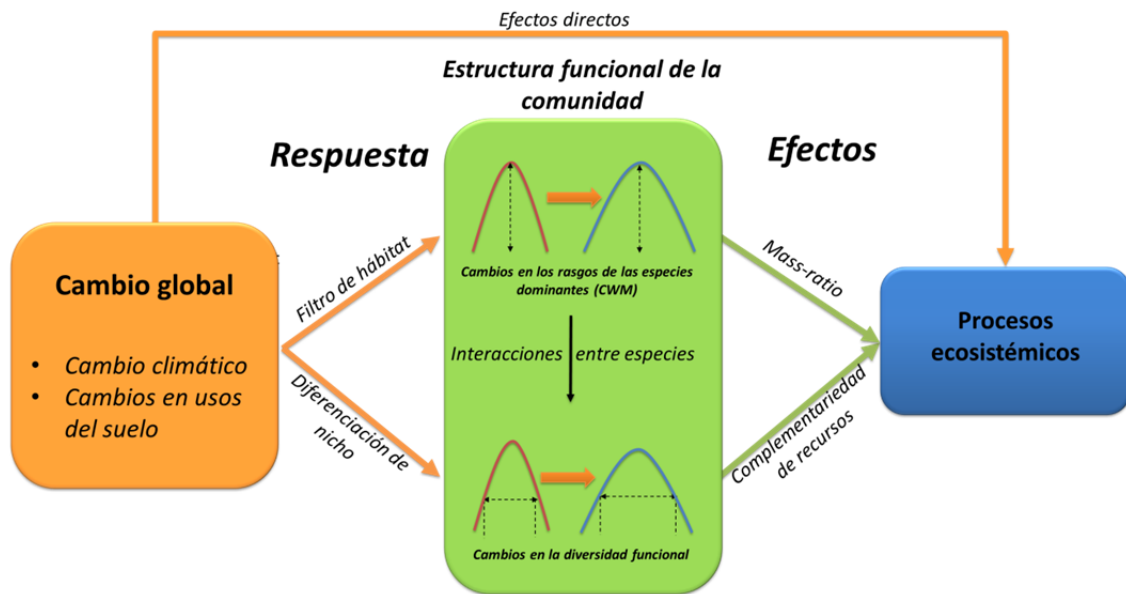


Fig. 3. Modelo teórico de los procesos que provocan la respuesta de la estructura funcional al cambio global y los procesos que dirigen los efectos de dicha estructura en los procesos ecosistémicos (modificada de Valencia *et al.*, 2015).

Ecosistemas áridos

Los ecosistemas de las zonas áridas, semiáridas y secas subhúmedas (zonas áridas en adelante) están entre los más sensibles a los efectos del cambio climático (Maestre *et al.*, 2012b). Estos ecosistemas constituyen el principal bioma terrestre, ya que ocupan el 41% de la superficie terrestre de la Tierra, albergan al 38% de la población global y representan un 25% de las reservas de carbono (C) orgánico de los suelos a nivel global (MEA, 2005; Safriel & Adeel, 2005). Se consideran zonas áridas aquellas en las que el índice de aridez (precipitación media / evapotranspiración media anuales, UNEP, 1992) está por debajo 0,65 y, por tanto, son muy vulnerables a una aumento de la sequía o de la temperatura (MEA, 2005). Los modelos climáticos predicen un aumento generalizado de la aridez a nivel global, que traerá como consecuencia que la extensión de las zonas secas aumente un 10% a finales del siglo XXI (Feng & Fu, 2013). Debido a la superficie actual y predicha de las zonas áridas a nivel global, a la dependencia de parte de la humanidad en los bienes y servicios que prestan, y a su sensibilidad al cambio

climático, es de capital importancia entender cómo estos ecosistemas se verán afectados por dicho cambio. Según el grupo intergubernamental de expertos sobre el cambio climático, se estima que la temperatura media de estas zonas incrementa entre un 2° y un 4°C para finales del siglo XXI (IPCC, 2013). Estos modelos también predicen cambios en las precipitaciones, con incrementos cercanos al 25% en China y descensos de alrededor del 30% en América o la cuenca Mediterránea (Bates *et al.*, 2008), junto con un aumento de su variabilidad, es decir, años extremadamente lluviosos, seguidos de años muy secos (Beniston *et al.*, 2007). El mismo informe del IPCC (2013) destaca que, entre los ecosistemas terrestres, los ecosistemas de tipo mediterráneo se encuentran entre los más vulnerables del mundo al cambio climático, siendo muy propensos a procesos de desertificación (Puigdefábregas & Mendizábal, 1998). A pesar de la importancia de los ecosistemas áridos, los efectos del cambio climático en su estructura y funcionamiento están mucho menos estudiados que en otros ecosistemas.

Estudios previos centrados en zonas áridas describen el papel fundamental de la diversidad vegetal en el mantenimiento de las múltiples funciones del ecosistema (Maestre *et al.*, 2012a), pudiendo amortiguar los posibles efectos negativos provocados por el cambio climático y/o por la desertificación. Por ello, entender los impactos del cambio climático sobre las especies y las comunidades y cómo estas responden es fundamental para la conservación y manejo de las zonas áridas. En este contexto, el esquema respuesta-efecto basado en rasgos funcionales puede ser una herramienta fundamental para ampliar el conocimiento de estos ecosistemas. A pesar de ello, esta metodología ha sido escasamente utilizada en las zonas áridas (de Bello *et al.*, 2010) y podría proporcionar avances en la comprensión de los efectos del cambio climático sobre los procesos ecosistémicos, mediados por los cambios en la estructura funcional de las comunidades de zonas áridas.

OBJETIVOS Y ESTRUCTURA DE LA TESIS

El objetivo general de esta tesis es la evaluación de la respuesta de la estructura funcional de la comunidad vegetal al cambio climático en zonas áridas, así como los efectos de estos cambios en la comunidad sobre los procesos ecosistémicos. Para la consecución de este objetivo general, se evaluaron comunidades a diferentes escalas espaciales: regional y local, desarrollando los siguientes objetivos específicos en cada escala:

A escala regional:

- Estudiar mediante una aproximación funcional la importancia relativa de los factores abióticos en la estructura funcional de comunidades de zonas Mediterráneas áridas. Además de evaluar el impacto relativo de los mecanismos que modulan la estructura funcional, es decir, el filtrado ambiental del hábitat, la diferenciación de nicho y las interacciones bióticas (competencia y/o facilitación).
- Evaluar en estas comunidades de zonas áridas cómo los cambios en la estructura funcional, producidos por los factores abióticos y el cambio en el uso del suelo (provocado por la matorralización), afectan a los procesos ecosistémicos.

A escala local:

- Evaluar los efectos del aumento de temperatura predichos por los modelos climáticos sobre la fotosíntesis y fenología y cómo se relacionan con diferentes rasgos morfo-fisiológicos, representantes de la forma de las hojas, economía foliar, y el tamaño de la planta de diferentes especies herbáceas de zonas áridas.
- Estimar los impactos directos del cambio climático y la diversidad vegetal sobre los procesos ecosistémicos, así como los efectos indirectos relacionados con

cambios en la estructura funcional de la comunidad vegetal y en la abundancia de otras comunidades (bacterias y protistas).

Conforme a estos objetivos, se ha estructurado la presente tesis en dos bloques, dedicados cada uno a las dos escalas espaciales mencionadas anteriormente. El primer bloque consta de dos capítulos, donde se realizaron estudios observacionales a escala regional. Este tipo de estudios son muy útiles para sugerir patrones generales en respuesta a los factores abióticos, pero ignora diferencias microclimáticas o de micrositios locales que provocan ruido en el estudio. El segundo bloque comprende tres capítulos en lo que se realizaron estudios experimentales mediante microcosmos, que ayudan a disminuir el ruido previamente mencionado. A continuación, se incluye una breve síntesis de la investigación llevada a cabo en cada uno de los capítulos.

Bloque I: Escala Regional

Capítulo 1. *Uncovering multi-scale effects of aridity and biotic interactions on the functional structure of Mediterranean shrublands*

Este capítulo está dedicado a estudiar el efecto de los factores abióticos sobre la estructura funcional de comunidades de matorral semiárido Mediterráneo muestreado a lo largo de un gradiente de aridez que va desde el centro al sureste de España (410 Km aproximadamente). Se evalúa la contribución tanto del filtrado ambiental del hábitat, la diferenciación de nicho y las interacciones bióticas mediante el uso de la información proporcionada por los rasgos funcionales de la comunidad. Los resultados de este capítulo han sido publicados en *Journal of Ecology* 101: 637-649.

Capítulo 2. *Functional diversity enhances the resistance of ecosystem multifunctionality to aridity in Mediterranean drylands*

Este capítulo evalúa la respuesta de la estructura funcional de la comunidad a la aridez y la matorralización (i.e., incremento en la cobertura de arbustos) y cómo los cambios en la comunidad afectan a los procesos ecosistémicos, en concreto los relacionados con los ciclos de nutrientes, en zonas áridas. Para lograrlo, se evalúa la importancia relativa tanto de la hipótesis de “mass-ratio”, como de la hipótesis de complementariedad de recursos en los procesos ecosistémicos. Los resultados de este capítulo han sido publicados en *New Phytologist* 206: 660–671.

Bloque II: Escala Local

Capítulo 3. *Functional traits determine the photosynthetic response of ten dryland species to warming*

El tercer capítulo estudia los efectos del calentamiento predichos por los actuales modelos de cambio climático en la respuesta fotosintética de especies perennes herbáceas de zonas áridas con rasgos funcionales contrastados. Se evalúa la respuesta fotosintética a lo largo del tiempo (2 periodos de crecimiento), comparándola con la identidad de las especies en esta respuesta. Este capítulo está en fase de revisión en *Environmental and Experimental Botany*.

Capítulo 4. *Warming modifies reproductive phenology in semiarid Mediterranean species*

En este capítulo se analizó el efecto del calentamiento sobre la fenología, tanto de flores como de frutos, de especies perennes herbáceas de zonas áridas. Se quiere determinar si

las respuestas fenológicas varían entre los grupos funcionales a los que pertenecen estas especies (gramíneas, leguminosas fijadoras de N y herbáceas no fijadoras de N) y si hay una respuesta fenológica diferenciada de los grupos funcionales al calentamiento. Además, se evalúan las relaciones entre las variables fenológicas y los rasgos funcionales representativos de los ejes de especialización de nicho explicados anteriormente. Este capítulo se encuentra en fase de preparación para su envío a *Basic and Applied Ecology*.

Capítulo 5. *Effects of plant species richness on multifunctionality are stronger than those of simulated climate change, being mediated by functional traits and soil microorganisms*

El quinto capítulo evalúa el efecto de los motores del cambio climáticos (incremento de temperatura y reducción de la precipitación) y la riqueza de especies sobre la estructura de las plantas y la abundancia de bacterias y protistas. Asimismo, se analizan los efectos directos del cambio climático y el número de especies vegetales, e indirectos mediados por la estructura funcional de las plantas y la abundancia de bacterias y protistas en los procesos ecosistémicos. Este capítulo se encuentra en fase de preparación para su envío a *Global Change Biology*.

METODOLOGÍA

En cada uno de los capítulos que prosiguen a este preámbulo, se desarrollan todos los materiales y métodos de forma detallada y sus respectivas referencias. Sin embargo, a continuación se describe de manera abreviada la metodología general utilizada en esta tesis doctoral, diferenciándose entre los dos bloques que la componen. El bloque I consta de parcelas observacionales obtenidas a escala regional, desde el centro al sureste de España. El bloque II fue desarrollado en las instalaciones en el Centro de Apoyo Tecnológico (CAT), de la Universidad Rey Juan Carlos, llevándose a cabo una serie de manipulaciones experimentales en condiciones de vivero.

Bloque I: Escala regional

Área de estudio

El estudio consta de 45 parcelas observacionales, repartidas lo largo de un gradiente de aridez que va desde el centro hasta el SE de la Península Ibérica, sumando 410 km (Fig. 4). La precipitación y temperatura media de estos sitios varía entre 294 y 479 mm, y entre 12 y 18°C, respectivamente. Con el fin de reducir la variabilidad entre las parcelas, se seleccionaron aquellas orientadas en solana (*sensu lato*) y con suelo calizo (Lithic Calciorthid; Soil Survey Staff, 1994). La vegetación de estas parcelas es espartal dominado por *Stipa tenacissima* L., o matorral dominados por arbustos no rebrotadores como *Rosmarinus officinalis* L. En ambos tipos de comunidades, se seleccionaron parcelas con y sin arbustos rebrotadores, fundamentalmente *Quercus coccifera* L, los cuales están incrementando su cobertura, como se ha venido observando en las imágenes históricas (Apéndice 1, Quero *et al.*, 2013) Los análisis del primer capítulo quedan restringidos a 12 parcelas de matorral. Por el contrario, el segundo capítulo tiene en cuenta las 45 parcelas.

a)



b)



Fig. 4. Ejemplos de los (a) espartales y (b) matorrales de las parcelas de muestreo.

Bloque II: Escala local

Área de estudio

En el caso de los capítulos 3, 4 y 5, el estudio se ha llevado a cabo en el Centro de Apoyo Tecnológico (CAT), de la Universidad Rey Juan Carlos, situado en Móstoles (40°20'2.37"N, 3°52'59.91"O, elevación de 650 m). La precipitación y temperatura media anuales ha sido de 14 °C y 449 mm, respectivamente (Estación Meteorológica de Cuatro Vientos, Madrid, 40° 22' 32" N - 3° 47' 10" O; 690 m; datos medios para el periodo 1971-2000; distancia al CAT de aproximadamente 10 km).

El experimento se llevó a cabo en microcosmos, que simulan pequeños ecosistemas para determinar cuál sería la perturbación que sufrirían estas comunidades artificiales y sus posibles respuestas ante el cambio climático. Estos microcosmos se establecieron en macetas de plástico redondas de 28 cm de diámetro y 38 cm de profundidad. Todas ellas contenían en el fondo 3 cm de arlita con el propósito de facilitar el drenaje, para luego rellenar con suelo procedente de un campo de cultivo situado en los alrededores de la zona experimental. Los microcosmos se sembraron a lo largo del mes de abril de 2011. Cada microcosmos contó con una población de entre 9 y 12 individuos, teniendo una densidad aproximada de 162 individuos/m².

Los distintos estudios se realizaron desde diciembre de 2011 hasta septiembre de 2013. La fecha de inicio corresponde con la fecha en la que se establecieron los tratamientos de cambio climático, es decir, incremento de temperatura (control vs. aumento de la temperatura media de ~3°C) y exclusión de lluvia (control vs. reducción de un 35% de la precipitación). Estas condiciones ambientales están dentro del rango predicho por los modelos climáticos para la segunda mitad de siglo XXI en el centro de la península ibérica.

Para el tratamiento de temperatura, se utilizaron cámaras de techo abierto, denominadas “Open top chambers” (OTCs; Fig. 5) o mini-invernaderos. Estas estructuras se han empleado en multitud de experimentos de simulación de calentamiento global a lo largo del mundo. Las OTCs son pirámides hexagonales truncadas, que se construyeron con 6 placas de metacrilato, material que mantiene las condiciones de luz visible de la zona experimental. Cada placa presenta un diseño hexagonal con lados inclinados de 42 cm 65 cm y 52 cm. Las OTCs cuentan con una apertura superficial y una separación con el suelo de 5 cm para permitir el flujo de lluvia y aire al sistema y evitar un aumento excesivo de la temperatura.



Fig. 5. Foto de detalle de un mini-invernadero (OTCs) empleados para el tratamiento de incremento de temperatura.

Para el tratamiento de reducción de la precipitación se construyeron casetas de exclusión de lluvia (Fig. 6), derivadas del modelo diseñado por Yahdjian & Sala (2002). Estas estructuras ocupan una superficie de $1,68 \text{ m}^2$ ($1,4 \text{ m} \times 1,2 \text{ m}$), con una altura media de 1 m. Los techos de cada una de ellas están compuestos por seis canalones de metacrilato, con una inclinación de 20° , cubriendo aproximadamente el 35 % de la superficie. Estos canalones están conectados a garrafas de plástico para recoger y medir el agua de lluvia excluida. Este tratamiento modifica la cantidad de agua caída en cada evento lluvioso, pero no la frecuencia de dichos eventos.



Fig. 6. Foto de detalle de una caseta de exclusión de lluvia empleada para el tratamiento de reducción de la precipitación.

Ya que el diseño experimental ha sido factorial completo, las casetas de exclusión de lluvia han sido combinadas con las OTCs (Fig. 7), de tal forma que las comunidades sometidas a este tratamiento sufrirán una disminución de precipitaciones y un aumento de temperatura a la vez.



Fig. 7. Foto de detalle de una caseta de exclusión de lluvia y mini-invernaderos (OTCs), empleados para el tratamiento conjunto de reducción de la precipitación e incremento de temperatura.

En el caso de los capítulos 3 y 4, los microcosmos de estos experimentos fueron comunidades de 9 individuos de la misma especie, es decir, monocultivos. En este caso, se situaron únicamente en tratamiento control e incremento de temperatura. En el caso del capítulo 5, los microcosmos tenían distintos niveles de riqueza de especies combinados con todos los tratamientos de cambio climático (i: control, ii: incremento de temperatura, iii: reducción de precipitación, y iv: combinación de los dos anteriores). Para ello, se han establecido comunidades compuestas por una, tres y seis especies, haciendo un total de 12 individuos por microcosmos.

Para todos los capítulos de este bloque, se colocaron sondas de humedad (10 cm de altura y enterrados 9 cm en el suelo) en una serie de microcosmos aleatoriamente elegidos entre cada tratamiento, obteniendo la humedad del suelo mediante la técnica de “Time-Domain Reflectometry” (TDR). Adicionalmente, se monitorearon los efectos de cada tratamiento, mediante sensores automatizados (HOBO U23 Pro v.2 Temp/RH, Onset Corporation, Bourne, MA, USA) que miden la temperatura y la humedad del aire.

Técnicas de obtención de datos en campo y laboratorio

En todos los capítulos se han empleado técnicas de campo sencillas tanto para muestrear la vegetación como el suelo, tal como la estimación visual de la cobertura u obtención de rasgos funcionales. La mayoría de los rasgos funcionales fueron medidos directamente en el campo (i.e., “soft traits” *sensu* Díaz *et al.*, 2004) o se recogieron muestras que fueron analizadas mediante tratamiento de imagen y pesadas en el laboratorio. Asimismo, en el capítulo 3 se midió la tasa fotosintética mediante un medidor de gases por infrarrojos, marca LI-COR, modelo 6400xt. La mayoría de las variables edáficas procedentes de las muestras extraídas fueron analizadas en el laboratorio de la Universidad Rey Juan Carlos (carbono orgánico, pentosas y hexosas,

nitrógeno y fosforo total, nitrógeno disponible, aminoácidos, proteínas, ratio de mineralización potencial, fósforo inorgánico disponible, fósforo Olsen, análisis de actividades enzimáticas tales como fosfatasa y β -glucosidasa y la abundancia de bacterias). Por otra parte, la abundancia de protistas se analizó en los laboratorios de la Universidad de Köln (Alemania).

Análisis estadísticos

En el capítulo 1 se emplearon diferentes técnicas como son análisis de componentes principales (siglas del inglés, Principal Component Analysis, PCA, modelos nulos y modelos lineales mixtos generalizados (siglas del inglés, General Linear Mixed Models, GLMMs). Los capítulos 2 y 5 se analizaron utilizando PCA combinado con una aproximación basada en relaciones causales (d-sep). En el caso del capítulo 3 se utilizaron análisis de regresión y GLMMs. Por último, PCA y GLMMs fueron utilizados para analizar el capítulo 4. Estos análisis han sido llevados a cabo mediante JMP v11 (SAS Institute, Cary, North Carolina, USA) o con diferentes paquetes de R (R Core Development Team, 2012).

CONCLUSIONES

1. La estructura de las comunidades vegetales de las zonas semiáridas mediterráneas está determinada por procesos como el filtrado del hábitat y la diferenciación de nicho. Además, las interacciones bióticas, tales como la competencia o la facilitación, juegan un papel fundamental en la estructura de dichas comunidades. La competencia entre especies promueve una alta diversidad de rasgos funcionales, y la facilitación incrementa la diversidad, al favorecer la presencia de especies raras y/o no dominantes.
2. Los rasgos relacionados con la arquitectura de la planta, como la altura vegetativa y la propagación lateral, y con la estructura de la hoja, como el área específica foliar y el porcentaje de materia seca, resumen los efectos de la matorralización sobre los procesos ecosistémicos de zonas semiáridas mediterráneas.
3. La estructura funcional de los ecosistemas semiáridos mediterráneos está determinada por la aridez y por cambios en los usos del suelo como la matorralización. Los rasgos funcionales de las especies dominantes y su diversidad funcional explican los procesos ecosistémicos en mayor medida que los efectos directos producidos por la aridez y la matorralización.
4. El mantenimiento y/o mejora de la diversidad funcional, por medio de la matorralización de especies rebrotadoras, puede paliar los efectos negativos sobre los procesos ecosistémicos que está provocando el cambio climático en las zonas semiáridas mediterráneas.
5. Las especies herbáceas de zonas semiáridas mediterráneas sometidas al calentamiento predicho por los modelos climáticos para la segunda mitad del siglo veintiuno, incrementaron sus tasas fotosintéticas al comienzo de la estación de crecimiento, lo que termina afectando a la floración. Los rasgos funcionales

afectaron a la respuesta fotosintética, siendo las especies de mayor tamaño pero con hojas más pequeñas, las que presentan mayores tasas fotosintéticas bajo condiciones de calentamiento.

6. El calentamiento provocó adelantos en la floración y fructificación de las especies, así como un descenso importante en el número de flores y frutos. Las diferentes respuestas observadas a nivel individual ayudarán a conocer cuáles podrían ser las respuestas de la comunidad, ya que los adelantos fenológicos pueden afectar la complementariedad entre especies o producir asincronías entre plantas y polinizadores. Asimismo, los cambios en la producción de flores y frutos pueden disminuir la abundancia de ciertas especies, modificándose el ensamblaje las comunidades.
7. El calentamiento y la exclusión de lluvia no afectaron ni a la estructura funcional de la comunidad de herbáceas ni a la abundancia de microorganismos del suelo. Sin embargo, los rasgos de las especies dominantes ligados a la arquitectura de la planta y a la estructura de la hoja, modulan la abundancia de microorganismos en el suelo y los procesos ecosistémicos. Es importante señalar también el efecto de la comunidad de microorganismos del suelo en los procesos ecosistémicos, ya que explicó gran parte de su variación, lo que refuerza su interés en futuros estudios.
8. El esquema de respuesta-efecto basado en los rasgos funcionales vegetales de zonas semiáridas mediterráneas ha proporcionado importantes avances en el conocimiento de los mecanismos que subyacen a los cambios en la estructura funcional de las comunidades frente a los motores del cambio global, así como del impacto de dichos cambios en los procesos ecosistémicos. Por tanto, este esquema se debería utilizar en la evaluación de zonas áridas, para mejorar los programas de restauración.

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BLOQUE I: ESCALA REGIONAL

Uncovering multi-scale
effects of aridity and biotic
interactions on the
functional structure of
Mediterranean shrublands

CAPÍTULO 1



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SUMMARY

- Habitat filtering (HF, trait convergence) and niche differentiation (ND, trait divergence) are known to impact upon plant community structure. Both processes integrate individual responses to the abiotic environment and biotic interactions. Thus, it is difficult to clearly identify the underlying abiotic and biotic factors that ultimately impact community structure by looking at community-level patterns of trait divergence or convergence alone.
- We used a functional trait-based and multi-scale approach to assess how biotic interactions and aridity determine the functional structure of semi-arid shrublands sampled along a large aridity gradient in Spain. At the regional scale, we investigated functional differences among species (axes of specialization) to identify important traits for community assembly. At the community scale, we evaluated the relative impact of HF and ND on community structure using a null model approach. Finally, at the plant neighbourhood scale, we evaluated the impact of biotic interactions on community structure by investigating the spatial patterns of trait aggregation.
- The shrub species surveyed can be separated along four axes of specialization based on their above-ground architecture and leaf morphology. Our community-scale analysis suggested that the functional structure of semi-arid communities was clearly non-random, HF and ND acting independently on different traits to determine community structure along the aridity gradient. At the plant neighborhood scale, the spatial distribution of species was also clearly not random, suggesting that competition and facilitation impacted on the observed changes in the functional diversity of shrubland communities along the aridity gradient.

- *Synthesis*: Our results demonstrated that HF and ND acted simultaneously on independent traits to jointly determine community structure. Most importantly, our multi-scale approach suggested that competition and facilitation interplayed with aridity to determine this structure. Competition appeared to be constant along the aridity gradient, and explained the high functional diversity observed in semi-arid shrublands. Facilitation affected subordinate and rare species and thus may act to enhance the biodiversity of these ecosystems. Finally, the framework employed in our study allows moving forward from the examination of patterns to the development of mechanistic trait-based approaches to study plant community assembly.

Key-words: aridity, community assembly, competition, determinants of plant community diversity and structure, facilitation, habitat filtering, Mediterranean shrubland, niche differentiation, null models, plant functional trait, spatial analyses.

INTRODUCTION

Deterministic processes structuring natural communities, which integrate individual responses to the abiotic environment and biotic interactions can be broadly separated into two categories: habitat filtering (HF) and niche differentiation (ND) (Keddy 1992; Weiher, Clarke & Keddy 1998; Kraft, Valencia & Ackerly 2008). At the community level, HF leads to trait convergence towards an optimum trait value in response to the local environment (Grime 2006). By contrast, ND leads to trait divergence and promotes the coexistence of species exploiting contrasted niches (Pacala & Tilman 1994; Silvertown 2004; Kraft, Valencia & Ackerly 2008).

Habitat filtering and ND are not mutually exclusive, despite their apparent opposite effects on community-level trait distributions (Cornwell & Ackerly 2009; Mason *et al.* 2011; Maire *et al.* 2012). One reason is that traits usually co-vary along independent axes of specialization, defining different trade-offs for plants to acquire and use local resources (Suding, Goldberg & Hartman 2003). Within communities, many independent axes have been described (Ackerly 2004; Wright *et al.* 2004; Gross, Suding & Lavorel 2007; Maire *et al.* 2009), each reflecting a different leading dimension of the species niche (Devictor *et al.* 2010). Consequently, HF can select species on a particular set of traits when ND can simultaneously occur on other independent traits (Spasojevic & Suding 2012). For instance, although competition for light may force plants to converge in height, other axes of specialization may allow competing species to coexist, for example by exhibiting contrasting phenological niches (Fargione & Tilman 2005) or by using soil resources differently (Maire *et al.* 2012).

Examining trait convergence and divergence at the community level constitutes an important first step to study community assembly (Kraft, Valencia & Ackerly 2008).

However, such an investigation does not suffice to infer the underlying mechanisms, as very different processes can lead to similar community trait distributions (Gross *et al.* 2009). Competition has been primarily hypothesized to promote trait divergence at the community level (see the “limiting similarity theory” MacArthur & Levins 1967), but superior competitors can also have a disproportionately large effect on local resources, and thus act as a habitat filter (e.g. in the case of competition for light, Grime 1973; 2006; Chesson 2000; Schamp, Chau & Aarssen 2008; Mayfield & Levine 2010). Facilitation has also been shown to increase trait divergence at the community level by promoting the coexistence of functionally contrasted species (Gross *et al.* 2009; Butterfield & Briggs 2011). Finally, abiotic factors are usually considered to cause community trait convergence (Grime 2006). They may also act as a disruptive force when different functional strategies exhibit equal fitness in response to similar abiotic constraints (e.g. stress-avoidance vs. tolerance strategies, Freschet *et al.* 2011). As a result, when focusing only on community trait distributions, our understanding of the effects of biotic interactions and abiotic factors on community structure remains unclear and very limited (Cleland *et al.* 2011).

Disentangling the relative contributions of the abiotic environment and biotic interactions in structuring natural communities would be especially interesting for arid, semi-arid and dry-subhumid ecosystems (drylands hereafter). Drylands are among the most globally significant terrestrial biomes (Reynolds *et al.* 2007). These ecosystems are currently threatened by climate change and desertification, with potentially irreversible impacts (Reynolds *et al.* 2007; Maestre *et al.* 2012). Changes in the composition of dryland vegetation in response to aridity are well documented (*e.g.* Alados *et al.* 2006). Nonetheless, important traits describing major axes of specialization in arid systems are not well established compared to more mesic

ecosystems (Kattge *et al.* 2011; Dussault *et al.* 2012). In addition, the impacts of HF and ND on community structure along large aridity gradients have never been documented (Maestre *et al.* 2009a). Species from drylands generally exhibit low values of specific leaf area, suggesting a prevalent role for HF, which results in the dominance of water stress-tolerant species (Dussault *et al.* 2012). Conversely, the high functional diversity observed within many dryland communities may indicate that ND-based processes are also important (Freschet *et al.* 2011). In this context, there is no clear understanding of how competition or facilitation interplay with aridity to determine local community structure (Tielborger & Kadmon 2000; Maestre, Valladares & Reynolds 2005), although they have been hypothesized to play a crucial role in these systems (Fowler 1986; Kefi *et al.* 2007; Pugnaire *et al.* 2011; Le Bagousse-Pinguet *et al.* 2012).

In this paper, we followed a functional trait-based and multi-scale approach (from the regional species pool, down to the plant-neighborhood scale, Fig. 1) to evaluate the relative importance of random processes, abiotic factors and biotic interactions in determining trait distributions of semi-arid Mediterranean communities. We first aimed to identify the major axes of functional differentiation across species within semi-arid shrublands to identify the important traits for community assembly (regional scale, Fig. 1). Then, we focused on the traits related to each axes of specialization. We evaluated the relative importance of non-random community assembly processes along a large aridity gradient using null models (community scale, Fig. 1). We hypothesized that HF and ND are not mutually exclusive due to the independence among different sets of traits. However, increasing aridity may impose sufficient constraints to force species to converge in the most stressed part of the gradient, decreasing ND among species (Freschet *et al.* 2011). Therefore, we hypothesized that the most arid communities will be mainly structured by HF. Finally,

we examined the spatial distribution of traits within communities to detect the impact of biotic interactions at the plant neighborhood scale, and to evaluate their consequences on community structure (Fig. 1). We hypothesized that facilitation can explain the high trait divergence observed within communities in semi-arid environments (Freschet *et al.* 2011). However, depending on the intensity of abiotic stress, the impact of facilitation on community structure may be modified, with competition prevailing at the wetter part of the gradient, and facilitation gaining in importance with increasing aridity, as predicted by the “Stress Gradient Hypothesis” (Bertness & Callaway 1994).

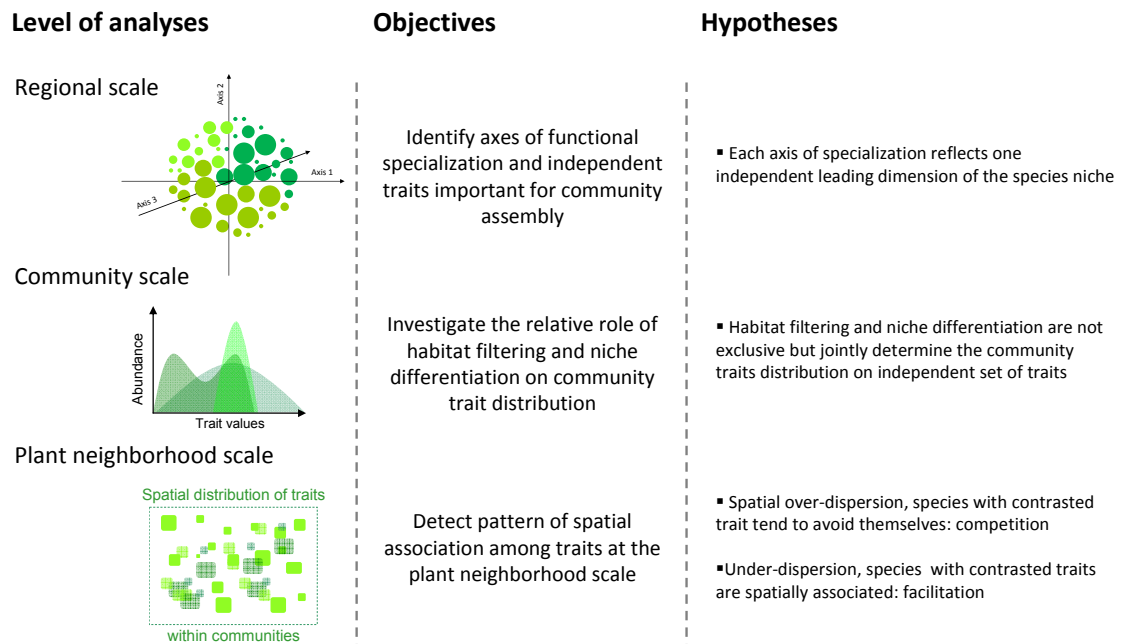


Fig. 1. Identifying the effect of abiotic factors and biotic interactions on the functional structure of plant communities using a multi-scale analysis. We graphically present the three levels considered: i) the species trait pool at the regional scale, ii) the trait distributions at the community scale, and iii) the patterns of spatial aggregation of traits at the plant neighborhood scale. For each level, we present the associated objectives and hypotheses (see details in the main text).

MATERIALS AND METHODS

Site selection and description of the gradient

We studied 12 experimental sites along a climatic gradient from central to south-east Spain (See Appendix S1 in Supporting Information). Site selection aimed to capture a significant range of the rainfall variability that is observed along this gradient and to reduce between-site variability associated with vegetation, slope, aspect, and soil type. To achieve this goal, all the sites were located on south-facing slopes, where shrublands are dominated by the shrubs *Rosmarinum officinalis* L. and *Quercus coccifera* L. and had similar abiotic within-site heterogeneity (*e.g.* % bare soil, stoniness, *data not shown*). Soils were in all cases derived from Lithic Calciorthid, (Soil Survey Staff, 1994). The sites were located along a clear aridity gradient (see Fig. S1); rainfall was negatively correlated with temperature along this gradient (Fig. S2 and Appendix S1).

Species abundance within communities

In 2011, we assessed the composition and structure of perennial vascular plants using 4 30 m long transects at each site, parallel to the slope and situated 8 m apart from each other. In each transect, 20 consecutive quadrats (1.5 m \times 1.5 m size) were placed to visually estimate the cover of each perennial species. We excluded annual plant communities from the analyses and restricted our study to perennial plants given their key role in maintaining ecosystem functioning and preventing desertification in drylands (Maestre & Escudero 2009; Maestre *et al.* 2012). Although annual plant communities constitute an important part of the diversity in arid systems, they may have little impact on the dynamic and the functioning of arid systems within the studied shrublands due to their low plant biomass (Wiegand *et al.* 1995; Cañellas & San Miguel

2000). We used the total number and relative cover of perennial species in each of the 80 quadrats as a surrogate for species richness and species abundance, respectively.

Trait measurements

To quantify the functional structure of the sampled communities, we measured plant functional traits on species that collectively represented 90% of the total cover at each site. Measurements were conducted in spring from 20th to 25th of March in order to measure traits during the growing period and to avoid late spring or summer drought. Ten individuals per species and per site were randomly selected to ensure that intraspecific trait variability (ITV) was taken into account in our sampling, a potential important factors when considering community assembly (Violle *et al.* 2012). On each individual, we measured the following above-ground traits according to standardized protocols (Cornelissen *et al.* 2003): (i) architecture and size-related traits related to competitive ability and/or plant water use efficiency (due to allometric relationships between plant size and the architecture of the root system, Westoby *et al.* 2002), branching density (number of main stems) and ramification (number of ramifications per stem), lateral spread, reproductive and vegetative height; (ii) phenology, measured using a phenology index (1 = no reproductive stem; 2 = reproductive stem starting to grow; 3 = flowering; 4 = flower fading; 5 = fruit present; and 6 = fruit absent and senescence of the reproductive stem); (iii) foliar traits related to light interception and water stress tolerance (Westoby *et al.* 2002; Gross, Suding & Lavorel 2007), leaf area, leaf length, leaf thickness and leaf width; and (iv) specific leaf area and leaf dry matter content, which are related with the leaf economic spectrum, i.e. the ability to acquire and use nutrient (Wright *et al.* 2004). These traits were selected because they reflect different plant species strategies for acquiring, using and conserving resources

(including light, nutrients and water), and for exploiting different temporal niches (Westoby *et al.* 2002; Maire *et al.* 2009). Overall, 36 out of 79 perennial species found at our study sites were sampled, and a total of 994 individuals were measured (Appendix S2).

Statistical analyses

Regional scale: identifying major axes of functional specialization - We considered here the species pool sampled along the full aridity gradient. To identify the main axes of specialization within semi-arid Mediterranean shrublands, we performed a principal component analysis (PCA) using the 12 traits measured on all species. This approach approximates the functional niche of species, defined as their position in a multi-dimensional trait space (Devictor *et al.* 2010). We used a VARIMAX procedure to maximize the correlations between the PCA components and the traits considered. We selected one trait for each PCA component with eigenvalue higher than 1 as a functional marker representative of each axis of specialization. We selected these traits and used them in subsequent analyses because they are independent variables at the species level and reflect important leading dimensions of the species niche (Gross, Suding & Lavorel 2007).

Community scale: Testing for HF and ND - We used a null model approach to evaluate the effects of HF and ND as drivers of community assembly (Gotelli & Graves 1996). The null assumption was that local communities should simply reflect a random distribution of individuals drawn from a regional pool, weighted by the abundance of each species present at the regional scale (Cornwell & Ackerly 2009). To generate the null assumption, we performed an individual-based randomization of species abundance across sites. We defined the community at the transect level and individuals at the

quadrat level, i.e. one ‘individual’ corresponded in our model to the sum of the cover of all individuals of a given species observed in a single quadrat. In total, we considered 4 community replicates (1 per transect) and 80 quadrats (20 per community) within each site.

A matrix describing the abundance of each individual species observed at the quadrat level was randomly shuffled (9999 times) across transect and sites using the “permatful” function in the R package *vegan* (Oksanen 2011). This procedure kept species abundance constant at the regional scale, but allowed species richness and abundance to randomly vary across quadrats within and between communities. As HF and ND may both modify the trait composition and the local species richness and density (Keddy 1992; Violle *et al.* 2011), we did not constrain the number and the local cover of species to be fixed within quadrat and communities during the randomization procedure. Our individual-based randomization had the advantage to directly reflect our sampling design by taking into account the pattern of local abundance of all sampled individuals at the quadrat level. This randomization procedure avoided any bias in the null model envelope size due to local variation in species number and cover observed at the quadrat level (Gotelli 2000). The size of the null envelope is only determined by species abundance at the regional scale, consistently with our null hypothesis.

For each of the 9999 randomizations, we used a second matrix containing the trait values of each individual species at the quadrat level, which was used to calculate the community-weighted trait values (CWT, Violle *et al.* 2007) and the functional distance (FD, adapted from Laliberté & Legendre 2010) at the community level. The CWT estimates the ‘mean trait value’ of the community weighted by the relative abundance of each species present in the community:

$$CWT_j = \sum_i^n p_i T_i \quad Eqn(1)$$

where p_i is the abundance of the species i in the community j and T_i the ‘mean trait value’ of the species i in the community j , respectively. The FD quantifies the degree of trait divergence within a community, weighted by the relative abundance of each species within the community:

$$FD_j = \sum_i^n p_i \left(\frac{|T_i - CWT_j|}{\sum_i^n |T_i - CWT_j|} \right) \quad Eqn (2)$$

where p_i is the abundance of the species i in the community j , T_i is the ‘mean trait value’ of the species i in the community j , and CWT_j is the community-weighted traits in the community j . Abundance data (p_i) were log transformed for all analyses. Both CWT and FD values were calculated for each selected trait from the PCA analysis.

Based on the 9999 randomizations, we calculated the 95 % confidence interval and compared the observed CWT and FD indices with null predictions to infer the effects of HF and ND on community trait distributions. Observed data outside the null envelope were significantly different from random expectations, indicating that deterministic processes led to less or more divergent community trait distribution than expected by chance. Specifically, observed FD values below the null envelope indicated that traits within communities were forced to converge more than expected by chance, suggesting for HF. Habitat filtering was also inferred when significant changes in CWT occurred along the rainfall gradient. CWT indicates which trait value was selected by HF (e.g. were tall or short species favored in a given site?). In contrast, the impact of ND was detected when communities exhibited high FD values above the null envelope, *i.e.* coexisting species showed stronger functional differences than expected under the

null hypothesis (Laliberté & Legendre 2010). Note that: (i) as we used weighted abundance indices (Violle *et al.* 2007; Laliberté & Legendre 2010), our study took into account not only the effect of species turnover along the aridity gradient, but also observed changes in species cover across sites; (ii) as multiple assembly processes can simultaneously affect community structure and influence different traits independently (Spasojevic & Suding 2012), we ran this analysis separately for each selected trait; (iii) intraspecific trait variability (ITV) can have a non-negligible, sometimes important, impact on community structure (Violle *et al.* 2012). To take the effect of ITV into account, we calculated CWT and FD using either the mean trait value per species at the regional scale, *i.e.* one value per species and trait along the whole gradient (without ITV); or the trait value of each species measured within each site (taking into account the observed ITV across sites).

We used linear mixed models to test for rainfall effects on community structure. We ran this analysis independently with and without ITV, *i.e.* with data calculated at the site or at the regional level respectively. The model had the following form:

$$CWT \text{ or } FD = f(Rain + Rain^2) \quad Eqn(3)$$

We introduced a quadratic term for rainfall as it has been shown that the functional response of communities to aridity is not necessarily linear (Kéfi *et al.* 2007; Cornwell & Ackerly 2009). Transect ID nested within sites was used as a random factor. We then tested if the slope of the relationship between rainfall and traits was affected by ITV using the same linear mixed model. When a significant interaction between rainfall and ITV was detected, we concluded that ITV modified the community response to aridity. To ensure models met the assumptions for parametric tests, we log-transformed data whenever appropriate and checked the residuals.

Within-community scale: evaluating the impact of biotic interactions - We used a similar null model approach to detect significant spatial patterns in trait distributions at the plant neighborhood scale (i.e. quadrat level). The study of such patterns has often been used to infer the impact of facilitation or competition on community structure (e.g. Pielou 1962; Fowler 1986; Pugnaire *et al.* 2011), e.g. by comparing the number of species present inside and outside nurse plant species (Cavieres & Badano 2009; Soliveres *et al.* 2010). Our trait-based analysis followed this approach by investigating the fine-scale spatial patterns of trait aggregation. Our analyses considered all sampled species in the community, and were conducted for each selected trait separately. The null prediction was that the spatial distribution of species within a given community was random, *i.e.* was not impacted by any biotic processes acting at the plant neighborhood scale.

Using the matrix of species abundance in each site (transect level), we randomized species distribution between quadrats independently along each transect (9999 permutations). For each randomization event, we calculated for each quadrat its weighted-trait value (quadrat-weighted trait value, QWT) similarly to the CWT (see Eq. 1). The QWT reflects the ‘mean trait value’ of directly interacting species at the plant-neighborhood scale weighted by the abundance of co-occurring species at the quadrat level. Using the QWT values predicted by the null model, we calculated the sum of the absolute pairwise differences between QWT values (PwD_k) for each trait in each transect k as following:

$$PwD_k = \sum_{ij}^n |(QWT_i - QWT_j)| \quad Eqn(4)$$

PwD is adapted from a standard index (the nearest-neighbor distance) generally used in community assembly studies to detect niche differentiation between co-occurring

species (see for instance Kraft, Valencia & Ackerly 2008). In our case, it corresponds to the degree of spatial dispersion of a trait across quadrats within a community, and we used it as a proxy of the impact of biotic interactions in structuring communities.

Based on the 9999 randomizations, we determined the mean and the 95 % confidence interval of PwD_k for each transect, which reflects the null prediction of spatial trait dispersion within each community. We compared the observed PwD values obtained in each transect with the null prediction (deviation from null prediction). When PwD was significantly higher than the null prediction, it implied that species with different trait values tended to spatially avoid one another. This pattern was indicative (but not the proof) of competition between species. For instance, if spatial overdispersion was observed on plant height, it implied that shorter plants avoid taller plants because of local competition for resources (Fowler 1986; Schamp, Chau & Aarssen 2008). When PwD was lower than the null prediction, it demonstrated that species with different trait values tended to be more spatially associated than expected by chance. This pattern could be interpreted as a sign of facilitation among species, as facilitation commonly occurs in closed spatial associations of species with contrasted trait values (Cavieres & Badano 2009). Note that:

- (i) we considered ITV observed between sites in the within community scale analyses, *i.e.* considering one trait value per species and per site. In our model, the trait value of a species can thus vary across sites but not within. Significant spatial pattern within a given community cannot be attributed to an effect of ITV but only reflected the spatial sorting of species within communities;
- (ii) the spatial dispersion of traits within a given community was statistically independent from the CWT and FD calculated at the community level, as for a given community trait distribution we can expect different spatial patterns. However, along

the aridity gradient spurious correlations between *PwD* and CWT or FD can be observed if the range of trait values observed within communities changed along the gradient. To facilitate the comparison of traits across communities, we thus standardized before analysis the trait value of each species *i* occurring within each transect *k*:

$$\text{SdTrait}_{ik} = \frac{(\text{trait}_i - \text{meantrait}_k)}{(R)} \quad \text{Eqn(5)}$$

where meantrait_k is the mean trait value of co-occurring species in a transect *k* and *R* is the range of trait values observed in a given transect *k* (maximum - minimum trait value). Consequently, SDtrait_{ik} is centered on zero and is independent from the range of trait value observed in a given site.

(iii) non-random spatial patterns can also be the consequence of abiotic heterogeneity within sites (Violle *et al.* 2012). As we selected sites exhibiting similar within-site environmental heterogeneity, this further supports that the observed spatial structure within communities might be a consequence of biotic interactions between plant species.

To test how biotic interactions changed in response to aridity, we tested whether the observed deviation from the null expectation of *PwD* changed along the aridity gradient:

$$\text{Deviation of } PwD = f(\text{polynomial}(\text{Rain})) \quad \text{Eqn(6)}$$

To ensure that our model results cannot be observed by chance alone, we ran a further round of randomizations (9999 times) and fitted the same model using randomized values and counted the number of times a significant value was obtained by chance for the polynomial relationship between rainfall and deviation of *PwD* detected for the observed data.

Combining effects of rainfall and biotic interactions on community structure - Our analysis using *PwD* can be related to the importance of biotic interactions in structuring the whole community, as spatial over- and underdispersion would denote competition or facilitation, respectively. To test how biotic interactions, quantified as above, changed along the rainfall gradient, we conducted a linear mixed model analysis which considered rainfall and *PwD* as the dependent variables, the transect ID as a random factor, and CWT or FD indexes calculated with ITV as the variables to be explained. The model considered each trait independently and had the following form:

$$CWT \text{ or } FD = f(Rain + Rain^2 + PwD + interactions) \quad Eqn(7)$$

The best models were selected using Akaike information criterion (AIC) for each trait and index. We then conducted a variance decomposition analysis based on the sum of squares of the models to evaluate the relative importance of rainfall and biotic interactions (estimated with observed *PwD* in each community) in explaining local community structure (either CWT or the FD). Statistical analyses were performed using R (R core Development team 2009) and JMP 7 (The SAS Institute, Cary, North Carolina, USA).

RESULTS

Regional scale

We identified 4 independent axes of specialization within the studied shrublands, which together explained up to 82 % of the total variance found in the data (Fig. 2). The first component was positively related to the plant size (VH, RH and LS, see trait abbreviations in Fig. 2), and was negatively correlated to the branching density. The second component was positively related to the leaf size (LL and LA), and negatively correlated with the number of stem ramifications and the phenology index. Note that the second component strongly contrasted grass species characterized by long leaves such as *Stipa tenacissima* L. from shrub species. The third and fourth components separated species based on their leaf morphology, where LT was positively correlated with the third component. SLA and LDMC were negatively correlated along the fourth component. Together, these results indicated that for a given SLA value, leaf morphology can vary independently across species through leaf density changes (via LDMC) and through the modification of the leaf thickness (LT). In addition, the fourth component separated exploitative species with high relative growth rate from conservative ones. We selected four traits to represent each independent component in the next analyses: vegetative height, LA, LT and SLA for the first, second, third and fourth components, respectively.

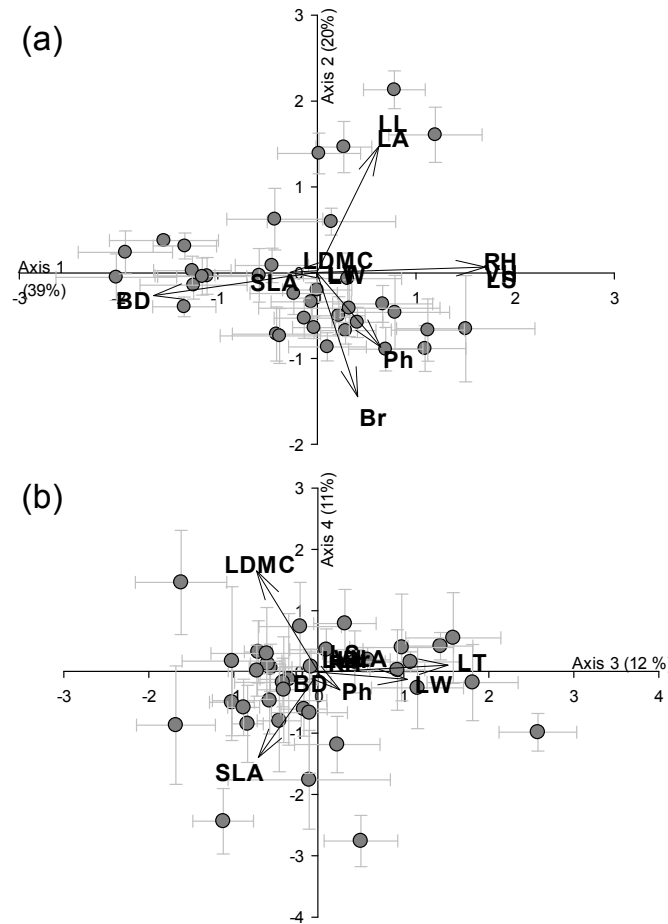


Fig. 2. Co-variation of plant functional traits along four components of a principal component analysis extracted from a trait \times species matrix. Included are 36 species measured along the aridity gradient and 12 traits (A, components 1 and 2; B, components 3 and 4). Trait abbreviations are: branching density (BD number of main stems), branching ramification (Br, number of ramification per stem), lateral spread (LS, m²), leaf dry matter content (LDMC, g g⁻¹), leaf area (LA, cm²), leaf length (LL, cm), leaf thickness (LT, mm), leaf width (LW, mm), phenology (Ph, index of phenology), reproductive height (RH, cm); specific leaf area (SLA, cm² g⁻¹); vegetative height (VH, cm).

Community scale

Functional differences between species translated at the community level into clear non-random community assembly patterns (Fig. 3). The values of the community-weighted traits (CWT) and functional distance (FD) considering all traits were in half of the cases outside the null envelope (59 % and 49 % for CWT and FD calculated with ITV, respectively). However, changes in CWT and FD observed in response to the rainfall gradient were trait-dependent. CW-Height decreased with increasing aridity, from an

average of 60 cm in the wettest sites to ~ 50 cm height in the driest part of the gradient (Fig. 3a). At high rainfall levels, we observed FD-H values above the null prediction, indicating a high community trait divergence, while plant height became less variable in the driest part of the gradient and converged below the null envelope toward low FD-H (Fig. 3b). Quadratic relationships between rainfall and CW-LA, and between rainfall and FD-LA were observed (Fig. 3c,d). We found values above the null-envelope for intermediate rainfall levels and below the null envelope at the two extremes of the gradient. Conversely, CW-LT increased with rainfall with values above and below the null envelope as aridity increased (Fig. 3e). No clear pattern was observed for FD-LT along the rainfall gradient, although some communities were outside the null envelope (Fig. 3f). The CW-SLA showed a strong prevalence of significantly lower values than expected by chance ($40 \text{ cm}^2\text{g}^{-1}$), with an indication for an abrupt change (happening only for the last site) below 300 mm rainfall toward higher SLA values (up to $100 \text{ cm}^2 \text{g}^{-1}$, Fig. 3g). The FD-SLA had a negative linear relationship in response to rainfall. It exhibited underdispersed values indicating a strong trait convergence toward particularly low SLA values at the wettest part and in the middle of the aridity gradient ($40 \text{ cm}^2 \text{g}^{-1}$, Fig. 3b).

Including the effect of ITV into the analysis only affected the response of community structure for some traits, i.e. height and SLA. FD-H had only a significant response to aridity when taking into account ITV (Fig. 3b). The community convergence toward lower values of plant height can be then mainly attributed to an intraspecific response of dominant shrubs. Similarly, ITV decreased the response of FD-SLA to increasing aridity, leading to trait convergence at the driest part of the gradient (Fig. 3h). This result indicates that SLA convergence toward low values was partly due to ITV.

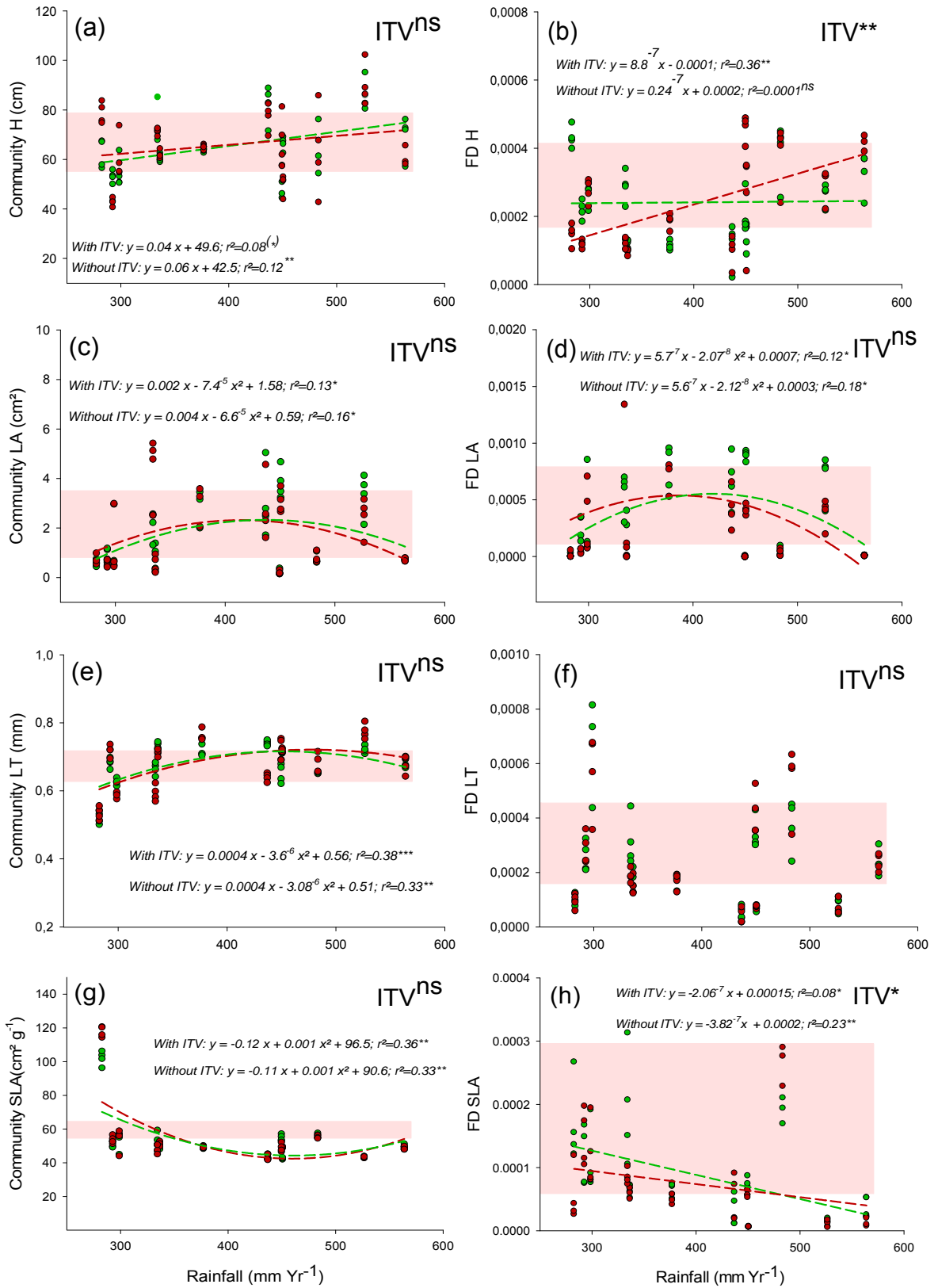


Fig. 3. Community-weighted trait (a, c, e, g) and associated functional distance (FD) (b, d, f, h) along the rainfall gradient. Red dots indicate the community traits and FD values taking into account for intra-specific trait variability (ITV) observed across sites. Green dots show values without ITV (one trait value per species only). The red zone represents the null model

envelopes. Dots outside the null envelopes are significantly different from the null prediction. The equations of the relationships with and without ITV are given in each panel. We indicated when ITV significantly impacted the relationships between traits and rainfall within panels: ns, non significant; (*) $P < 0.1$, *, $P < 0.05$; ** $P < 0.001$; ***, $P < 0.0001$. See Fig. 2 for trait abbreviations.

Plant neighborhood scale

Significant relationships between rainfall and the deviation of the sum of the pairwise distances (PwD) from the null expectation were observed for all traits with the exception of LT (Fig. 4c). The spatial dispersion of height within communities shifted from a spatially overdispersed pattern at high levels of rainfall to a spatially underdispersed pattern under drier conditions. Species with contrasted sizes spatially avoided themselves at the wettest part of the gradient, whereas they tended to be spatially associated at the driest part (Fig. 4a). An inverse pattern was observed along the rainfall gradient for SLA, where the spatial overdispersion of SLA peaked in the driest part of the gradient whereas an underdispersed spatial pattern was observed in the wettest part (Fig. 4d). For LA, we found a cubic relationship with a spatial overdispersion of traits occurring at intermediate level of rainfall (Fig. 4 b) while traits tended to be spatially underdispersed at the very dry end of the gradient.

The probability of observing by chance significant relationships between the deviation of PwD from the null expectation and rainfall was very low. Based on 9999 simulation runs, we found significant relationships in less than 3% of the cases for height and SLA and less than 1% for LA. These results indicate that variations of spatial distribution of traits within communities were clearly not random along the aridity gradient and provide evidence for the effect of competition and facilitation acting on the spatial structure observed within communities.

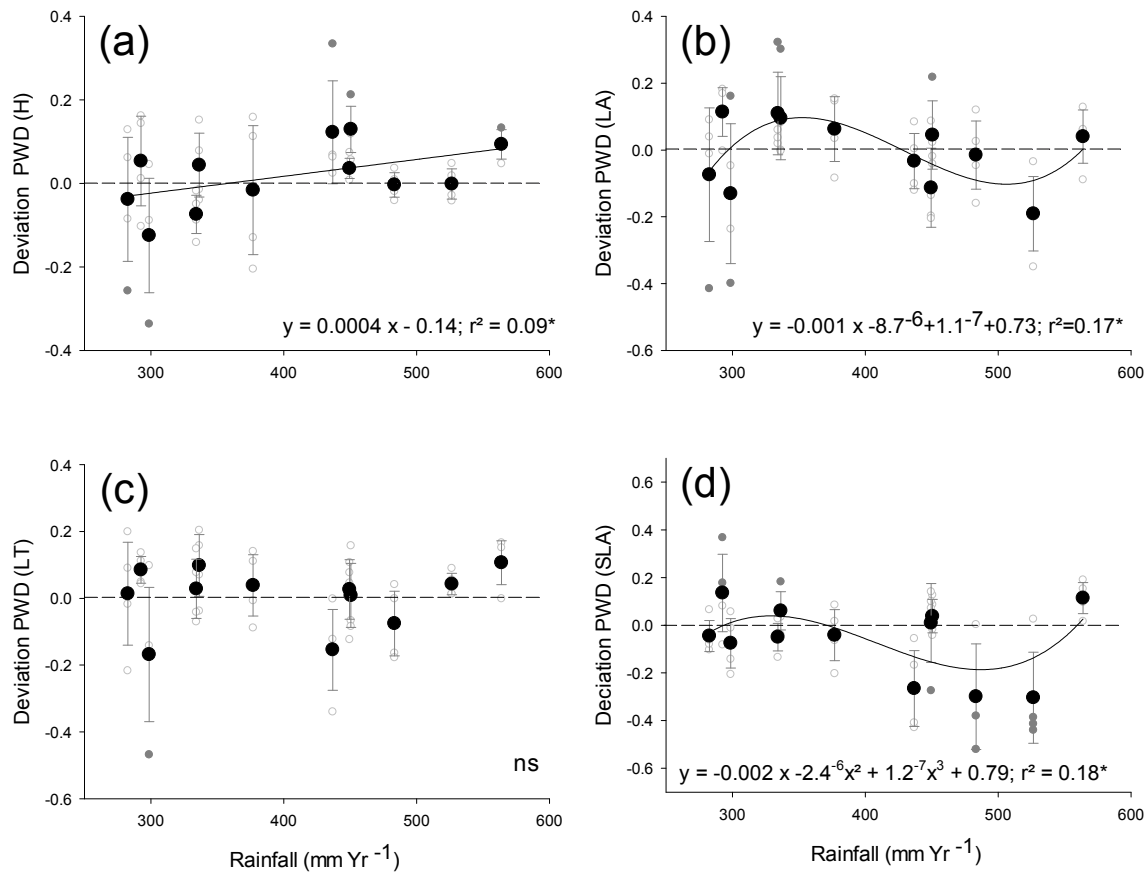


Fig. 4. Deviation of pairwise differences (PWD) from null predictions within communities for vegetative height (H, a), leaf area (LA, b), leaf thickness (LT, c) and specific leaf area (SLA, d) along the rainfall gradient. The mean deviation (black dots), associated standard deviation per site, and observed data at the transect level (grey dots) are graphed. Open grey dots are located within the null envelop, filled grey dots are significantly different from null predictions. The equations of the relationships are given for each trait in each panel; ns: $P > 0.05$; *: $P < 0.05$.

Combining effects of biotic interactions and rainfall on community structure

Our results suggested that the community structure is determined by the concomitant effects of rainfall and local biotic processes (estimated by PwD), and that these effects varied with the trait considered (see Table S2 and Fig. 5). CW-H was mainly affected by biotic interactions, while spatial overdispersion was positively correlated with taller plant types (see coefficient in Table S2, Fig. 5a). A similar pattern was observed for FD-H, where biotic interactions and higher rainfall increased the FD-H within communities (30% and 70% of model r^2 , respectively). Both CW-LA and FD-LA were impacted by the concomitant effects of rainfall and biotic interactions (80 % and 71 %

of model r^2 , respectively), where biotic interactions tended to increase the CW- and FD-LA, respectively (Fig. 5b). CW-LT was mostly affected by rainfall (60%), while FD-LT was mainly influenced by biotic interactions (even if the total model r^2 was low in that case, $r^2 = 0.16$; Fig. 5c). Finally, CW-SLA and FD-SLA were impacted by rainfall, with positive quadratic relationships (55% and 41% of model r^2 , respectively) indicating an increase in CW-SLA and FD-SLA at the end of the aridity gradient studied. Biotic interactions interacted positively with rainfall to increase the CW-SLA and FD-SLA (Table S2 and Fig. 5 d).

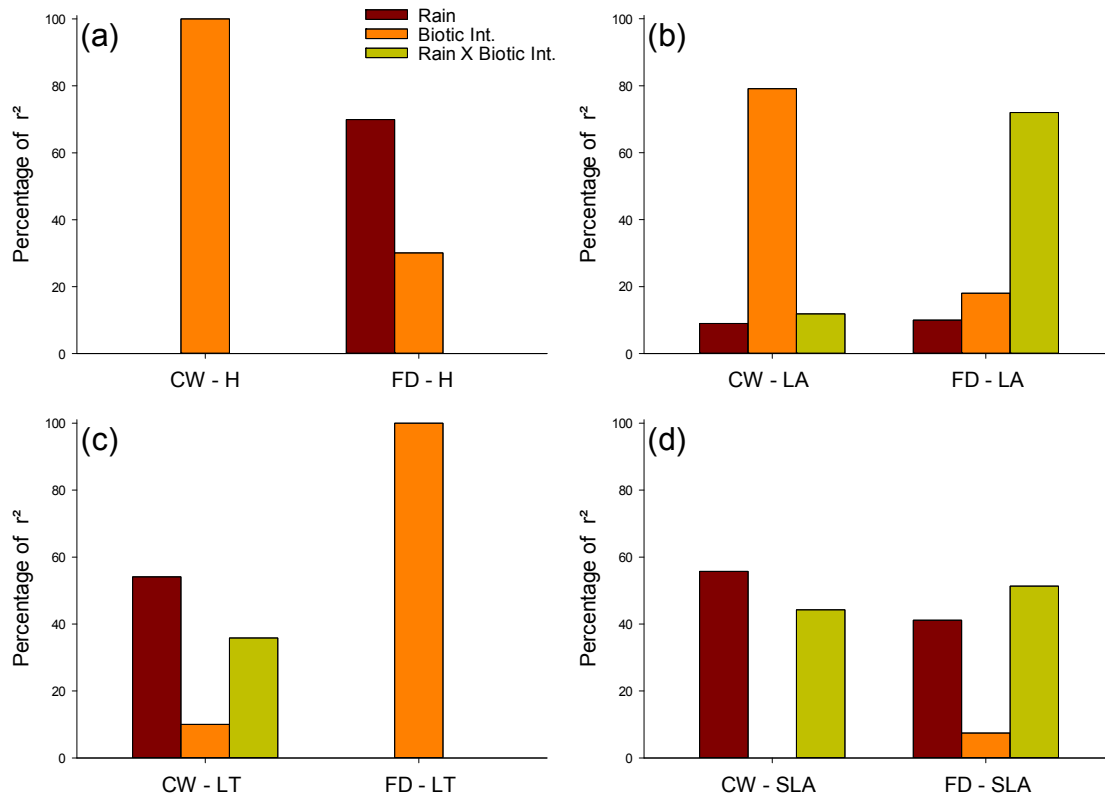


Fig. 5. Relative importance (percentage of model r^2) of rainfall (rain), biotic interactions (Biotic int.) and their interactions as predictors of the community-weighted trait (CWT) and the functional distance (FD) for (a) vegetative height (H), (b) leaf area (LA), (c) leaf thickness (LT), and (d) specific leaf area (SLA). See Table S2 for additional information on the models fitted.

DISCUSSION

Most of the CWT and FD values found at our sites were located outside the null model envelopes, with a relatively high frequency compared to recent trait-based community analyses carried out in more mesic environments (*e.g.* Cornwell & Ackerly 2009; Freschet *et al.* 2011; Spasojevic & Suding 2012). This result indicated that the functional structure of the semi-arid shrublands is strongly determined by non-random processes. Consistently with our main hypothesis, we found that HF and ND act jointly to determine plant community structure along the aridity gradient. These two key community assembly processes operated together, but not necessarily in a similar way on the four axes of functional specialization identified in this study. Our results highlight the need to take multiple assembly processes into account when investigating the mechanisms that drive community structure (see also Spasojevic & Suding 2012). More importantly, our multi-scale analysis helps to understand how biotic interactions interact with aridity to determine the community structure of Mediterranean shrublands.

Community assembly along contrasted axes of specialization

The first axis of specialization is related to plant size, and reflects a trade-off for biophysical constraints in determining water fluxes within the plant (Enquist 2002). It corresponds to an allometric relationship, which coordinates the architecture of above- and below-ground plant parts (Westoby *et al.* 2002; Kerkhoff & Enquist 2006). At the community level, we observed a strong decrease in plant size and FD-H as a direct response to increasing aridity, suggesting that HF is taking place (Fig. 3, Díaz, Cabido & Casanoves 1998; Thuiller *et al.* 2004). Increasing water stress at the plant level is known to increase the risk of cavitation, which imposes the plant to exhibit lower

stature as rainfall decreases (Enquist 2002). Plant height's response to aridity was mediated by ITV (as indicated by the different regression lines in Fig. 3b). The ITV effect might be due to the response of tall dominant shrubs such as *Q. coccifera*, for which the phenotype is altered along the aridity gradient, likely because of the genetic differentiation between populations of this species (Rubio De Casas *et al.* 2009). This result highlights the importance of taking ITV into account as an important factor when investigating trait-based community assembly (Violle *et al.* 2012).

The spatial trait dispersion found for height, with consistent spatial overdispersion and underdispersion of traits at the wettest and driest parts of the gradient, respectively, suggested a shift from competition to facilitation with increasing aridity (Fig. 4a), in accordance with the main prediction of the “Stress-Gradient Hypothesis” (SGH, Bertness & Callaway 1994). Spatial overdispersion patterns were systematically associated with high trait divergence at the community level, indicating that competition is likely to translate at the community level by promoting high ND and a spatial coexistence between functionally contrasted competitors (King & Woodell 1973; Fowler 1986; Haase *et al.* 1996). Our result contrasts with general observations from more mesic ecosystems (Grime 1973; Wedin & Tilman 1993; Schamp, Chau & Aarssen 2008; Gross *et al.* 2009), where asymmetric competition often leads to species exclusion and community-level trait convergence (Grime 1973; Tilman 1988; Schamp, Chau & Aarssen 2008; Gross *et al.* 2009). In semi-arid environments, inter- and intra-specific competition is an important factor in explaining vegetation patterns (Tielborger & Kadmon 2000; Gilad, Shachak & Meron 2007; Rietkerk & Van de Koppel 2008). Competitive interactions for water may limit the spatial aggregation of tall competitors, reinforcing a patchy habitat where only less competitive species with small stature were able to persist in the remaining open areas (*e.g.* *Thymus vulgaris*; Fowler 1986; Haase *et*

al. 1996; Gilad, Shachak & Meron 2007). On the contrary, facilitation did not translate into high trait divergence at the community level as expected (e.g. Gross *et al.* 2009). This result suggests that facilitation impacted only on subordinate or rare species (Bruno *et al.* 2003; Liancourt, Michalet & Callaway 2005). Facilitation results from a cost-benefit relationship (Liancourt, Michalet & Callaway 2005) and is likely to occur only when the cost of having neighbors is lower than the benefits they can provide. Under dry conditions, small plants are more likely to benefit from the facilitation of taller nurse plants, as the competitive abilities of the latter should decrease with increasing abiotic stress, while the benefit of being close to a nurse plant might be maximized under high aridity levels (Bertness & Callaway 1994; Liancourt, Michalet & Callaway 2005; Maestre *et al.* 2009b).

The second axis reflected a tradeoff for hydrological constraints at the leaf level according to the corner's rule: highly branched species cannot develop large and long leaves to avoid leaf overlapping and maximize light interception (Westoby *et al.* 2002; Swenson & Enquist 2008). High leaf area generally leads to low water stress tolerances whereas highly branched species with small leaves are better suited to arid environments (Westoby *et al.* 2002). At the community level, CW-LA and FD-LA followed quadratic responses to rainfall, indicating that HF is driven by contrasted mechanisms along the gradient (Figs. 3 c,d). On one hand, this quadratic relationship can be explained by the limitation of grass species abundances (e.g. *S. tenacissima*) due to competition with tall shrub at the highest rainfall levels, which would lead to the exclusion of tall grass species from the community (Gasque & Garcia-Fayos 2004). On the other hand, the abundance of grasses such as *S. tenacissima* may decrease due to the increasing abiotic stress at the driest part of the aridity gradient (Armas, Kikvidze & Pugnaire 2009) where an underdispersed spatial pattern was recorded for leaf area (Fig.

4b). Similarly to what was observed for plant height, spatial overdispersion of LA (Fig. 4) may indicate that competition is also important under intermediate levels of abiotic stress, and translated into an observed spatial coexistence between shrub and grass species and high ND.

For the third and four PCA axes, CW-LT decreased while CW-SLA increased with aridity (Figs. 3e to h). These results indicated a shift in the dominant strategy along the rainfall gradient, from stress-tolerant strategies with thick leaves (Grime 1973; Dussault *et al.* 2012) to stress-avoidance strategies characterized by thin leaves and high SLA (Niinemest 2001; Poorter *et al.* 2009; Freschet *et al.* 2011). Sites located in the driest part of the gradient are dominated by fast growing summer-deciduous species (e.g. *Lavandula multifida* L., *Satureja obovata* Lag.). These species are well adapted to dry environments as they can exploit the short growing season during late winter and spring, when cool temperatures and lower water stress allow plants to grow (Poorter *et al.* 2009). The decrease in abundance of stress-tolerant species at the driest end of the aridity gradient may point out that water-stress becomes too strong for these perennial leaf species to support summer drought (Lillis & Fontanella 1992; Poorter *et al.* 2009).

Interestingly, we observed that the spatial distribution of SLA within communities changed from underdispersion in wetter sites to overdispersion in dry ones, suggesting a shift from facilitation to competition with increasing aridity (Fig. 4d). This result contrasted with predictions from the “Stress gradient hypothesis” (Bertness & Callaway 1994) and results observed on plant height (Fig. 4a). They, however, agreed with other empirical and theoretical studies conducted in water-limited systems (Maestre, Valladares & Reynolds 2005; Maestre *et al.* 2009b; Gross *et al.* 2010). Facilitation is likely to benefit only to those species that are not well adapted to local abiotic conditions (Liancourt, Callaway & Michalet 2005; Gross *et al.* 2010). Under low

aridity conditions, facilitation might only concern those exploitative species characterized by high SLA and perennial leaves, which cannot tolerate high aridity levels (Dussault *et al.* 2012). These species (*e.g.* *Brachypodium retusum*, *Dactylis hispanica*) may persist under shrub canopies due to facilitation (Maestre *et al.* 2004), and explain the observed spatial underdispersion of SLA observed under less arid conditions. The spatial overdispersion of SLA observed at the driest part of the gradient studied suggested that competition might be the prevailing force in dry conditions between well adapted summer-deciduous species and stress-tolerant shrubs.

We did not observe a clear response of FD-LT along the aridity gradient, although most of the communities exhibited a significant trait divergence (Fig. 3f). This suggests a coexistence of contrasted functional strategies with similar responses to aridity (*i.e.* two stress-tolerant strategies either with thick leaves filled with water vs. dense leaves strategies, Poorter *et al.* 2009, Dussault *et al.* 2012), which led to high trait divergence at the community level.

Community level impact of competition and facilitation in drylands

An important result from our study is that a shift from competition to facilitation appears to be trait-dependent (See Fig. 6 for a synthesis of our results). While patterns observed on some traits (*e.g.* height) supported the “SGH” (Bertness & Callaway 1994), others showed contrasting responses to aridity (Fig. 4). The trait dependency of the relationship between aridity vs the outcomes of biotic interactions may explain contrasted patterns of competition/facilitation previously observed along aridity gradients (*e.g.* Tielbörger & Kadmon 2000; see also Maestre, Valladares & Reynolds 2005 for a meta-analysis). Thus, our result provide a mechanistic explanation helping to solve this issue (see also Brooker *et al.* 2008; Maestre *et al.* 2009b for reviews): (i)

competition and facilitation co-occurred within each community along the gradient but acted independently on different traits (Maire *et al.* 2012); and (ii) competition mainly impacted dominant species while facilitation benefited subordinate or rare species (Bruno *et al.* 2003).

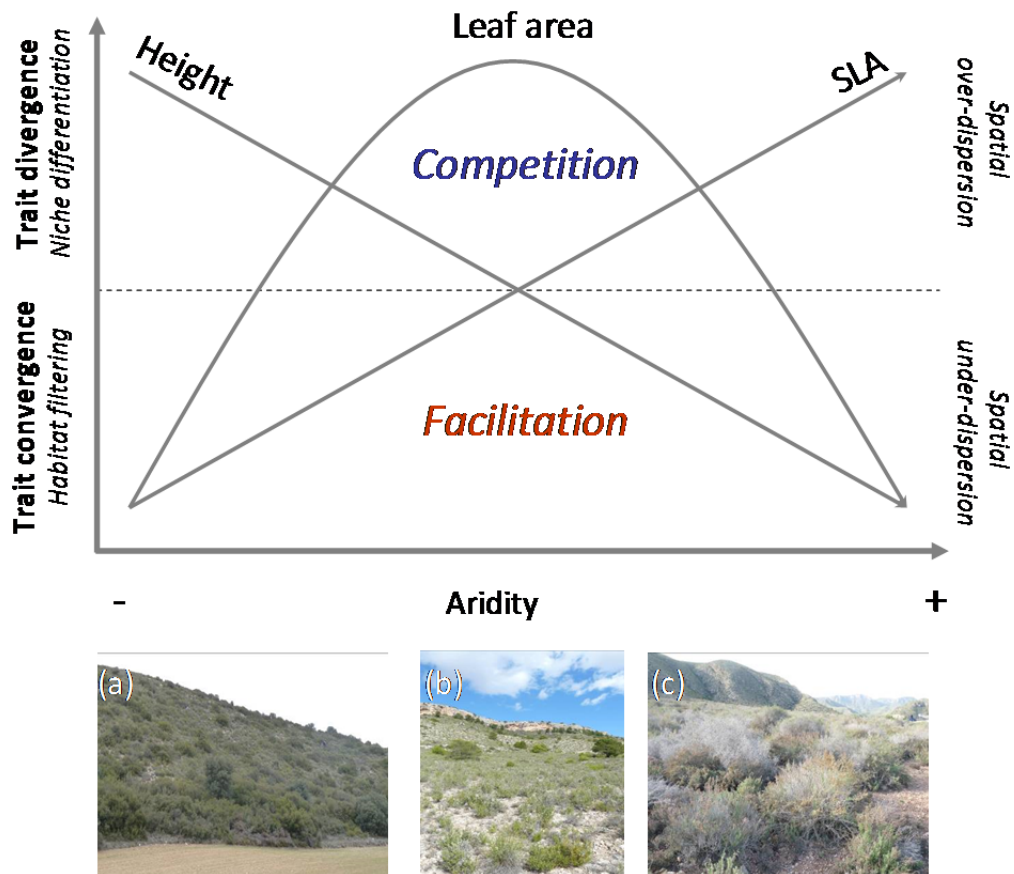


Fig. 6. The functional response of Mediterranean shrublands to aridity suggests an impact of biotic interactions on community structure. High trait convergence indicates the effect of habitat filtering (HF) along the aridity gradient. High trait divergence indicates niche differentiation (ND) among co-occurring species. Coexistence between functionally contrasted species is also suggested by the spatial dispersion of traits within communities. Along the aridity gradient, HF and ND jointly determined the functional structure of Mediterranean shrublands, acting independently on contrasted axes of plant specialization. Importantly, spatial analysis at the plant neighborhood scale suggests that competition and facilitation impacted the whole community structure but acted differently on contrasted sets of traits. Competition impacted mostly the dominant plant species leading to high trait divergence at the community level while facilitation impacted only subordinate and rare species (see main text for details).

The competition between dominant plant types seems to be constant along the aridity gradient while the traits on which competition may act changed with aridity (Fig. 6). These results suggest that the type of resources for which competitors interact may change along the aridity gradient, consistently with theoretical predictions (Tilman 1988; Tilman & Pacala 1993) and previous empirical work in temperate grasslands (Wedin & Tilman 1993). At the wettest part of the gradient, tall shrubs may have a strong competitive effect for light availability within their neighborhood (Gasque & Garcia-Fayos 2004; Armas & Pugnaire 2005). Under these conditions, smaller drought-tolerant species are only likely to persist in open areas (Fig. 4a). With increasing stress, the size of the plant and the functional divergence for height decreased, and the abundance of medium-size grass species (e.g. *S. tenacissima*) increased. This translated into an intense competition, likely for soil resources (e.g. water, Armas & Pugnaire 2005), and a spatial avoidance between grasses and shrubs within communities is observed (Armas, Kikvidze & Pugnaire 2009)(Fig.4b). At the driest part of the gradient, stress-avoidant and summer-deciduous species increased in abundance (Poorter *et al.* 2009), increasing the SLA of the shrubland communities. Under these conditions, competition during the growing season may favor the development of exploitative plant types, with high SLA and faster growth rate, which can outcompete locally slow growing and more stress-tolerant shrubs (Fig. 4d). For all traits, competition between dominant plant types translated into high functional divergence at the community level indicative of high ND between competitors (Fig. 5).

Similar to competition, facilitation occurred all along the aridity gradient by acting on contrasted traits, but did not promote high functional divergence at the community level (Fig. 6). This result implies that facilitation likely impacted subordinate and rare species (Bruno *et al.*, 2003; Liancourt, Michalet & Callaway

2005). Thus, facilitation can be considered as an important factor maintaining high species diversity in semi-arid environments (Pugnaire *et al.* 2011). For instance, in semi-arid *S. tenacissima* steppes from Spain, Soliveres *et al.* (2011) found that facilitation by grasses (*S. tenacissima*) and shrubs (*Q. coccifera*) increased local species richness, and that, similarly to our results, the community-wide importance of these nurse plants remained constant along a rainfall gradient similar to the one studied here.

Together, our results emphasize that biotic interactions are likely to explain a non negligible part of the functional variation observed along the aridity gradient (Fig. 5). In general, aridity seems to act as a convergence force clustering trait values of co-occurring species (e.g. low SLA and height value at the wettest and the driest part of the gradient respectively). In contrast, competition (but not facilitation) appeared to explain the large functional diversity observed within drylands (Freschet *et al.* 2011). The effect of competition led, in some cases, to FD values above the null envelope (Fig. 3), indicating the effect of ND, while aridity led to FD values below the null envelope, suggesting the effect of HF. However, it is important to keep in mind that CWT and FD values within the null envelope are not necessarily random but may highlight the opposite effect of aridity and competition on community trait distributions: the former clustered species traits toward an optimum value; the latter increased trait divergence between competitors. Together, these results highlighted the importance of identifying the relative effect of abiotic and biotic factors on community structure as their opposite effects may explain apparent random patterns which are often found in trait-based community analyses (Cornwell & Ackerly 2009; Spasojevic & Suding 2012).

CONCLUDING REMARKS

Our knowledge on the mechanisms driving the functional structure of arid and semi-arid ecosystems remains incomplete (Maestre *et al.* 2009a; Freschet *et al.* 2011; Eldridge *et al.* 2011; Dussault *et al.* 2012), despite their ecological importance, extent, and the ecosystem services that they provide to human populations (Safirel & Adeel 2005). Our community level analysis indicated the overwhelming impact of deterministic process in structuring shrublands across a large aridity gradient in Spain. Consistent with our leading hypothesis, HF and ND appeared to be non-exclusive mechanisms which together shape the functional structure of semi-arid communities (see Fig. 6). Together, our results highlighted the need to consider multiple traits to understand contrasted processes that jointly operate to shape natural communities (see also Spasojevic & Suding 2012).

By considering multiple scales to study community assembly, our approach also offers a practical way to separate the role of biotic interactions from the effect of abiotic factors in explaining the community level patterns of trait divergence and convergence (Fig. 1). As such, our study constitutes an important step forward in the attempt to link biotic interactions to community structure (see also Gross *et al.* 2009; Cavieres & Badano 2009). Our results observed at the plant-neighborhood scale suggest that biotic interactions have an important impact on the structure of semi-arid communities; modifying species realized niches and abundances, and thus affecting the structure of the whole community. Overall, our study calls for a more mechanistic approach of community functional assembly (e.g. Savage, Webb & Norberg 2007; Gross *et al.* 2009), i.e. to move from patterns of community trait divergence and convergence toward the examination of the underlying mechanisms acting at the individual scale.

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SUPPORTING INFORMATION

Appendix S1: Site selection and gradient information

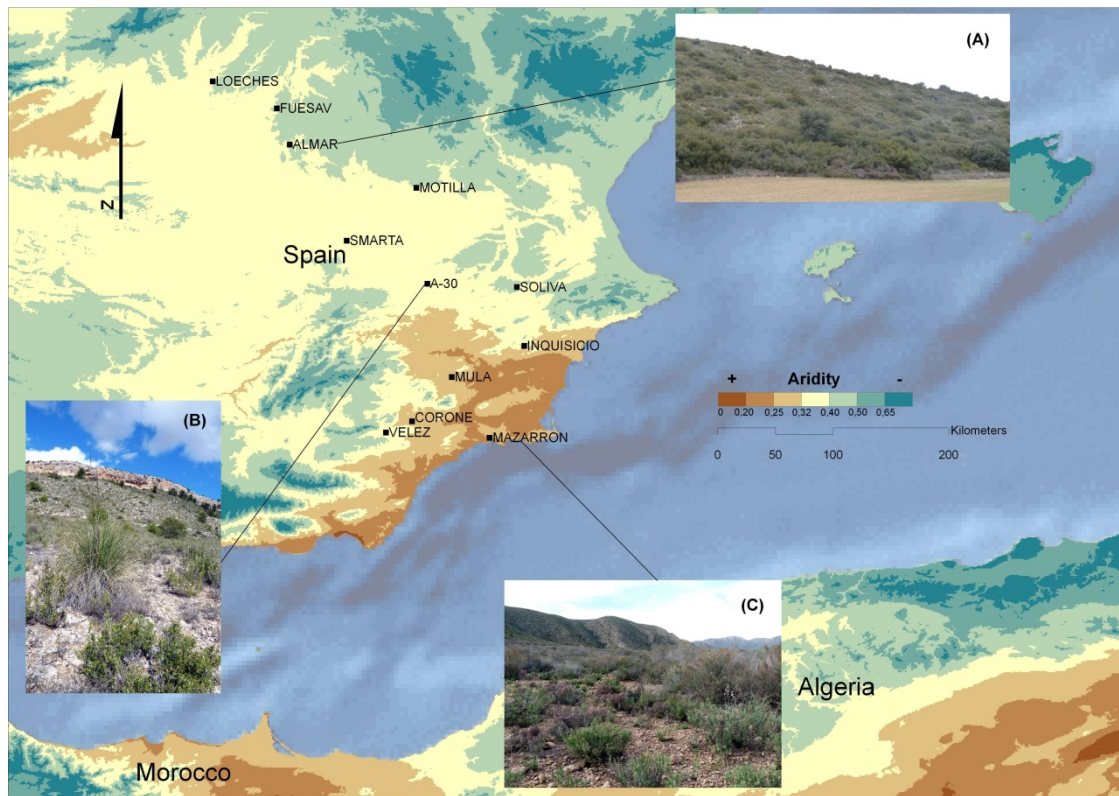


Fig. S1. The Mediterranean shrublands sampled along an aridity gradient from Central to South-eastern Spain. Aridity is expressed as the ration between precipitation and potential evapotranspiration. Pictures indicated three communities which characterize the response of the studied shrublands to aridity: (a) Tall shrubland: in the wettest part of the gradient, vegetation is dominated by tall shrubs (*Rhamnus lycioides*, *Quercus cocciferra*) with scattered trees (*Quercus ilex*), characterized by low specific leaf area (stress tolerant strategies); (b) Small shrubland: as aridity increases, tall shrubs become scarcer, and are replaced by small shrubs (*Thymus vulgaris*, *Rosmarinus officinalis*) and tall grass species (*Stipa tenacissima*); and (c) Summer deciduous shrubland: at the end of the gradient, most of stress tolerant shrub species have decreased in abundance and summer deciduous shrubs producing fast growing leaves (e.g. *Satureja obovata*) become dominant.

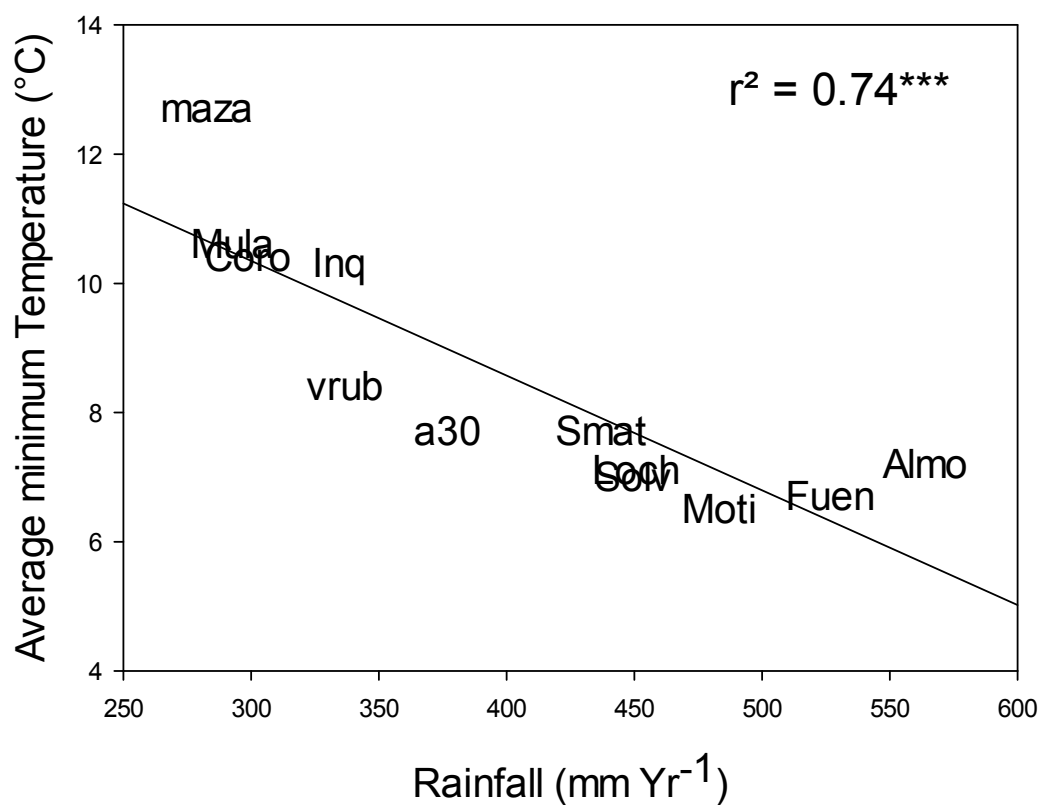


Fig. S2. Aridity gradient evaluated. X-axis corresponded to the mean annual rainfall at each site. Y-axis is the minimum average temperature observed in each site. See Fig. S1 for site locations and names.

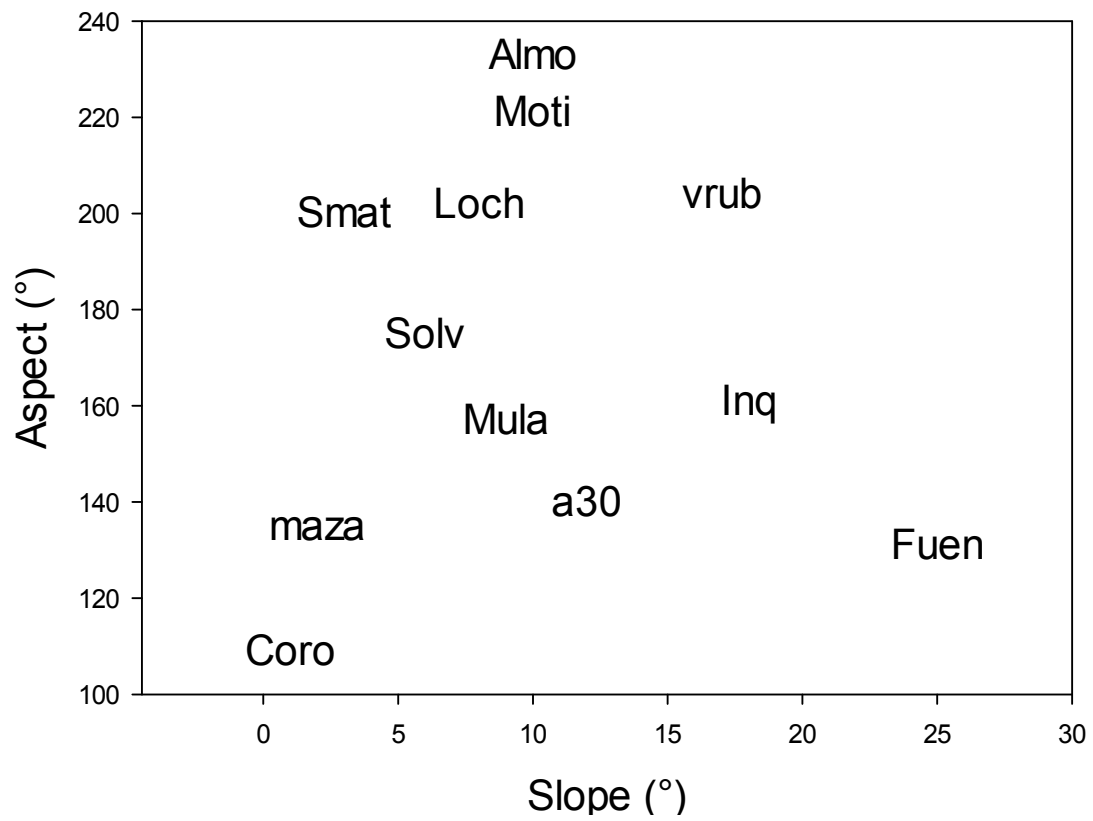


Fig. S3. Slope and aspect across selected sites.

Appendix S2: species trait values and abundance at the regional scale

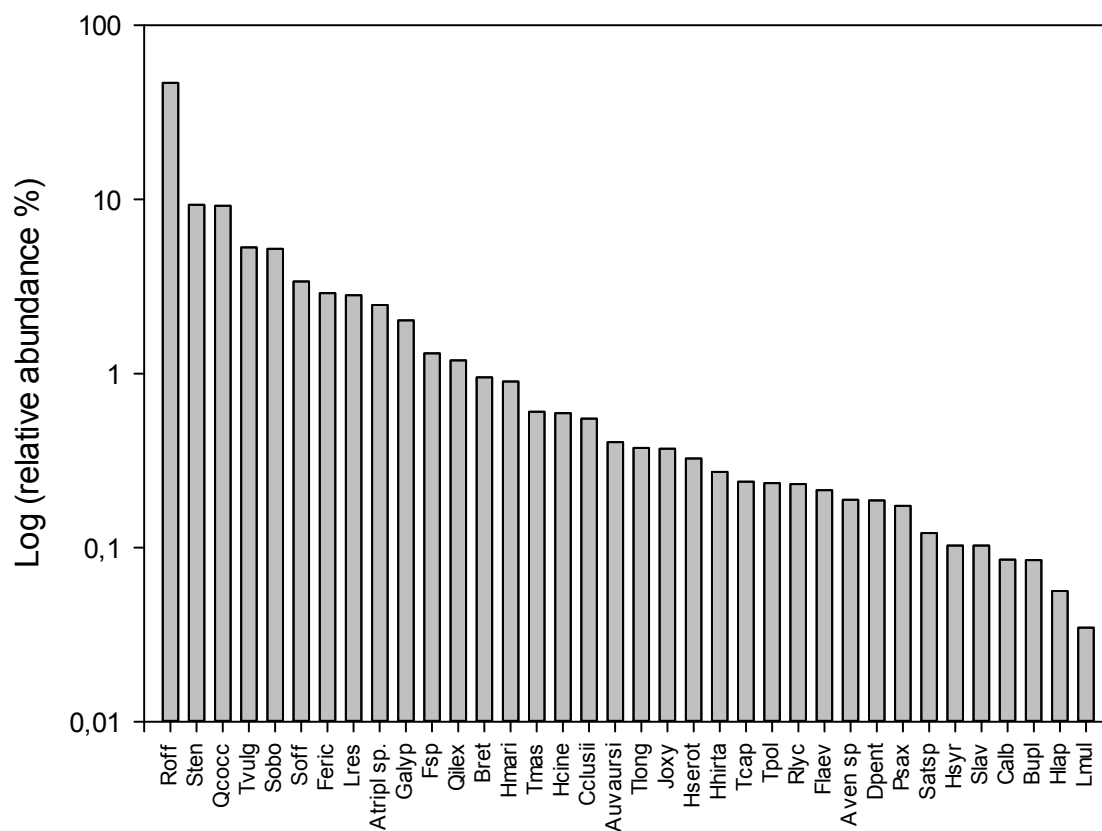


Fig. S4. Species relative abundance at the regional scale. The distribution follows a lognormal distribution (Poisson Law). See Table A for species names.

Table S1. Species name and trait values for maximum vegetative height, average leaf area, leaf thickness and specific leaf area.

Gender	Species	Height	Leaf Area	Leaf Thickness	Specific Leaf Area
		(cm)	(cm ²)	(mm)	(cm ² g ⁻¹)
<i>Arctostaphylos</i>	<i>uva-ursi</i>	23.1	1.23	0.85	37.29
<i>Atriplex</i>	<i>spp</i>	38.9	0.62	0.56	113.01
<i>Avena</i>	<i>spp</i>	58.3	6.7	0.28	227.55
<i>Brachypodium</i>	<i>retusum</i>	30.6	0.23	0.28	144.49
<i>Bupleurum</i>	<i>spp.</i>	19.9	0.65	0.51	92.66
<i>Cistus</i>	<i>albidus</i>	51.2	3.74	1.20	68.66
<i>Cistus</i>	<i>clusii</i>	54.2	0.23	0.69	45.82
<i>Dorycnium</i>	<i>pentaphyllum</i>	9.6	0.06	0.37	124.90
<i>Fumana</i>	<i>ericoides</i>	27.8	0.07	0.58	45.32
<i>Fumana</i>	<i>laevipes</i>	4.5	0.04	0.42	92.24
<i>Fumana</i>	<i>spp</i>	6.8	0.02	0.23	72.49
<i>Globularia</i>	<i>alypum</i>	43.4	0.75	0.62	44.66
<i>Helianthemum</i>	<i>cinereum</i>	5.3	0.68	0.67	67.54
<i>Helianthemum</i>	<i>marifolium</i>	31.8	0.08	0.47	87.35
<i>Helianthemum</i>	<i>syriacum</i>	28.5	0.55	1.22	52.93
<i>Helichrysum</i>	<i>serotinum</i>	32.8	0.26	0.57	76.53
<i>Hormathophylla</i>	<i>lapeyrousiana</i>	14.8	0.20	0.92	47.08
<i>Hyparrhenia</i>	<i>hirta</i>	41	2.28	0.54	89.48
<i>Juniperus</i>	<i>oxycedrus</i>	116.4	0.15	0.54	58.06
<i>Launaea</i>	<i>resedifolia</i>	113	1.17	0.63	136.23
<i>Lavandula</i>	<i>multifida</i>	26.4	1.18	0.54	216.93
<i>Phagnalon</i>	<i>saxatile</i>	32.5	0.10	0.62	58.27
<i>Quercus</i>	<i>coccifera</i>	135.1	2.67	0.77	38.11
<i>Quercus</i>	<i>ilex</i>	132.4	1.54	0.70	44.20
<i>Rhamnus</i>	<i>lycioides</i>	75.2	0.20	0.52	58.93
<i>Rosmarinus</i>	<i>officinalis</i>	70.4	0.29	0.80	44.89
<i>Salvia</i>	<i>lavandulifolia</i>	22.6	1.06	0.73	54.79
<i>Satureja</i>	<i>obovata</i>	44.22	0.26	0.51	84.19
<i>Satureja</i>	<i>spp</i>	46.2	0.10	0.44	63.22
<i>Stipa</i>	<i>offneri</i>	33.1	1.78	0.51	57.17
<i>Stipa</i>	<i>tenacissima</i>	78.7	15.37	0.68	35.22
<i>Teucrium</i>	<i>capitatum</i>	8.5	0.18	0.44	94.29
<i>Teucrium</i>	<i>polium</i>	13.7	0.05	0.31	115.67
<i>Thymus</i>	<i>longiflorus</i>	31.4	0.04	0.41	97.32
<i>Thymus</i>	<i>mastichina</i>	18.6	0.08	0.38	80.62
<i>Thymus</i>	<i>vulgaris</i>	21.5	0.03	0.36	89.28

Appendix S3 Predicting community structure in response to rainfall and biotic interactions

Table S2. Predicting community-weighted traits (CW) and community functional distance (FD) in response to annual rainfall (rain) and biotic interactions (estimated with the degree of spatial trait dispersion within communities, VarTi) using stepwise regression models.

CW-Height					CW-Leaf Area				CW-Leaf Thickness				CW-Specific Leaf Area			
Model r ²	0.35				0.75				0.60				0.35			
	df	est.	Fratio	Pvalue	df	est.	Fratio	Pvalue	df	est.	Fratio	Pvalue	df	est.	Fratio	Pvalue
Rain					1	-	9.31	0.004	1	+	25.9	0.0001				
Rain ²									1	-	13.6	0.0006	1	+	20.5	0.0001
PWD	1	+	25.1	0.0001	1	+	82	0.0001	1	+	7.3	0.009				
Rain x PWD					1	-	12.3	0.001	1	+	7	0.01	1	+	5.6	0.02
Rain ² x PWD									1	-	19.3	0.0001	1	+	10.6	0.002
error	46				44				42				44			

FD-Height					FD-Leaf Area				FD-Leaf Thickness				FD-Specific Leaf Area			
Model r ²	0.43				0.43				0.16				0.22			
	df	est.	Fratio	Pvalue	df	est.	Fratio	Pvalue	df	est.	Fratio	Pvalue	df	est.	Fratio	Pvalue
Rain	1	+	23.1	0.0001	1	-	2.31	0.1					1	+	4.2	0.04
Rain ²					1	-	4.16	0.04					1	+	3.9	0.05
PWD	1	+	9.9	0.003	1	+	16.6	0.0002	1	+	9.9	0.004	1	-	1.4	0.23
Rain x PWD													1	+	0.9	0.33
Rain ² x PWD													1	+	9.2	0.004
error	45				44								42			

Selected models have the lowest AIC. We indicated model parameter including model total r², df, Fratio the sign of the estimates (Est.) and P value.

Functional diversity enhances
the resistance of ecosystem
multifunctionality to aridity in
Mediterranean drylands

CAPÍTULO 2



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SUMMARY

- We used a functional trait-based approach to assess the impacts of aridity and shrub encroachment on the functional structure of Mediterranean dryland communities (functional diversity and community-weighted mean trait values [CWM]), and to evaluate how these functional attributes ultimately affect multifunctionality (i.e., the provision of several ecosystem functions simultaneously).
- Shrub encroachment (the increase in the abundance/cover of shrubs) is a major land cover change that is taking place in grasslands worldwide. Studies conducted on drylands have reported positive or negative impacts of shrub encroachment depending on the functions and the traits of the sprouting or non-sprouting shrub species considered.
- Functional diversity and CWM were equally important as drivers of multifunctionality responses to both aridity and shrub encroachment. Size traits (e.g., vegetative height or lateral spread) and leaf traits (e.g., specific leaf area and leaf dry matter content) captured the effect of shrub encroachment on multifunctionality with a relative high accuracy ($r^2=0.63$). Functional diversity also improved the resistance of multifunctionality along the aridity gradient studied.
- Maintaining and enhancing functional diversity in plant communities may help to buffer negative effects of ongoing global environmental change on dryland multifunctionality.

Keywords: aridity, drylands, functional biogeography, functional diversity, global change, multifunctionality, shrub encroachment, traits.

INTRODUCTION

Global change is altering biodiversity worldwide at an unprecedented rate, with important consequences for the functioning of natural ecosystems (Vitousek *et al.*, 1997; Chapin *et al.*, 2000). A response-effect framework based on plant functional traits has been proposed to explore the ecosystem-level consequences of local changes in biodiversity in response to ongoing global environmental change (global change hereafter; Lavorel & Garnier 2002; Suding *et al.*, 2008). This approach states that changes in the functional structure of communities can partly affect ecosystem functioning (“indirect” effects, sensu Suding *et al.*, 2008), although global change drivers also alter such functioning directly (Asner *et al.*, 2004; Austin *et al.*, 2004; Zepp *et al.*, 2007). The influential “mass-ratio hypothesis” (Grime, 1998) considers that the traits of dominant species largely determine the effects of plant communities on ecosystem functioning. As such, trait-based studies have mainly focused on community-weighted mean values (CWM hereafter; Garnier *et al.*, 2004; Violle *et al.*, 2007; Suding *et al.*, 2008; see de Bello *et al.*, 2010 for a review). However, global change drivers can also affect the variance of the trait distributions within communities (here defined as “functional diversity” [FD], see Laliberté & Legendre, 2010). High FD may reflect an increase in complementarity in resource use between species (Gross *et al.*, 2007b), thus improving ecosystem functioning (Díaz *et al.*, 2007).

Most studies investigating the relationship between the community functional structure and ecosystem functioning have studied one or a few ecosystem functions (see de Bello *et al.*, 2010 for a review). However, ecosystems are primarily valued because they provide multiple functions and services simultaneously (i.e. multifunctionality hereafter; Zavaleta *et al.*, 2010). Therefore assessing how global change drivers may

impact multifunctionality is crucial to understand the ecological consequences of global change (Reiss *et al.*, 2009; Zavaleta *et al.*, 2010; Cardinale *et al.*, 2012). In this context, high levels of FD have been hypothesized as crucial for maintaining high multifunctionality (Mouillot *et al.*, 2011).

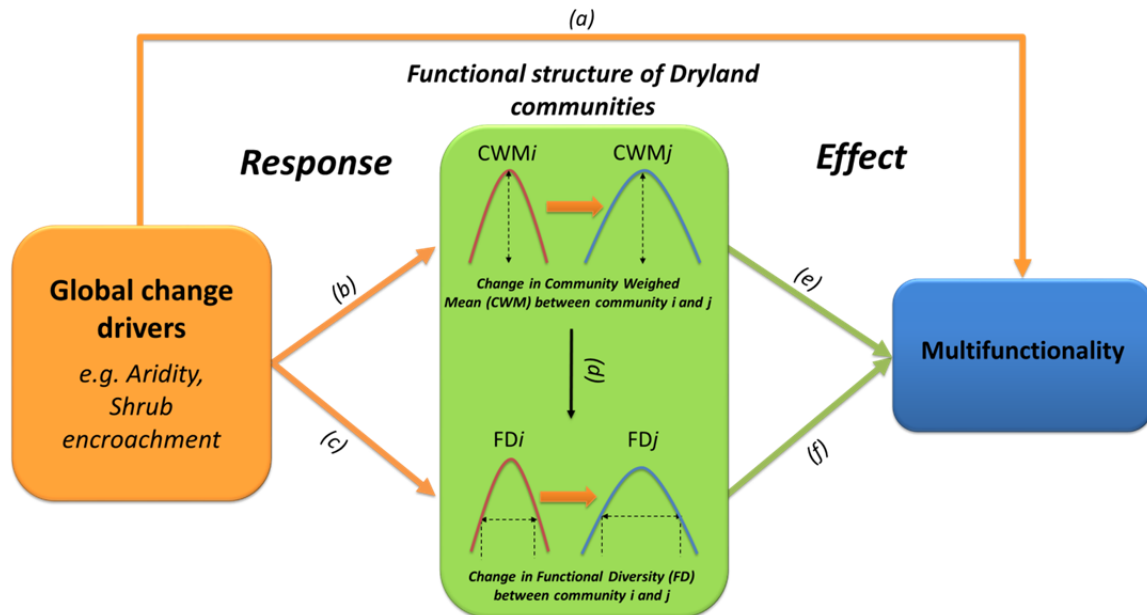


Fig. 1. Hypothetical relationships between aridity, shrub encroachment and the functional structure and multifunctionality of dryland communities. Aridity and shrub encroachment directly impact multifunctionality (a). The left part of the figure represents the response of the plant community to both variables (CWM: mean trait distribution [b]; FD: functional diversity [c]; and co-variation between CWM and FD [d]). Changes in community functional structure promote effects on multifunctionality via two non-exclusive mechanisms, mass-ratio process (e) and niche complementary (f). The red unimodal distributions represent a hypothetical trait distribution within a community *i* in a given environmental condition. The blue distributions represent the impact on trait distribution produced by contrasting amounts of aridity and shrub encroachment in a community *j* compared to community *i*. See Supporting Information Notes S1 for a detailed rationale of the different relationships depicted in the figure.

Arid, semi-arid and dry-subhumid ecosystems (drylands hereafter) are currently impacted by climate change (Maestre *et al.*, 2012b) and shrub encroachment (Eldridge *et al.*, 2011). Shrub encroachment, i.e. an increase in abundance and/or density of shrub species in grasslands (Schlesinger *et al.*, 1990), is a major land cover change that is occurring in drylands worldwide (Knapp *et al.*, 2008; Maestre *et al.*, 2009; Li *et al.*, 2013). This phenomenon has been found to promote dryland desertification by reducing

plant biomass and species richness (Knapp *et al.*, 2008), increasing fire risk (Mitchley & Ispikoudis, 1999) and enhancing soil erosion (Schlesinger *et al.*, 1990). However, other studies have found positive effects of shrub encroachment on the richness of different organisms and on ecosystem functioning (see Eldridge *et al.*, 2011 for a review). Maestre *et al.* (2009) hypothesized that the functional traits of encroaching shrubs relative to those of the grasses being replaced are key determinants of the outcomes of shrub encroachment. Size-related traits of shrub species might be particularly important, as the replacement of grass species by tall and large shrubs (i.e. sprouting shrubs) enhances species richness and ecosystem functions such as nutrient/carbon cycling and storage (Eldridge *et al.*, 2011; Quero *et al.*, 2013).

Climate change has been projected to increase aridity in drylands worldwide during this century (Feng & Fu, 2013). This can modify the traits of dominant shrub species, as well as the functional diversity within communities (Gross *et al.*, 2013), because aridity generally favors small stress-tolerant and slow-growing shrub species with particular leaf traits, e.g. thick evergreen leaves with low specific leaf area and high leaf dry matter content (Ackerly *et al.*, 2002). However, these species can be replaced in the most arid Mediterranean ecosystems by stress-avoidant species characterized by summer deciduous leaves and an opposite trait strategy (Ackerly *et al.*, 2002; Gross *et al.*, 2013). Changes in leaf trait values may have important consequences on ecosystem functioning, as they determine the rate of resource capture and utilization (Garnier *et al.*, 2004) as well as litter decomposition (Kazakou *et al.*, 2006). Also, increasing aridity may select for small shrub species (Gross *et al.*, 2013) and limit the ability of tall sprouting shrubs to enhance multifunctionality. Finally, increasing aridity may also alter multifunctionality by modifying the FD within drylands (Gross *et al.*, 2013) because: (i) a positive effect of species diversity on multifunctionality has been

found in global drylands (Maestre *et al.*, 2012a), and (ii) high FD has been hypothesized to improve the resistance of dryland ecosystems to aridity (Volaire *et al.*, 2014); this is so because FD could increase the probability that some species will survive if environmental conditions change, and thus could maintain ecosystem functioning (Díaz & Cabido, 2001).

In this paper, we evaluated how aridity and shrub encroachment affect the functional structure of Mediterranean drylands, and assessed how changes in their functional structure ultimately drive variations in multifunctionality. We also quantified the relative contribution of mass ratio (reflected by CWM) vs. niche complementarity (reflected by FD) processes on multifunctionality for multiple traits (size and leaf traits) using a confirmatory path analysis (Shipley, 2013; Fig. 1). We tested the following hypotheses: (i) mass ratio and niche complementarity processes are important drivers of multifunctionality (Mouillot *et al.*, 2011), (ii) high functional diversity will improve multifunctionality (Mouillot *et al.*, 2011), (iii) high functional diversity will minimize the negative effects of aridity on multifunctionality (Cardinale *et al.*, 2012; Maestre *et al.*, 2012a), and (iv) the effects of aridity on multifunctionality will be modulated by the traits of shrubs which determine the outcome of shrub encroachments.

MATERIALS AND METHODS

Study area

We surveyed 45 sites along an aridity gradient from central to south-east Spain (Fig. S1). Mean annual precipitation and temperature along this gradient ranged from 294 mm to 479 mm and from 12 °C to 18 °C, respectively. Aridity (1- aridity index [precipitation/potential evapotranspiration]; Delgado-Baquerizo *et al.*, 2013) values range from 0.57 to 0.76, and are strongly correlated to both annual mean precipitation ($R^2 = 0.97$) and temperature ($R^2 = 0.89$) in the studied sites. Climatic data were extracted from the WorldClim global database (Hijmans *et al.*, 2005), while data to calculate the aridity index were obtained from Trabucco and Zomer (2009). All the studied sites were located on south-facing slopes, with slope values ranging from 1° to 22° (measured in situ with a clinometer), and had soils derived from limestone (Lithic Calciorthid; Soil Survey Staff, 1994). Vegetation at these sites was either a grassland dominated by *Stipa tenacissima* or a shrubland dominated by obligate-seeder shrubs such as *Rosmarinus officinalis* (hereafter non-sprouting shrubs, Fig. S2). Within grasslands and shrublands, we selected sites with and without tall sprouting shrubs (such as *Quercus coccifera*; Fig. S2; Table S1).

Vegetation sampling

We established a 30 m x 30 m plot at each study site. Total plant cover within each plot was sampled by using four 30 m long transects located 8 m apart from each other, which were extended parallel to the slope. In each transect, the cover of every perennial species in 20 consecutive quadrats (1.5 m x 1.5 m) was visually recorded. We focused on perennial plants as they represent most of the plant biomass in drylands (Whitford,

2002), and their cover is a good predictor of ecosystem functioning in these areas (Maestre & Escudero, 2009; García-Gómez & Maestre 2011; Gaitán *et al.*, 2014). Species abundance per site was calculated as the sum of the cover measured in the 80 quadrats.

At each plot, we measured the traits of all the perennial plant species that accounted for at least 80% of the total plot cover, in a decreasing order of relative abundance. These measurements were conducted on ten randomly selected individuals per species during the peak of the vegetation growth season (spring). We assigned to each species and plot the average value of the individuals measured in that plot. In the case of the species for which we did not have local trait values we used the average trait values observed in the three nearest sites. Ten traits were measured following standardized protocols (Cornelissen *et al.*, 2003): (i) plant architecture traits: vegetative height VH (cm), lateral spread LS (cm²), branching density (BD, number of main stems) and ramification (Br, number of ramifications per stem). These traits are related to plant water use efficiency and/or competitive ability (Westoby *et al.*, 2002); (ii) leaf traits, including leaf area (LA, cm²), leaf length (LL, cm), leaf width (LW, cm) and leaf thickness (LT, mm), all reflecting light interception and water stress tolerance (Westoby *et al.*, 2002), and specific leaf area (SLA, cm² g⁻¹) and leaf dry matter content (LDMC, g g⁻¹), which correlate with plant relative growth rate and nutrient acquisition and utilization (Wright *et al.*, 2004).

Soil sampling and analyses

Soil cores (0–7.5 cm depth) were sampled during the peak of the dry season (July–August) under the canopy of five randomly selected *S. tenacissima* and *R. officinalis* individuals, and five other in randomly selected open areas devoid of vascular

vegetation. In those sites with sprouting shrubs, additional soil cores were sampled under the canopy of five randomly selected individuals of these shrubs. Hence, 10 or 15 soil samples, respectively, were collected per site.

Soil samples were sieved by a 2 mm mesh and air dried for one month prior to laboratory analyses. For each soil sample, the following variables were quantified as described in Maestre *et al.* (2012a) and Delgado-Baquerizo *et al.* (2013): organic C, pentoses, hexoses, total N, total available N, aminoacids, proteins, net potential mineralization rate, total P, available inorganic P, Olsen P (inorganic P – HCL 1M) and the activities of phosphatase and β -glucosidase. These variables constitute a good proxy for processes such as nutrient cycling, biological productivity, and buildup of nutrient pools, which are important determinants of ecosystem functioning in drylands (Whitford, 2002). Most of these processes are also considered to support ecosystem services, as other types of ecosystem services depend on them (MEA, 2005; Isbell *et al.*, 2011).

Data management

Community trait distribution

For each measured trait, we calculated two complementary indices of functional structure: CWM and FD. CWM corresponds to the mean trait value of a community weighted by the relative abundance of each species, and reflects the trait values of the most dominant plant species in a given community. It was calculated with the following equation (Violle *et al.*, 2007):

$$CWM_j = \sum_i^n p_{ij} T_{ij} \quad (\text{eqn. 1})$$

where p_{ij} is the abundance of the species i in the community j and T_{ij} the mean trait value of the species i in the community j .

Functional diversity quantifies the degree of trait dispersion within a community (adapted from Laliberté & Legendre, 2010). Calculated for each trait separately, FD is similar to the variance of the community trait distribution weighted by the relative abundance of each species within the community. It was calculated as:

$$FD_j = \sum_i^n p_{ij} \left(\frac{|T_{ij} - CWM_j|}{\sum_i^n |T_{ij} - CWM_j|} \right) \quad (\text{eqn. 2})$$

where p_{ij} is the abundance of the species i in the community j , T_{ij} is the mean trait value of the species i in the community j , and CWM_j is the community-weighted trait of the community j . High FD values suggest higher complementarity in resource used between species within a given community (Maire *et al.*, 2012).

Multifunctionality index

Multifunctionality was estimated from all the soil variables measured using the M index of Maestre *et al.* (2012a). To obtain a M value for each site, Z scores were first calculated for each of the 13 soil variables estimated at the scale of each $30 \text{ m} \times 30 \text{ m}$ plot surveyed. These estimates were obtained by using a weighted average of the mean values observed in bare ground and vegetated areas, and weighted by their respective cover at each plot (Maestre *et al.*, 2012a). Raw data were normalized prior to calculations; a square root transformation normalized most of the variables evaluated. Following this, the Z scores of the 13 soil variables were averaged to obtain M . This index provides a straightforward and easily interpretable measure of the ability of different communities to sustain multiple ecosystem functions simultaneously (Byrnes *et al.*, 2014). It is also statistically robust (Maestre *et al.*, 2012a), and is being

increasingly used when assessing multifunctionality (Quero *et al.*, 2013; Pendleton *et al.*, 2014; Wagg *et al.*, 2014; Bradford *et al.*, 2014). We acknowledge that the use of M may preclude a detailed analysis of how particular species differ in their importance for different functions (e.g., Hector & Bagchi, 2007; Gotelli *et al.*, 2011), and that in this index declines in a particular process/function can theoretically be compensated for by increases in another process/function (something that has been criticized in the past; e.g. Gamfeldt *et al.*, 2008). However, we did not find that particular sites with high values of a single or a few functions had consistently low values for other functions (Table S2). Moreover, the relatively large number of variables employed to calculate M makes it relatively robust to outliers or atypical values. We also acknowledge that having variables that are highly correlated among them could make them somewhat redundant (albeit this also simplifies the interpretation of the values of M). However, in our dataset only nine out of the 78 correlations between the soil variables evaluated had r values higher than 0.7, suggesting that redundancy is not very high within our data (Table S2). Finally, our estimates of M are highly related to other multifunctionality indices (Fig. S3). Thus, our results and conclusions are robust to the choice of metric used to estimate multifunctionality.

Statistical analyses

Functional variation between dryland communities

We conducted a principal component analysis (PCA) with Varimax rotation using the CWM and FD values of all the traits measured. These analyses were done separately for CWM and FD. We used the PCA coordinates in those components with an eigenvalue higher than one to measure the CWM and the FD of each community. This procedure allowed us to identify the plant strategy spectrum along which traits co-vary across

species and communities (Maire *et al.*, 2012). It has also the advantage to consider only independent variables in further analyses. CWM values were normalized using log-transformation before PCA analyses.

Community responses to aridity and shrub encroachment

We used a stepAICc procedure (following Grace, 2006) to evaluate the relationship between either CWM or FD (response variables) and aridity, abundance of sprouting shrubs and abundance of non-sprouting shrubs (predictors). Since the functional response to aridity is not necessarily linear (e.g., Gross *et al.*, 2013), a quadratic term was introduced if needed. The best model was selected based on the Akaike information criterion (AICc; Akaike, 1973). To evaluate the relative importance of aridity and shrub encroachment as drivers of the functional structure of the studied communities, we conducted a variance decomposition analysis based on the sum of squares of the selected models. Note that we also initially included slope in our models, as it has important effects on water availability in drylands (Gómez-Plaza *et al.*, 2001). However, this variable was not retained in any model based on an AICc model selection ($P > 0.05$ in all cases, data not shown). Therefore, we removed slope as a predictor in our models because it does not explain additional variation over that explained by aridity and functional structure.

Direct and indirect effects of aridity and shrub encroachment on multifunctionality

To test for relationships between CWM, FD, aridity, shrub encroachment, and multifunctionality, we conducted a confirmatory path analysis using a d-sep approach (Shipley, 2009; Laliberté & Tylianakis, 2012). This methodology allows relaxing some of the limitations of standard structural equation models, including non-normal data

distribution, non-linear relationships between variables and small sample sizes (Grace, 2006; Shipley, 2009). The d-sep approach is based on an acyclic graph that depicts the hypothetical relationships and independence claims between variables, where the latter are tested using the C statistic (See Fig. 1 and Supporting Information Notes S1 for the detailed rationale of our analyses). We tested three main alternative hypotheses, where multifunctionality responses to aridity and shrub encroachment are driven by: (i) CWM only (mass ratio hypothesis); (ii) FD only (niche complementary hypothesis); and (iii) the interplay of mass-ratio and niche complementarity processes (combined hypothesis). To simplify the *a priori* models used, a stepAICc procedure was first conducted to select the predictors that explained most of the variability found in multifunctionality (see Table S3). When several models were not rejected, we used the AICc procedure adapted for confirmatory path analysis to select the best model (Shipley, 2013). Finally, standardised path coefficients were used to measure the direct, indirect, and total effects of the predictors (Grace & Bollen, 2005).

As multiple traits can potentially act simultaneously on multifunctionality through contrasted mechanisms (e.g. mass ratio and niche complementarity in the case of the combined hypothesis), their respective effects on multifunctionality responses to aridity might be difficult to isolate. Thus, we ran a sensitivity analysis on the best selected model to highlight the relative contribution of multiple traits to the observed multifunctionality in response to aridity and shrub encroachments. To do so, we used the parameters of the best model to estimate multifunctionality values along the aridity gradient studied under different scenarios, which were created by manipulating the abundances of non-sprouting and sprouting shrubs. The first scenario considered only the effects of aridity by fixing the abundance of both shrub types at 0%. The second scenario focused on the interplay between aridity and encroachment by non-sprouting

shrubs. To do so, we fixed the abundance of sprouting shrubs at 0%, and simulated the effects of aridity on non-sprouting shrubs. In the third scenario we simulated the interactive effects of aridity and encroachment by sprouting shrubs. We fixed the abundance of sprouting shrubs at 30% (an average abundance that can be observed along the aridity gradient), and that of non-sprouting shrubs at 0% (to remove their effect from the simulation).

Principal component analysis and stepAICc analyses were carried out using JMP 11 (The SAS Institute, Cary, North Carolina, USA); d-sep analyses were conducted using the `lm` function in R (R Core Development Team, 2012).

RESULTS

Functional structure of studied communities

The CWM of the studied communities segregated along two PCA components, which accounted for 62% of the total variance found in the data (Fig. 2a, Table S4a and Table S5a). The first component (36% of the variance) separated communities according to their leaf trait values (hereafter CW-leaf trait), with SLA and Br being negatively correlated to LDMC, LL and LA. The first PCA component was negatively correlated with the abundance of *S. tenacissima* ($r^2=0.82$, P value < 0.001; dot scale in Fig. 2), and discriminated grasslands from shrublands. The second PCA component discriminated communities according to plant size traits (hereafter CW-size trait), with VH, LW and LS being negatively correlated to BD.

Similarly to what was observed with CWM, the FD of the studied communities was explained by the two first PCA components, which accounted for 55% of the total variance in the data (Fig. 2b, Table S4b and Table S5b). The first component (31% of the variance) discriminated communities according to the FD of traits related to plant size (hereafter FD-size trait), such as FD-Br, FD-LS, FD-LW and FD-VH. The second PCA component (24% of the variance) segregated communities according to the FD of leaf traits (hereafter FD-leaf traits), such as FD-SLA, FD-LDMC, and FD-LT.

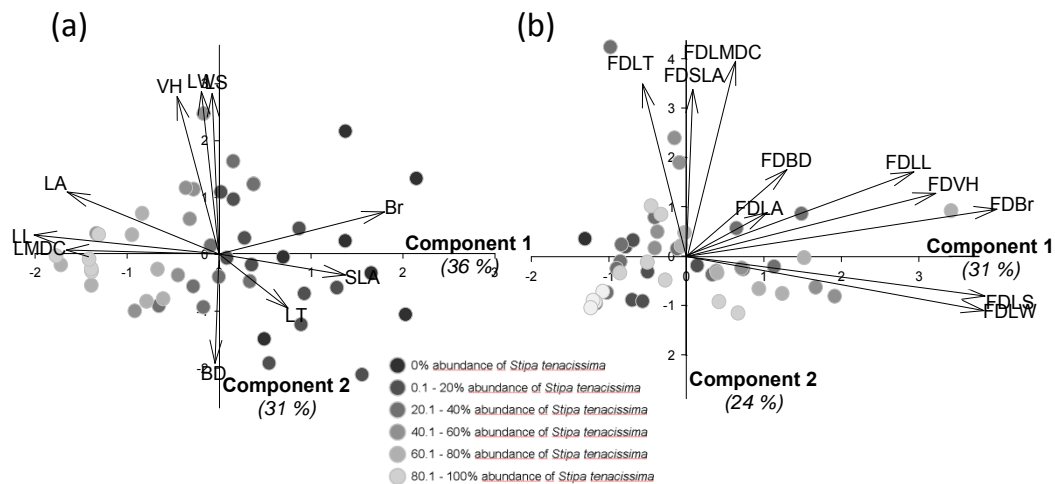


Fig. 2. Principal component analysis (PCA) of Community-Weighted Mean Trait (a) and Functional Diversity values (b). Light grey dots represent communities dominated by grass species, dark dots are communities dominated by shrub species. BD = branching density (number of main stems); Br = number of ramifications per stem; LA = leaf area; LDMC = leaf dry matter content; LL = leaf length; LS = lateral spread; LT = leaf thickness; LW = leaf width; SLA = specific leaf area; VH = vegetative height. For each component we indicate the % of variance explained. See Table S4a for correlations among Community-Weighted Trait values and Table S4b for correlations among Functional Diversity Trait values. Furthermore, see Table S5a for correlation between Community-Weighted Traits values and two mean components of a PCA (a) and Table S5b for the different Functional Diversity Traits value traits values and two mean components of the PCA (b).

Community response traits to aridity and shrub encroachment

The abundance of non-sprouting shrubs largely determined CW-leaf traits (73% of the explained variance, Table 1) and the communities dominated by these species had higher CW-SLA and -Br, and lower CW-LDMC, -LL, and -LA. These traits were also significantly impacted by aridity and the abundance of sprouting shrubs, although to a lesser extent (8% and 19% of the explained model variance, respectively; Table 1). A quadratic relationship was observed between aridity and CW-leaf traits (Table 1 and Fig. S4). In contrast, CW-size traits were mostly driven by the abundance of sprouting shrubs (Table 1). Communities with high CW-size traits were those dominated by tall sprouting shrubs.

Table 1. Results of the stepwise procedure to evaluate functional community responses to aridity and shrub encroachment for community weighed (CW) mean and functional diversity (FD) of leaf and size-related traits. We included a quadratic term when significant. Aridity: (1- aridity index [precipitation/potential evapotranspiration]); Sprouting: Sum of abundances of all sprouting shrubs; Non-sprouting: Sum of abundances of all shrubs except the sprouting shrubs; est: Estimate; DF: degree of freedom; % of r^2 : variance decomposition analysis based on the sum of squares. See Fig. S4 for details of the different relationships.

	CW-leaf traits					CW-size traits					FD-leaf traits					FD-size traits				
Model r^2	0.9					0.63					0.32					0.72				
	est	DF	Fratio	Pvalue	% of r^2	est	DF	Fratio	Pvalue	% of r^2	est	DF	Fratio	Pvalue	% of r^2	est	DF	Fratio	Pvalue	% of r^2
Aridity	-6.842	1	28.08	<0.0001	6.5						-7.755	1	5.39	0.0255	21.1					
Aridity ²	65.8141	1	8.35	0.0063	1.9						113.7509	1	3.72	0.0611	14.6					
Non-sprouting	0.0301	1	294.35	<0.0001	68.2						0.0041	1	0.78	0.3837	3	-0.0001	1	0	0.958	0
Non-sprouting ²	0.0002	1	19.6	<0.0001	4.5						-0.0005	1	10.52	0.0024	41.2	-0.0002	1	3.6	0.0651	2.8
Sprouting	0.0273	1	80.92	<0.0001	18.8	0.0523	1	76.93	<0.0001	100	-0.0207	1	5.11	0.0295	20	0.0817	1	78.1	<0.0001	60.1
Sprouting ²																-0.0019	1	48.19	<0.0001	37.1
Error		39					43					39					40			

The abundance of sprouting shrubs largely impacted FD-size traits (97% of the variance explained), whose values peaked at intermediate levels of non-sprouting shrub abundance (Table 1). Finally, variations in FD-leaf traits were driven by the interplay of aridity and shrub abundance (Table 1). A positive quadratic relationship between aridity and FD-leaf traits ($r^2=36\%$) indicated that the FD values of these traits peaked at low and high aridity levels. Sprouting shrubs tended to have a negative impact on FD-leaf traits ($r^2=20\%$), while non-sprouting shrubs increased FD-leaf traits ($r^2=44\%$).

Linking community response traits to effect traits on multifunctionality

The model including the combined effects of CWM and FD (combined hypothesis) was the only model not rejected by the data (Fig. 3 and Table S6). This model explained 62% of the variation in multifunctionality. Importantly, it highlighted that the effects of shrub encroachment on multifunctionality were mostly indirect via its effects on the functional structure of the plant community (Fig. 4).

While aridity had a direct effect on multifunctionality, it also had a large cascading effect by altering the functional structure of the studied communities. Aridity favoured the abundance of non-sprouting shrubs, which resulted in higher values of CW-leaf traits (Fig. 3). Shifting leaf trait values toward higher SLA had a strong adverse effect on multifunctionality. In contrast, the abundance of sprouting shrubs was independent from aridity. Increasing the abundance of these shrubs changed the value of CW-size traits towards higher plant height. Such an increase did not directly impact multifunctionality, but had an indirect effect via the changes it promoted in FD (Fig. 3). Increasing the average size of the species in the community augmented the FD of size traits, though decreased the FD of leaf traits, especially for intermediate values of CW-size traits (quadratic relationship). It must be noted that communities showing a high variance in size traits were also characterized by high FD values of leaf traits. Increasing FD values of both leaf and size traits generally increased multifunctionality. However, a significant interaction between aridity and FD leaf traits was observed (Fig. 3). This indicates that the effect of these traits on multifunctionality shifted from positive to negative under high aridity conditions. Finally, sprouting and non-sprouting shrubs did not have a direct effect on multifunctionality (Fig. 4), suggesting that all their effects on multifunctionality were explained by the functional traits measured.

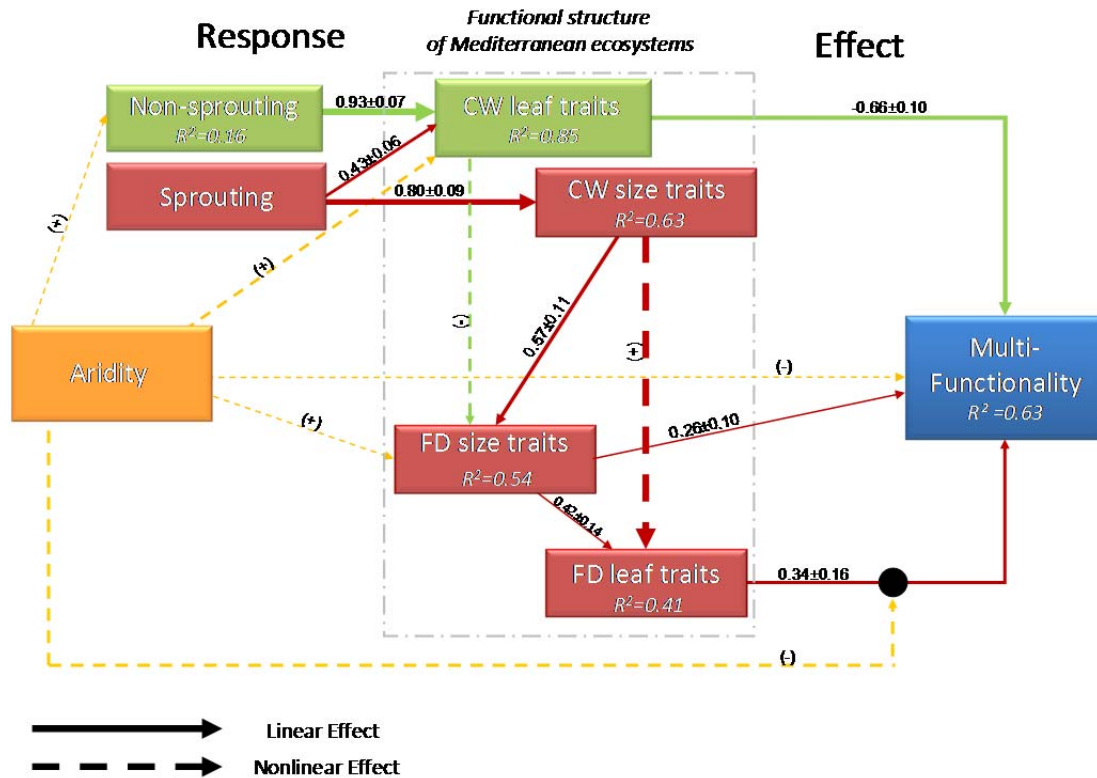


Fig. 3. Directed acyclic graph depicting the causal relationships between aridity (1- aridity index [precipitation/potential evapotranspiration]), the abundance of sprouting and non-sprouting shrubs, the functional structure of communities and multifunctionality. The width of each arrow is proportional to the standardized path coefficients. The continuous arrows represent linear effects and the discontinuous arrows non-linear effects (quadratic relationships). The black dot represents the interactive effects of aridity and FD leaf traits on multifunctionality. For the non-linear effects the sign of the quadratic relationships is shown (positive (+) or negative (-), whereas the standardized path coefficients for the latter are not available. CW = Community Weighted Mean, and FD = Functional Diversity.

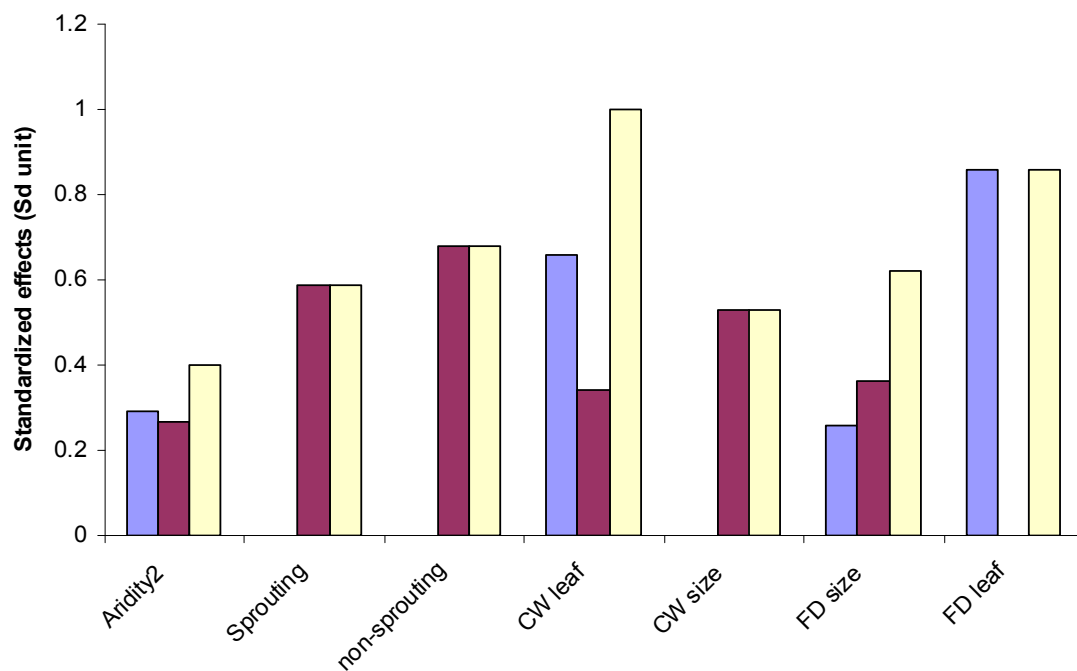


Fig. 4. Absolute effect sizes of the direct (blue bars), indirect (fuchsia bars), and total effects (sum of direct and indirect, yellow bars) of aridity (1- aridity index [precipitation/potential evapotranspiration]), the abundance of sprouting and non-sprouting shrubs and the functional structure of communities (CW = Community Weighted Mean and FD = Functional Diversity) on multifunctionality.

Model scenarios

In the sensitivity analyses of our final path model (Fig. 3), scenario 1 modelled the direct effect of aridity on multifunctionality as it had fixed zero abundance of both types of shrubs. In this case, multifunctionality directly decreased with increases in aridity (orange line, Fig. 5). In scenario 2, we modelled the effects of aridity on the abundance of non-sprouting shrubs (significant link in Fig. 3) and its consequences for multifunctionality. Increasing the abundance of non-sprouting shrubs augmented CW-leaf traits, and strongly decreased multifunctionality, along the aridity gradient (green line, Fig. 5). Finally, in scenario 3 we fixed the abundance of sprouting shrubs to 30% to maintain high values of functional diversity along the aridity gradient and to model its effects on multifunctionality. In this scenario, multifunctionality values remained

high for most of the aridity gradient, declining only under high aridity conditions (red line, Fig. 5).

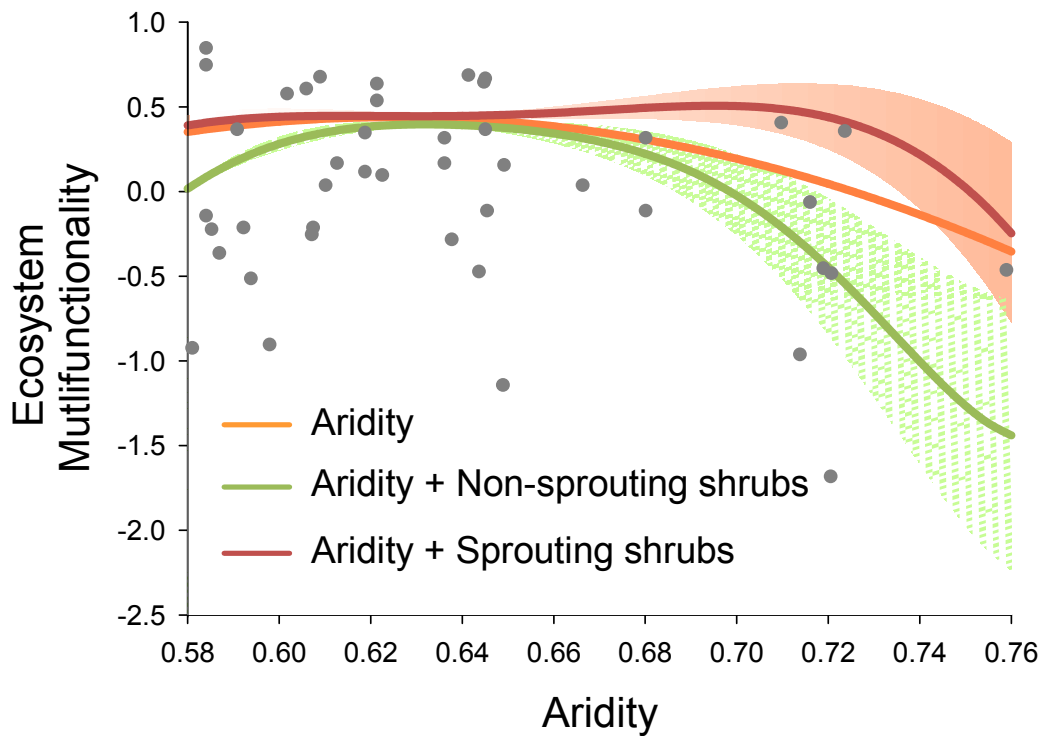


Fig. 5. Results of the sensitivity analysis carried out to evaluate the relationships between multifunctionality and aridity (1- aridity index [precipitation/potential evapotranspiration]) under different scenarios varying the abundance of shrub species. Scenario 1 (orange line) = grassland multifunctionality responses to aridity without shrubs; Scenario 2 (green line) = the abundance of non-sprouting shrubs is increased in grasslands and the abundance of sprouting shrubs is fixed at 0%; and Scenario 3 (red line) = the abundance of sprouting and non-sprouting shrubs is fixed at 30% and 0%, respectively. The shaded areas surrounding the lines are the 95% confidence intervals. The grey dots represent the position of the communities sampled around the aridity gradient.

DISCUSSION

Our study represents a first attempt to evaluate how multiple traits mediated dryland multifunctionality responses to two major global change drivers, i.e. aridity and shrub encroachments. Dryland multifunctionality largely depends on the functional structure of the plant communities. Our results indicate that mass ratio and niche complementarity processes, as reflected by CWM and FD, respectively, were equally important as drivers of multifunctionality responses to both aridity and shrub encroachment (Fig. 3). Specifically, the two key findings from our study are: (i) high FD improved the resistance of multifunctionality (i.e., the ability not to be affected by something, especially adversely; Oxford Dictionaries, 2014) to increases in aridity; (ii) two sets of simple plant functional traits (size traits like VH and leaf traits like SLA or LDMC) can capture the effect of shrub encroachment on multifunctionality with a relative high accuracy. Thus, this set of traits can be particularly helpful to identify when and where shrub species affect multifunctionality positively or negatively, and to clarify the contrasted results previously found in the literature regarding the effects of shrub encroachment on ecosystem functioning (Eldridge *et al.*, 2011).

Functional diversity enhances multifunctionality in drylands

Functional diversity within dryland communities improved ecosystem multifunctionality, and accounted for a large fraction of the variation across communities (42% of the effect on multifunctionality, Fig. 4). This result contrasts with studies conducted in more mesic ecosystems, which highlighted the importance of CWM as a driver of ecosystem functioning (Garnier *et al.*, 2004; Díaz *et al.*, 2007; Mokany *et al.*, 2008). However, most studies addressing the relationship between FD

and ecosystem functioning conducted so far have considered single ecosystem functions (e.g. productivity, Garnier *et al.*, 2004 or soil carbon, Laliberté & Tylianakis, 2012, see also de Bello *et al.*, 2010 for a review). Our results suggest that FD and the associated niche complementarity might be particularly important when considering multiple ecosystem processes simultaneously (Mouillot *et al.*, 2011).

In temperate ecosystems, the effect of high FD on ecosystem functioning has been generally associated to higher resource acquisition rates (Van Ruijven & Berendse, 2005) and resource use efficiency (Gross *et al.*, 2007a), temporal niche variability (Maire *et al.*, 2012) and plant soil feedbacks (Van der Heijden *et al.*, 2008). While future experiments are needed to identify the underlying mechanisms supporting the positive relationship between FD and ecosystem multifunctionality reported here, our results suggest that FD may improve multifunctionality in drylands via two distinct pathways:

1. Increasing the functional diversity of size traits can lead to regular spatial distributions of plants according to their size (Gross *et al.*, 2013), with tall individuals being regularly spaced between each other. Such spatial distributions, which are characteristic of dryland communities (Fowler, 1986), can limit runoff and maximise soil infiltration and heterogeneity (Valentin *et al.*, 1999), thus enhancing species diversity (Soliveres *et al.*, 2011) and maximising plant growth and ecosystem functioning (Puigdefábregas *et al.* 1999).
2. High leaf trait diversity indicates the occurrence of contrasting leaf strategies (Westoby *et al.*, 2002) commonly found in Mediterranean systems (e.g., stress avoidance vs. tolerance; Ackerly *et al.*, 2002; Freschet *et al.*, 2011). Differences in the leaf strategy of co-occurring species may have strong positive effects on ecosystem processes such as productivity (Gross *et al.*, 2007a), carbon cycling

(Milcu *et al.*, 2014), and litter decomposition rates (Bardgett & Shine, 1999; Cornwell *et al.*, 2008). For instance, some studies have shown that increasing the FD of litter positively influences microbial communities (Zak *et al.*, 2003) and litter decomposition rates (Vos *et al.*, 2013), two potentially important factors for maintaining and improving dryland multifunctionality.

Multiple traits mediate the impact of mass ratio processes on multifunctionality

By considering multiple traits, our study showed that the outcomes of shrub encroachment can be explained by size-related and leaf traits. Shrubs species (such as *Q. coccifera*) had a positive cascading effect on multifunctionality, which was mediated by increasing CW-size trait values (Fig. 3), in accordance with Maestre *et al.* (2009). Increasing plant size in dryland communities has been shown to be strongly associated with an increase in FD size traits locally, and with a high spatial heterogeneity of plant biomass within communities (Gross *et al.*, 2013), two features that can have potential positive effects on ecosystem functioning as discussed above. Maestre *et al.* (2009) showed how large *Quercus* species can increase the availability of local soil resources under their canopy in semi-arid *S. tenacissima* grasslands. The positive effects of these shrubs on local resources have been shown to increase species diversity of the whole community (Maestre *et al.*, 2009; Soliveres *et al.*, 2011), an important parameter reinforcing the positive effect of sprouting shrubs on multifunctionality (Quero *et al.*, 2013). Our results complement previous findings by illustrating how sprouting shrubs can enhance functional diversity within dryland communities, ultimately affecting multifunctionality.

CW-leaf trait values increased with an increase in the abundance of non-sprouting shrubs (Table 1 and Fig. S4). This had a negative impact on

multifunctionality, particularly in the most arid part of the gradient. The negative effect of fast growing species on multifunctionality can be explained by a negative plant soil feedback, as suggested by Garnier *et al.* (2004). Negative relationships between SLA and soil nutrient contents have previously been found in Mediterranean French grasslands (Garnier *et al.*, 2004) and along successional vegetation stages, where fast-growing species are replaced by slow-growing species (Berendse, 1990). Higher growth and nutrient acquisition rates may accelerate nutrient uptake from the soil (Lavorel & Garnier, 2002). At the same time, plants with higher SLA may produce litter with higher decomposition rates (Kazakou *et al.*, 2006). Together with the reduction of litter accumulation per unit of soil surface, these effects may accelerate nutrient loss at the scale of the whole ecosystem (Garnier *et al.*, 2004). This may be particularly true in the most arid part of the aridity gradient, where the typical characteristics of the semi-arid Mediterranean climate are worsened. For instance, the high variability of interannual precipitation distribution promotes increases in water runoff during short periods (Martínez-Mena *et al.*, 2001) and increases soil erosion that might accelerate nutrient loss (Martínez-Mena *et al.*, 2002). In addition, the negative effect of fast growing summer deciduous species on multifunctionality can be amplified via an effect on FD size traits (e.g. the negative link between CW-leaf traits and FD-size traits in Fig. 3). Summer deciduous species with a stress avoidance strategy can outcompete the more stress-tolerant grass and shrub species (Gross *et al.*, 2013) by producing allelopathic compounds (as has been found for species such as *Artemisia herba-alba*, Escudero *et al.*, 2000). Competition between fast- and slow-growing species may decrease the abundance of slow-growing sprouting shrubs and modify the size and spatial distribution of plant biomass within communities (Gross *et al.*, 2013). This situation may decrease the positive effects of sprouting shrubs on functional diversity,

accelerating species loss and affecting the functioning of the whole ecosystem (Maestre *et al.*, 2009).

Importance of functional diversity for ecosystem resistance to increasing aridity

The sensitivity analysis allowed us to explore how aridity interplays with plant functional community structure to determine multifunctionality (Fig. 5). While aridity had a direct detrimental effect on multifunctionality (scenario 1, Fig.5, Delgado-Baquerizo *et al.*, 2013), this negative effect was further reinforced by the increase in abundance of non-sprouting shrubs, as favoured by increasing aridity (scenario 2, Fig. 5). Moreover, we found an interactive effect of aridity and FD-leaf traits on multifunctionality (Fig. 3), suggesting that the effects FD-leaf traits shifted from positive to negative as aridity increased. At low aridity levels, high FD-leaf traits may reflect the coexistence between fast growing species characterized by perennial leaves (e.g. *Brachypodium retusum*), and stress-tolerant shrub or grass species (Frenette-Dussault *et al.*, 2012) that maximised ecosystem multifunctionality. In contrast, under high aridity conditions, the increase in FD-leaf traits observed reflected the increasing in abundance of non-sprouting shrubs (see selection effect in Loreau & Hector, 2001), characterized by high value of leaf traits (i.e. fast growing species with summer deciduous leaves, Gross *et al.*, 2013) that may negatively affect ecosystem functioning.

An important result of our study was that high FD (enhanced by the occurrence of sprouting shrubs in grasslands) strongly delayed the collapse of multifunctionality under high aridity conditions. This was suggested by our sensitivity analysis (Fig. 5) where high FD size traits were generally able to buffer the negative effects of aridity on multifunctionality, hence increasing the ecosystem resistance to aridity. Our results agree with previous experimental studies showing how higher species or functional

diversity can improve ecosystem resistance to global change drivers such as climate or land use changes (Hooper *et al.*, 2005; Isbell *et al.*, 2011; Cardinale *et al.*, 2012). Understanding how the attributes of biotic communities mediate the resistance of ecosystem structure and functioning to global change drivers is a major current ecological research. By identifying how fundamental attributes of biotic community predict ecosystem multifunctionality, our findings can be particularly useful for developing mechanistic models aiming to predict ecosystem resistance to climate change in drylands, which will increase the degree of aridity experienced by these ecosystems worldwide (Feng & Fu, 2013).

We standardized our sampling design by selecting sites with similar soil, slopes and aspect (south-facing slopes). Local variation in topo-edaphic conditions could however alter plant community structure (Fonseca *et al.*, 2000; Gross *et al.*, 2008) and multifunctionality. For instance, while we did not find any significant effect of slope on multifunctionality, other local factors such as slope aspect, soil texture or bedrock type could affect water availability (Fonseca *et al.*, 2000; Gómez-Plaza *et al.*, 2001; Delgado-Baquerizo *et al.*, 2013). Evaluating how local topo-edaphic factors interplay with climatic/land use factors to determine the functional structure of dryland communities and their effect on multifunctionality represents an important research front for the future.

CONCLUDING REMARKS

Our work suggests that the functional traits of dominant species and their diversity within communities modulate changes in multifunctionality in Mediterranean ecosystems along gradients of aridity and shrub encroachment. We showed that maintaining and enhancing FD (promoted by sprouting shrubs) in these ecosystems may help to buffer negative effects of climate change on multifunctionality. We also identified key traits that can predict accurately the outcome of shrub encroachment. Our results contribute to resolve the existing debate in the literature on the contrasted effects of shrub encroachment in drylands worldwide (e.g. Schlesinger *et al.*, 1990; Maestre *et al.*, 2009). On the one hand, traits related to the size of the plant species reflected the abundance of sprouting shrubs, which positively feedback on multifunctionality via their positive effect of FD. On the other hand, leaf traits such as SLA were related to the abundance of non-sprouting shrubs, which negatively impacted multifunctionality (particularly at the driest part of the aridity gradient studied). These results suggest that high values of SLA may typify those shrub species that are commonly associated with land degradation and desertification in drylands (Eldridge *et al.* 2011).

Our results can be used to develop specific trait-based management and restoration programs (Sandel *et al.*, 2011; Laughlin, 2014) aiming to buffer the effects of climate change and shrub encroachment on multifunctionality. For instance, reintroducing/favouring the development of plants with low SLA and/or large size, such as sprouting shrubs, and enhancing local functional diversity would reverse or limit the negative effects of increasing aridity and seasonal fast growing summer deciduous plant species on multifunctionality.

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SUPPORTING INFORMATION

Supporting Information Notes S1. Rationale of the different relationships depicted in Figure 1.

In the main text, we proposed a conceptual figure (Fig. 1) that summarizes causal relationship between aridity, shrub encroachment, dryland functional community structure and multifunctionality. This figure is adapted to drylands from the “response effect framework” developed by Lavorel & Garnier (2002). Below we describe the *a priori* justifications for all links (arrows) leading to endogenous variables (box with one or more arrows leading to it) present in Fig. 1 in the main text.

While aridity can directly alter ecosystem functioning (arrow a; Delgado-Baquerizo *et al.*, 2013), the response-effect framework states that ecosystem responses to global change drivers are largely mediated by ‘indirect effects’ via changes in functional community structure, i.e. both community weighted mean (CWM) (Suding *et al.*, 2008) and functional diversity (Díaz *et al.*, 2007). We first detail how aridity and shrub encroachment may impact the functional structure of dryland communities via two non-exclusive mechanisms (habitat filtering, Keddy, 1992; Maire *et al.*, 2012 and niche differentiation, Maire *et al.*, 2012) (RESPONSE), then explain how those changes may cascaded on multifunctionality (EFFECT). Justifications for each arrow are grouped together for each endogenous variable.

RESPONSE

Arrow b: In drylands, aridity has been reported to decrease the size of the plants and to select species with slow growth rate (Cornwell & Ackerly, 2009; Freschet *et al.*, 2011). However, for extreme aridity values, slow-growing and stress-tolerant species can be replaced by fast growing summer deciduous species (stress avoidance species; (Freschet

et al., 2011; Gross *et al.*, 2013). Shrub encroachment in grasslands impacts on the functional structure of drylands by increasing plant size (Eldridge *et al.*, 2011), especially when encroachers are sprouting shrub species (Quero *et al.*, 2013). Shrub encroachment can also impact leaf traits such as specific leaf area and C:N ratio compared to grasslands. The impact in leaf traits depends on the species implies in shrub encroachment, i.e. *Quercus coccifera* decrease C:N ratio compared to *Stipa tenacissima* (dominant species in grassland) (Ferran *et al.*, 2005).

Arrow c: aridity can favor high functional diversity (FD) by promoting contrasted functional strategies to deal with stress, i.e. stress avoidance vs. stress tolerant species.

Arrow d: co-variation between changes in CWM and FD can be observed in drylands due to the effect of biotic interactions (Gross *et al.*, 2013). High functional diversity can occur because dominant competitors with contrasted functional traits values can spatially coexist within communities (Gross *et al.*, 2013). Facilitation has also been proposed to promote high FD (Gross *et al.*, 2009) since well adapted dominant plant species promote the persistence of mal-adapted plants under their canopy (Valiente-Banuet *et al.*, 2006; Butterfield & Briggs, 2011; Gross *et al.*, 2013). For instance, tussock grass species such as *Stipa tenacissima* and tall sprouting shrubs (e.g. *Quercus coccifera*) have been shown to increase species richness (Maestre *et al.*, 2009; Soliveres *et al.*, 2011; Quero *et al.*, 2013) and functional diversity (Gross *et al.*, 2013) in drylands as a result of facilitation (Maestre *et al.*, 2001; García-Fayos & Gasque, 2002; Amat *et al.*, 2014).

EFFECT

Arrow e: in contrast, sprouting shrubs have been shown to increase multifunctionality because their particular functional traits maximize multiple processes such as water acquisition, water use efficiency, litter accumulation, and carbon storage (Maestre *et al.*,

2009; Eldridge *et al.*, 2011). Non-sprouting shrubs have been shown to decrease multifunctionality, and in some cases accelerate desertification processes (Kefi *et al.*, 2007; Quero *et al.*, 2013). However, which traits mediate these effects is currently unknown.

Arrow f: recent studies have shown that species diversity may increase ecosystem multifunctionality (e.g. Gamfeldt *et al.*, 2008; Zavaleta *et al.*, 2010; Maestre *et al.*, 2012; Vos *et al.*, 2013). Mediterranean ecosystem are generally characterized by high functional diversity respective to other biomes (Freschet *et al.*, 2011) which, together with the fact that functional diversity may increase ecosystem multifunctionality, suggests that functional diversity might be important in driving multiple ecosystem processes in drylands.

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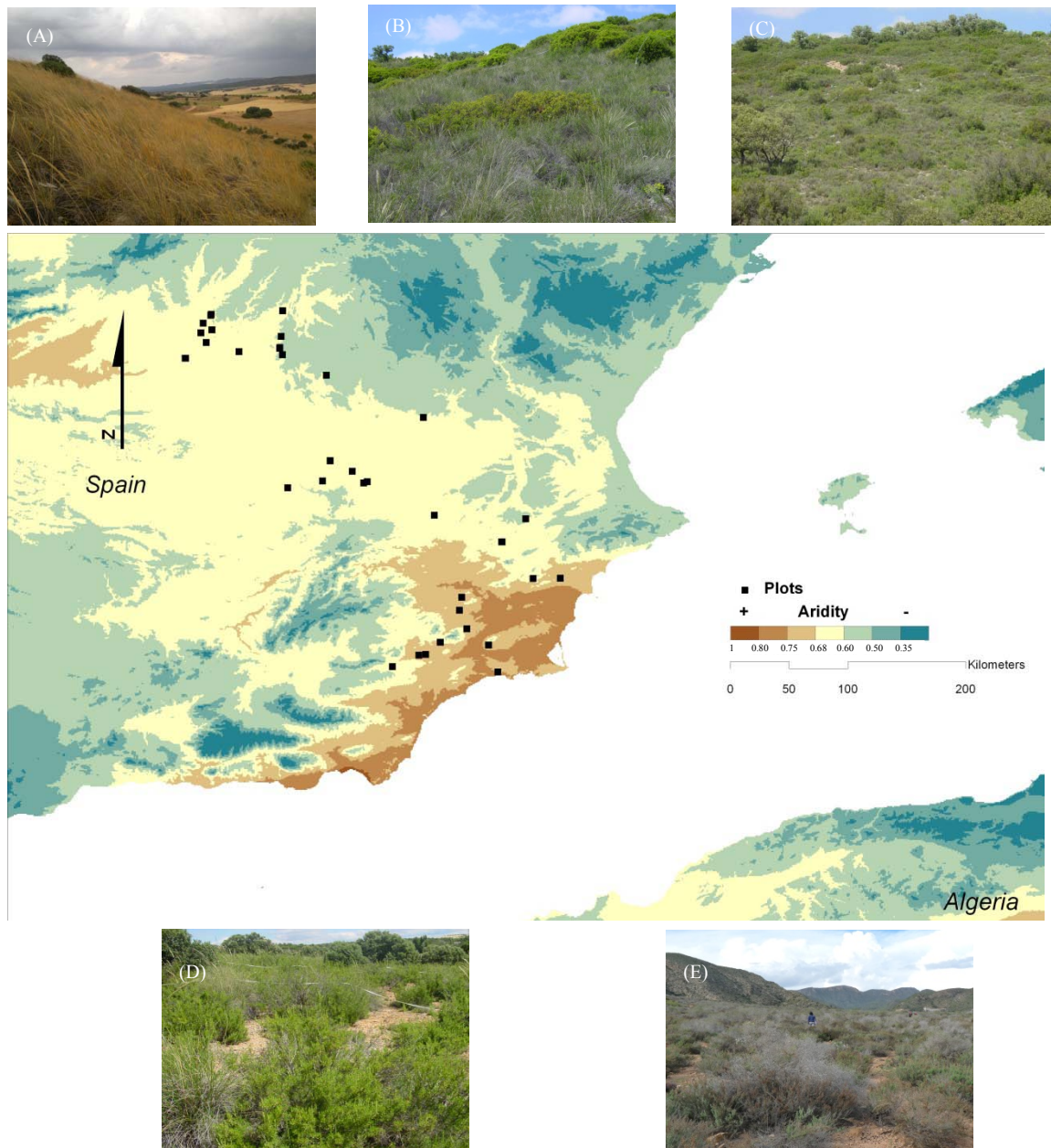


Figure S1. Map with the location of the study sites along the aridity gradient evaluated. Pictures indicate examples of the different types of communities along the aridity gradient (1-aridity index [precipitation/potential evapotranspiration]): (A) Grassland dominated by *Stipa tenacissima*; (B) Grassland with sprouting shrubs; (C) Shrublands dominated by *Rosmarinus officinalis* with sprouting shrubs; (D) Shrublands dominated by *Rosmarinus officinalis* with *Stipa tenacissima*; (E) Shrublands dominated by summer deciduous shrubs species.



<p>Sprouting shrubs:</p> <ul style="list-style-type: none">- Resprout after a fire- Long-lived species- They have a well-developed root system <p>because they occupy the same space for a long time.</p> <p>Examples of species:</p> <p><i>Quercus coccifera</i>, <i>Juniperus oxycedrus</i> <i>Pistacia lentiscus</i> and <i>Rhamnus lycioides</i></p>  <p><i>Quercus coccifera</i></p>	<p>Non-sprouting shrubs:</p> <ul style="list-style-type: none">-Obligate seeders (survive fire as a dormant seed pool in the soil)-Suffer mortality after fires- Do not have a well-developed root system <p>Examples of species:</p> <p><i>Rosmarinus officinalis</i>, <i>Artemisia herba-alba</i> and <i>Globularia alypum</i></p>  <p><i>Rosmarinus officinalis</i></p>
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Figure S2. Differences between non-sprouting and sprouting shrubs (Information based on Keeley & Zedler 1978).

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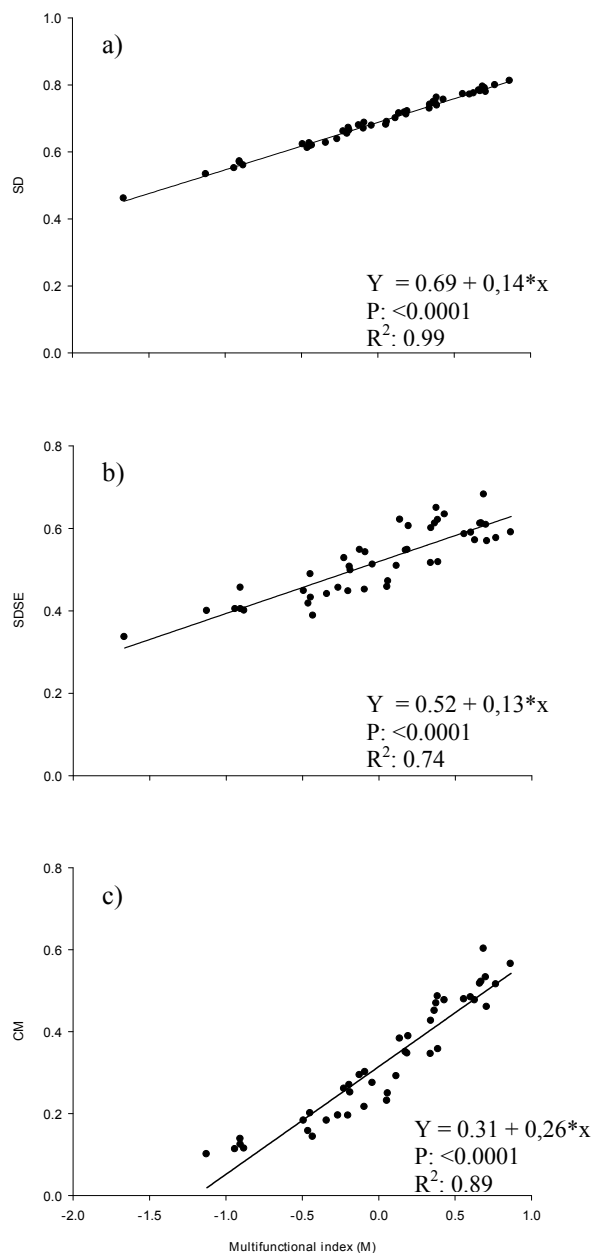


Figure S3. Relationships between our multifunctionality index based on the average of Z-scores of ecosystem functions and other multifunctionality indices: a) the average of multiple functions, previously standardized by dividing by the maximum (SD, Mouillot *et al.*, 2011); b) this average minus the standard deviation (SDSE, Pasari *et al.*, 2011); and (c) the cross-multiplied ten soil variables previously standardized by dividing by the maximum (CM, Bowker *et al.*, 2013).

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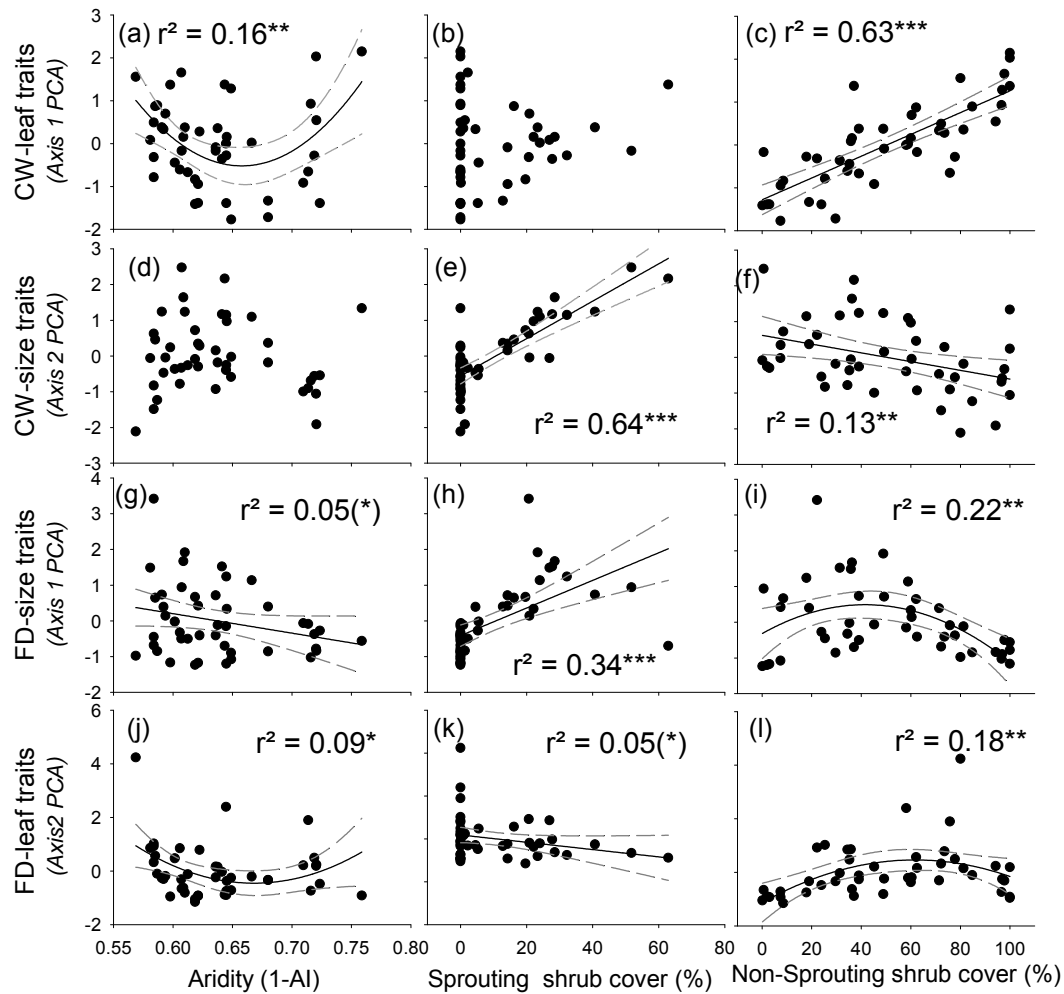


Figure S4. Responses to aridity (1- aridity index [precipitation/potential evapotranspiration]) and shrub encroachment of community weighed mean traits (CWM) and functional diversity (FD) evaluated with leaf and size related traits. Co-variations among traits are shown in Fig. 2 for CWM and FD. Community responses to: aridity (1- Aridity Index [AI]) are shown in panels (a, d, g, j); responses to shrub encroachment separating sprouting shrub and non-sprouting shrub are shown in panels (b, e, h, k) and (c, f, i, l) respectively. The black solid line indicates significant ($P < 0.05$) relationships; the grey dash lines show 95% confidence intervals in these cases. We provided the R^2 of the linear regression in each panel. (*) $P < 0.09$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table S1. Main characteristics of the study sites. Climate variables were extracted from the Worldclim global database (www.worldclim.org) and Global Aridity Index (Trabucco and Zomer, 2009).

COD	LAT	LON	ELE	SLO	AMT	RAI	AI	ARIDITY	CBA	TCT	NSS	SS
1	37°35'35.04"N	1°13'56.57"W	76	4.25	17.8	294	0.241	0.759	55.750	44.250	100.000	0.000
2	37°47'57.23"N	1°18'13.80"W	405	21.00	16.5	339	0.277	0.724	60.875	36.792	23.920	5.234
3	38°03'55.91"N	1°31'37.33"W	445	7.75	16.1	341	0.279	0.721	65.568	34.432	94.271	1.301
4	38°09'54.31"N	1°30'32.36"W	446	14.00	16.0	345	0.280	0.720	70.417	28.500	100.000	0.000
5	37°55'23.46"N	1°28'12.62"W	443	2.34	16.1	344	0.281	0.719	41.208	57.875	77.811	0.000
6	37°43'36.22"N	1°47'04.56"W	476	9.15	15.9	339	0.284	0.716	48.250	46.708	96.607	0.000
7	37°43'16.79"N	1°50'13.15"W	487	0.50	15.9	341	0.286	0.714	41.208	27.083	75.812	0.000
8	37°48'01.14"N	1°18'19.54"W	406	15.75	16.1	353	0.290	0.710	50.917	36.750	45.242	0.101
9	37°49'16.16"N	1°40'25.49"W	654	2.88	15.1	378	0.320	0.680	50.250	48.750	18.977	12.824
10	37°49'17.92"N	1°40'20.26"W	651	1.25	15.1	378	0.320	0.680	55.083	44.917	29.648	0.000
11	38°18'30.88"N	0°57'47.68"W	504	15.40	15.5	398	0.334	0.666	47.944	27.621	58.922	23.991
12	39°02'54.74"N	2°13'49.56"W	779	4.50	13.7	415	0.351	0.649	53.167	46.500	7.421	0.000
13	37°38'02.03"N	2°02'22.61"W	863	15.96	14.4	405	0.351	0.649	62.146	37.686	96.874	0.000
14	39°07'39.03"N	2°20'42.50"W	780	11.75	13.8	420	0.355	0.645	58.333	40.583	60.038	22.109
15	39°59'31.33"N	3°37'08.21"W	597	14.50	14.5	412	0.355	0.645	16.075	66.758	17.909	32.187
16	39°59'33.27"N	3°37'21.13"W	579	10.25	14.5	412	0.355	0.645	43.417	48.708	1.998	0.000
17	39°12'27.14"N	2°30'53.16"W	750	1.75	13.8	422	0.355	0.645	50.000	48.250	58.179	0.000
18	39°02'17.65"N	2°15'28.45"W	806	8.25	13.6	423	0.357	0.644	58.070	41.930	37.077	62.923
19	39°12'31.60"N	2°30'50.71"W	752	3.50	13.8	426	0.359	0.641	31.850	62.775	31.337	27.736
20	38°47'30.67"N	1°43'03.70"W	845	16.25	13.5	422	0.362	0.638	56.000	31.167	81.305	0.850
21	40°21'17.25"N	2°52'38.77"W	625	10.25	13.6	405	0.364	0.636	49.625	45.208	62.574	0.000
22	40°21'16.74"N	2°52'42.07"W	629	8.00	13.6	405	0.364	0.636	41.250	57.417	49.258	14.229
23	39°03'10.77"N	2°34'20.10"W	851	4.75	13.4	446	0.378	0.622	42.500	57.500	73.618	0.000
24	40°12'31.61"N	3°25'08.99"W	621	14.75	14.0	432	0.379	0.621	18.558	57.858	7.506	14.238
25	40°12'31.82"N	3°25'05.49"W	616	22.00	14.0	432	0.379	0.621	18.167	63.877	2.924	0.000
26	40°11'09.64"N	3°30'10.31"W	615	17.75	14.1	429	0.381	0.619	40.825	56.633	8.584	19.673

27	40°11'09.96"N	3°30'12.47"W	617	17.50	14.1	429	0.381	0.619	26.100	67.233	0.116	0.000
28	40°19'11.97"N	3°25'36.61"W	631	18.75	13.9	436	0.388	0.613	29.292	49.800	39.086	0.000
29	40°15'36.74"N	3°29'07.82"W	667	21.75	13.9	437	0.390	0.610	34.250	62.625	48.925	23.337
30	40°19'36.26"N	3°25'26.11"W	650	20.75	13.8	439	0.391	0.609	30.375	53.917	36.285	28.510
31	40°06'43.61"N	3°27'46.77"W	735	18.50	13.7	441	0.393	0.607	35.583	64.417	0.667	51.772
32	39°00'05.34"N	2°50'17.28"W	903	3.25	13.2	467	0.393	0.607	73.188	24.134	97.801	2.199
33	38°35'18.98"N	1°11'58.12"W	815	18.50	13.9	444	0.394	0.606	50.125	42.292	34.445	0.000
34	38°35'17.60"N	1°12'13.62"W	833	14.50	13.7	454	0.398	0.602	51.661	36.191	35.269	5.469
35	39°00'35.82"N	2°39'46.40"W	945	6.23	13.0	468	0.402	0.598	79.500	15.167	100.000	0.000
36	39°52'38.65"N	2°47'17.05"W	833	14.75	12.9	458	0.406	0.594	41.205	55.267	60.353	20.866
37	40°22'09.69"N	3°23'19.23"W	715	12.75	13.1	453	0.408	0.592	43.792	56.208	71.422	4.502
38	40°09'29.88"N	2°53'20.75"W	811	21.00	12.8	448	0.409	0.591	35.845	63.977	39.060	40.724
39	38°45'54.99"N	1°01'12.60"W	770	10.75	14.0	457	0.413	0.587	55.333	38.500	84.806	0.000
40	39°32'19.20"N	1°48'08.23"W	905	8.50	12.4	466	0.415	0.585	57.729	34.195	62.174	16.180
41	40°04'5.62"N	2°54'02.61"W	899	18.75	12.6	462	0.416	0.584	21.958	67.833	22.202	20.648
42	40°04'24.98"N	2°53'56.71"W	895	21.50	12.5	465	0.416	0.584	29.833	60.500	25.365	0.000
43	39°51'42.62"N	2°32'37.48"W	870	14.75	12.5	479	0.416	0.584	49.750	50.000	72.257	0.000
44	40°01'04.90"N	2°52'45.67"W	878	20.00	12.5	470	0.419	0.581	43.178	56.155	35.845	26.909
45	37°53'32.49"N	1°42'11.46"W	1024	21.75	13.1	468	0.431	0.569	42.919	36.424	80.084	0.000

COD = Code of the plot, LAT = latitude (WGS84 datum), LON = longitude (WGS84

datum), ELE = elevation (m), SLO = slope (°), AMT = annual mean temperature, RAI =

annual precipitation, AI = Aridity index (precipitation/potential evapotranspiration),

ARIDITY = 1-AI, CBA= Cover of bare areas (%), TCT = total perennial cover (%),

NSS= Relative cover of non-sprouting shrubs (%), SS= Relative cover of sprouting shrubs (%).

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Table S2. Results of Pearson correlation coefficients between the different soil variables (our surrogates of ecosystem functions) used to calculate the multifunctionality index.

	ORC	HEX	PEN	BGL	TP	P_HCL	AVP	FOS	TON	AVN	AMI	PRO
ORC												
HEX	0.45**											
PEN	0.03	0.02										
BGL	0.48**	0.64**	-0.20									
TP	-0.09	-0.25	-0.11	-0.03								
P_HCL	-0.41**	-0.21	-0.24	0.07	0.12							
AVP	0.00	0.16	-0.29	0.36*	0.45**	0.10						
FOS	0.72**	0.55**	-0.03	0.67**	-0.09	-0.17	0.10					
TON	0.88**	0.46**	-0.10	0.64**	0.10	-0.29	0.14	0.76**				
AVN	0.52**	0.60**	-0.06	0.84**	-0.01	-0.12	0.24	0.63**	0.69**			
AMI	0.52**	0.72**	-0.21	0.80**	-0.03	-0.10	0.28	0.60**	0.66**	0.82**		
PRO	0.17	0.64**	0.00	0.55**	-0.19	-0.13	0.07	0.30*	0.26	0.47**	0.42**	
MIN	0.40**	0.50**	-0.29	0.71**	0.10	0.06	0.35*	0.47**	0.53**	0.61**	0.77**	0.16

Significance levels are as follows: * $p < 0.05$ and ** $p < 0.01$.

ORC: organic carbon; HEX: hexoses; PEN: pentoses; BGL: β -glucosidase extracellular activity; TP: total phosphate; P_HCL: Olsen phosphate (inorganic P – HCL 1M); AVP: available inorganic phosphate; FOS: phosphatase activity; TON: total nitrogen; AVN: total available nitrogen; AMI: aminoacids; PRO: proteins and MIN: net potential mineralization rate.

Table S3. Stepwise procedure to evaluate the responses of community weighed mean (CWM) and functional diversity (FD) evaluated with leaf and size related traits to aridity (1- aridity index [precipitation/potential evapotranspiration]) and shrub encroachment. We evaluated a quadratic term when it was significant. We also assessed the effect of slope on multifunctionality; this variable was not selected in any *a priori* models (data not shown) and thus was removed from the final analyses. Est: direction of relationship; DF: degree of freedom; % of r^2 : variance decomposition analysis based on the sum of squares.

	model mass ratio				model niche complementary				model combined hypothesis			
	(hypothesis i)				(hypothesis ii)				(hypothesis iii)			
Model r^2	0.46				0.25				0.63			
	Est	DF	Fratio	Pvalue	Est	DF	Fratio	Pvalue	Est	DF	Fratio	Pvalue
Aridity(Ari)	-2.4	1	3.2	0.0824	1.8	1	0.6	0.4394	-1.2	1	0.5	0.4817
Ari ²					-110.7	1	8.2	0.0069	-47.7	1	2.7	0.1090
CW-leaf traits	-0.4	1	32.8	<0.0001					-0.4	1	39.7	<0.0001
CW-size traits	0.1	1	4.8	0.0345								
Ari*CW-leaf traits												
Ari*CW-size traits												
Ari ² *CW-leaf traits												
Ari ² *CW-size traits												
FD-leaf traits					0.1	1	1.0	0.3213	0.2	1	4.3	0.0460
FD-size traits					0.2	1	5.2	0.0288	0.1	1	7.1	0.0111
Ari*FD-leaf traits					-1.8	1	1.2	0.2775	-2.1	1	3.3	0.0762
Ari*FD-size traits												
Ari ² *FD-leaf traits					-65.8	1	4.7	0.0368	-84.8	1	15.4	0.0004
Ari ² *FD-size traits												
error		41				38				37		

Table S4. Results of Pearson correlation coefficients among a) community-weighted trait values and b) functional diversity (FD) trait values.

a)	SLA	VH	LA	LT	LW	LMDC	Br	BD	LS
SLA									
VH	-0.30*								
LA	-0.49**	0.41**							
LT	0.05	-0.34*	-0.30*						
LW	-0.09	0.71**	0.40**	-0.28					
LMDC	-0.72**	0.17	0.56**	-0.33*	0.09				
Br	0.35*	-0.04	-0.56**	0.31*	0.13	-0.54**			
BD	0.22	-0.48**	-0.33*	0.18	-0.41**	0.00	-0.20		
LS	-0.10	0.78**	0.25	-0.24	0.83**	0.02	0.17	-0.32*	
LL	-0.54**	0.29	0.77**	-0.31*	0.19	0.70**	-0.83**	0.09	0.14

b)	FDSL	FVDH	FDLA	FDLT	FDLW	FDLMD	FDBr	FDBD	FDSL
FDSL									
FVDH	0.27								
FDLA	0.23	-0.05							
FDLT	0.43**	0.08	-0.02						
FDLW	-0.13	0.43**	0.25	-0.16					
FDLMD	0.35*	0.22	0.18	0.64**	-0.06				
FDBr	0.14	0.58**	0.06	0.06	0.58**	0.23			
FDBD	0.25	0.51**	-0.16	0.20	-0.07	0.21	0.27		
FDSL	-0.13	0.47**	0.01	-0.14	0.71*	-0.07	0.57**	0.26	
FDDL	0.27	0.25	0.43**	0.06	0.37*	0.45**	0.59**	-0.04	0.28

Significance levels are as follows: * $p < 0.05$ and ** and $p < 0.01$.

SLA = specific leaf area; VH = vegetative height; LA = leaf area; LT = leaf thickness; LW = leaf width; LDMC = leaf dry matter content; Br = number of ramifications per stem; BD = branching density (number of main stems); LS = lateral spread; LL = leaf length.

Table S5. Results of Pearson correlation coefficients between a) different community-weighted (CW) traits values and two mean components of a Principal Component Analysis (component 1: CW-leaf traits and component 2: CW-size traits); b) different functional diversity (FD) traits values and two mean components of a Principal Component Analysis (component 1: FD-size traits and component 2: FD- leaf traits).

a)	CW-leaf traits	CW-size traits
SLA	0.69**	-0.14
VH	-0.23	0.87**
LA	-0.77**	0.36*
LT	0.38*	-0.34*
LW	-0.08	0.90**
LMDC	-0.85**	0.02
Br	0.84**	0.23
BD	-0.02	-0.64**
LS	-0.01	0.89**
LL	-0.94**	0.08

b)	FD-size traits	FD- leaf traits
FDSL	0.02	0.74**
FDVH	0.69**	0.29
FDLA	0.19	0.20
FDLT	-0.14	0.78**
FDLW	0.84**	-0.22
FDLMDC	0.09	0.83**
FDBr	0.84**	0.21
FDBD	0.26	0.39**
FDLS	0.84**	-0.19
FDLL	0.57**	0.38*

Significance levels are as follows: * $p < 0.05$ and ** and $p < 0.01$.

SLA = specific leaf area; VH = vegetative height; LA = leaf area; LT = leaf thickness; LW = leaf width; LDMC = leaf dry matter content; Br = number of ramifications per stem; BD = branching density (number of main stems); LS = lateral spread; LL = leaf length.

Table S6. Conditional independence tests applied in the different hypothesis of the d-sep model implied by the hypothesized path models.

D-sep claim of	Model formula S	Ho	P	C	P of C
Independence				statistic	(df)
Mass ratio (hypothesis i)				43.01	0.058
					(30)
(NSS;SS) {ARI ² }	SS ~ NSS + ARI ²	NSS =0	0.0623		
(NSS; CWM2) {SS, ARI ² }	CWM2 ~ NSS + SS + ARI ²	NSS=0	0.3794		
(NSS; FD1) {ARI ² , CWM1 ² , CWM2}	FD1 ~ NSS + ARI ² + CWM1 ² + CWM2	NSS=0	0.5485		
(NSS; FD2) {ARI ² , CWM2 ² , FD1}	FD2 ~ NSS + ARI ² + CWM2 ² + FD1	NSS=0	0.428		
(NSS; M) {ARI ² , CWM1, CWM2}	M ~ NSS + ARI ² + CWM1 + CWM2	NSS=0	0.7582		
(ARI;SS) {Ø}	SS ~ ARI	ARI=0	0.1553		
(SS; FD2) {CWM2 ² , FD1}	FD2 ~ SS + CWM2 ² + FD1	SS=0	0.7035		
(SS; FD1) {ARI ² , CWM1 ² , CWM2}	FD1 ~ SS + ARI ² + CWM1 ² + CWM2	SS=0	0.4695		
(SS; M) {ARI ² , CWM1, CWM2}	M ~ SS + ARI ² + CWM1 + CWM2	SS=0	0.3703		
(ARI;CWM2) {SS}	CWM2 ~ ARI + SS	ARI=0	0.226		
(ARI; FD2) {CWM2 ² , FD1}	FD2 ~ ARI + CWM2 ² + FD1	ARI=0	0.4835		
(CWM1; CWM2) {SS, NSS, ARI}	CWM2 ~ CWM1 + SS + NSS + ARI	CWM1=0	0.1478		
(CWM1; FD2) {SS, NSS, ARI, CWM2 ² , FD1}	FD2 ~ CWM1 + SS + NSS + ARI + CWM2 ² + FD1	CWM1=0	0.7359		
(FD1; M) {CWM1 ² , CWM2, ARI ² }	M ~ FD1 + CWM1 ² + CWM2 + ARI ²	FD1=0	0.5956		
(FD2; M) {CWM1, CWM2 ² , ARI ² , FD1}	M ~ FD2 + CWM1 + CWM2 ² + ARI ² + FD1	FD2=0	0.0008		
Niche complementary (hypothesis ii)				86.34	<0.0001
					(30)
(NSS;SS) {ARI ² }	SS ~ NSS + ARI ²	NSS =0	0.0623		
(NSS; CWM2) {SS, ARI ² }	CWM2 ~ NSS + SS + ARI ²	NSS=0	0.3794		
(NSS; FD1) {ARI ² , CWM1 ² , CWM2}	FD1 ~ NSS + ARI ² + CWM1 ² + CWM2	NSS=0	0.5485		
(NSS; FD2) {ARI ² , CWM2 ² , FD1}	FD2 ~ NSS + ARI ² + CWM2 ² + FD1	NSS=0	0.428		

(NSS; M) {ARI ² , FD1, FD2+(AI*FD ²)+(AI2*FD ²)}	M ~ NSS + ARI ² + FD1 + FD2 + (AI*FD ²) + (AI2*FD ²)	NSS=0	<0.0001
(ARI;SS) {Ø}	SS ~ ARI	ARI=0	0.1553
(SS; FD2) {CWM2 ² , FD1}	FD2 ~ SS + CWM2 ² + FD1	SS=0	0.7035
(SS; FD1) {ARI ² , CWM1 ² , CWM2}	FD1 ~ SS + ARI ² + CWM1 ² + CWM2	SS=0	0.4695
(SS; M) {ARI ² , FD1, FD2+(AI*FD ²)+(AI2*FD ²)}	M ~ SS + ARI ² + FD1 + FD2 + (AI*FD ²) + (AI2*FD ²)	SS=0	0.1372
(ARI;CWM2) {SS}	CWM2 ~ ARI + SS	ARI=0	0.226
(ARI; FD2) {CWM2 ² , FD1}	FD2 ~ ARI + CWM2 ² + FD1	ARI=0	0.4835
(CWM1; CWM2) {SS,NSS, ARI}	CWM2 ~ CWM1 + SS + NSS + ARI	CWM1=0	0.1478
(CWM1;FD2) {SS,NSS, ARI, CWM2 ² , FD1}	FD2 ~ CWM1 + SS + NSS + ARI + CWM2 ² + FD1	CWM1=0	0.7359
(CWM1; M) {ARI ² , NSS, SS, FD1, FD2+(AI*FD ²)+(AI2*FD ²)}	M ~ CWM1 + ARI ² + NSS + SS + FD1 + FD2 + (AI*FD ²) + (AI2*FD ²)	FD1=0	<0.0001
(CWM2; M) {ARI ² , SS, FD1, FD2+(AI*FD ²)+(AI2*FD ²)}	M ~ CWM2 + ARI ² + SS + FD1 + FD2 + (AI*FD ²) + (AI2*FD ²)	FD2=0	0.2424
Combined hypothesis (hypothesis iii)		26.97	0.520
(28)			
(NSS;SS) {ARI ² }	SS ~ NSS + ARI ²	NSS =0	0.0623
(NSS; CWM2) {SS, ARI ² }	CWM2 ~ NSS + SS + ARI ²	NSS=0	0.3794
(NSS; FD1) {ARI ² ,CWM1 ² ,CWM2}	FD1 ~ NSS + ARI ² + CWM1 ² + CWM2	NSS=0	0.5485
(NSS; FD2) {ARI ² , CWM2 ² , FD1}	FD2 ~ NSS + ARI ² + CWM2 ² + FD1	NSS=0	0.428
(NSS; M) {ARI ² , CWM1, FD1, FD2+(AI*FD2)+(AI2*FD2)}	M ~ NSS + ARI ² + CWM1 + FD1 + FD2 + (AI*FD2) + (AI2*FD2)	NSS=0	0.5217
(ARI;SS) {Ø}	SS ~ ARI	ARI=0	0.1553
(SS; FD2) {CWM2 ² , FD1}	FD2 ~ SS + CWM2 ² + FD1	SS=0	0.7035
(SS; FD1) {ARI ² , CWM1 ² , CWM2}	FD1 ~ SS + ARI ² + CWM1 ² + CWM2	SS=0	0.4695
(SS; M) {ARI ² , CWM1, FD1, FD2+(AI*FD2)+(AI2*FD2)}	M ~ SS + ARI ² + CWM1 + FD1 + FD2 + (AI*FD2) + (AI2*FD2)	SS=0	0.974
(ARI;CWM2) {SS}	CWM2 ~ ARI + SS	ARI=0	0.226
(ARI; FD2) {CWM2 ² , FD1}	FD2 ~ ARI + CWM2 ² + FD1	ARI=0	0.4835

(CWM1; CWM2) {SS,NSS, ARI}	CWM2 ~ CWM1 + SS + NSS + ARI	CWM1=0	0.1478
(CWM1;FD2) {SS,NSS, ARI,	FD2 ~ CWM1 + SS + NSS + ARI +	CWM1=0	0.7359
CWM2 ² , FD1}	CWM2 ² + FD1		
(CWM2; M) {ARI ² , SS, CWM1,FD1,	M ~ CWM2 + ARI ² + SS + CWM1 +	FD2=0	0.8082
FD2+(AI*FD2)+(AI ² *FD2)}	FD1 + FD2 + (AI*FD ²) + (AI ² *FD ²)		

Notes: Key to variables: NSS = non-sprouting shrubs, SS = sprouting shrubs, ARI = (1-aridity index [precipitation/potential evapotranspiration]), CWM1 = community weighted leaf trait, CWM2 = community weighted size trait, FD1 = functional dispersion size trait, FD2 = functional dispersion leaf trait, M = Multifunctionality index. \$ = The associated mixed model regression for each d-sep claim using the lm function in R to test the independence claims. The *P* value is obtained by comparing the value of the C statistic for each hypothesis to a chi-square distribution with the same degrees of freedom – note that a model is rejected if the C statistic is significantly different from the χ^2 value.

BLOQUE II: ESCALA LOCAL

Functional traits determine the
photosynthetic response of ten
dryland species to warming

CAPÍTULO 3



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En fase de revisión en *Environmental and
Experimental Botany*

SUMMARY

Relatively few studies so far have assessed how ongoing global warming will affect the photosynthetic performance of dryland plant species. We conducted a common garden experiment over two years with contrasting environmental conditions (dry vs. wet) to evaluate how warming affected the photosynthetic capacity of ten dryland species with contrasting functional attributes, and to test whether their functional traits modulated warming effects on their photosynthetic performance. In the dry year, warming increased photosynthetic rates at the beginning of the growing season, suggesting a modification in the growing period (earlier spring). In the wet year, functional traits modulated photosynthetic responses to warming. Larger species with small leaves had higher photosynthetic rates under warming compared to smaller species with higher leaf lengths. Our results highlight the importance of studying photosynthetic responses along different years and considering functional traits when evaluating photosynthetic responses to climate change, particularly in stressful environments such as drylands.

Keywords: Photosynthesis; climate change; drylands; Mediterranean; functional traits; open top chambers

INTRODUCTION

The increase in temperature is a major driver of ongoing global climate change (Hanson *et al.*, 2005, IPCC, 2013). Climatic models predict an increase in global mean temperature of 1.4-5.8°C by the end of the XXIth century, with important regional and seasonal heterogeneity (IPCC, 2013). Global warming is already affecting species, communities and ecological processes in terrestrial ecosystems worldwide (Hughes, 2000; Rosenzweig *et al.*, 2007; Maestre *et al.*, 2012; Delgado-Baquerizo *et al.*, 2013; Peñuelas *et al.*, 2013). Plant physiological variables, such as photosynthesis, are potentially among the most affected by climate change due to their dependency on temperature (Berry and Björkman 1980; Luo 2007). Many studies have evaluated the photosynthetic responses of vascular plants to warming, with contrasting results so far; while warming increases the photosynthetic rate of many species, decreases or lack of variation with warming have also been reported (see Liang *et al.*, 2013 and references therein). Multiple explanations have been provided to account for these discrepancies. It has been suggested that species from habitats with small temperature variations could have less ability to acclimate to warming compared to species living in habitats with higher temperature ranges, such as arid, semi-arid and dry-subhumid environments (drylands hereafter; Billings *et al.*, 1971; Xiong *et al.*, 2000; Holmgren *et al.*, 2012). On the other hand, species living in temperate or cold climates would mainly benefit from warming more than those already living in warm climates because temperature is a limiting factor for plant photosynthesis in cold habitats (Parmesan, 2007; Liang *et al.*, 2013). Alternatively, differences between studies may be explained by reductions in soil moisture caused by warming (Luo *et al.*, 2001; Dermody *et al.*, 2007). Such reductions can be particularly important in water-limited environments such as drylands, and

would decrease photosynthetic activity (De Valpine and Harte 2001; Rodgers *et al.*, 2012). Warming may also exceed the metabolic optima of dryland species, which can reduce their photosynthetic performance (Valladares *et al.*, 2005) and even increase their mortality rate (Bai *et al.*, 2010).

Methodological issues can also explain part of the discrepancies in the photosynthetic responses to warming found so far. Some studies have subjected species to a short warming period conducted with constant temperatures (Strand *et al.*, 1997; Atkin and Tjoelker, 2003; Campbell *et al.*, 2007), while others have measured photosynthetic activity once or twice during the growing season (Llorens *et al.*, 2004; Atkin *et al.*, 2006, 2007), and thus could have not properly captured the variability in the photosynthetic rate observed throughout the growing season. Another important factor that could explain the discrepancies alluded above is the functional traits of the species studied, as they largely determine photosynthetic responses from local to global scales (Reich, 1998). For example, Sullivan *et al.* (2008) showed that warming increased aboveground biomass of *Salix arctica* (Pall.), a response that had a positive feedback on its photosynthetic activity. A recent meta-analysis has shown that photosynthetic and respiration responses to warming varied among functional groups (Liang *et al.*, 2013). For example, warming increased the photosynthesis of perennial, but not annual herbs, and enhanced that of grasses, but not forbs (Liang *et al.*, 2013).

To further advance in our understanding of plant physiological responses to warming, studies should: i) explicitly evaluate the role of functional traits in determining such responses, ii) measure the photosynthetic activity of multiple species throughout the growing season over different years, and iii) use experimental designs that capture daily and seasonal variations in temperature induced by warming. We aimed to do so by evaluating the photosynthetic responses to warming of ten species from Mediterranean

semi-arid habitats showing high variation in their functional traits. We used an experimental design that involved the use of open top chambers (OTCs) to simulate climatic conditions expected by the late XXIth century, and conducted multiple photosynthetic measurements during the growing season (spring and the beginning of summer) of two years with contrasting environmental conditions. Specifically, we evaluated whether the photosynthetic responses to warming varied among the studied species, and assessed how their functional traits modulated them. Studies evaluating the physiological responses of dryland species with contrasting functional attributes to warming are very scarce (but see Niu *et al.*, 2008). Furthermore, and to our knowledge, no previous study has assessed how functional traits affect temporal variations in photosynthetic responses to warming. We hypothesized that warming would stimulate photosynthesis during the first stages of the growing season because there are not water limitations during this period. This effect would vanish as the growing season progresses due to a reduction in soil moisture and to the increase in air temperature, which would exceed the photosynthetic optimum in Mediterranean species (Shaver *et al.*, 2000). We also expect that the magnitude of photosynthetic responses to warming will depend on the functional traits of the species considered.

MATERIALS AND METHODS

Experimental design

We conducted a microcosm experiment at the Climate Change Outdoor Laboratory (CCOL), located at the facilities of Rey Juan Carlos University (URJC, Móstoles, Spain: 40°20'37''N, 3°52'00''W, 650 m a.s.l.; Fig. S1), between April 2011 and July 2013. The experiment was designed as a randomized block design, with three treatments: block, warming (control vs. ~2.9 °C annual temperature increase), and species (*Agropyron cristatum* (L.) Gaertn, *Festuca ovina* (L.), *Lygeum spartum* (L.) Kunth, *Medicago sativa* (L.), *Plantago lanceolata* (L.), *Psoralea bituminosa* (L.), *Sanguisorba minor* (Scop.), *Hedysarum coronarium* (L.), *Dorycnium pentaphyllum* (Scop.) and *Phlomis herba-venti* (L.)). We selected these species because they can be found in grasslands developing in abandoned fields and restored roadside slopes in semi-arid areas from central Spain (García-Palacios *et al.*, 2010; Mola *et al.*, 2011). The selected species belong to three main functional groups (grasses, nitrogen-fixing legumes and forbs), which reflect differences in traits that are potentially relevant to the physiological variables of interest for this study, such as biomass production, resource use and N-fixation ability (Reich *et al.*, 2001; Gross *et al.*, 2007; McLaren and Turkington, 2010).

Microcosms consisted of plastic containers (depth 38 cm, internal diameter 28 cm, volume 0.023 m³) filled from the base with 3 cm of expanded clay for drainage and then 32 cm of natural soil coming from an orchard located in the surroundings of the CCOL (pH: 7.6; conductivity: 1128 µS/cm; sand content: 73.5%, silt content: 18.5%, clay content: 8.0%; total carbon: 0.82%, total nitrogen: 0.08%, organic carbon: 0.52%). All the microcosms were initially irrigated with 500 mL of a soil microbial inoculum to

recreate realistic microbial communities, as described in Maestre *et al.* (2005). We planted a monoculture in each microcosm. For doing so we used seeds obtained from a commercial supplier (Intersemillas Ltd, Valencia, Spain). The seeds of each species were randomly sown within each microcosm during the first week of April, 2011. Six weeks after sowing, some individuals were removed to reach a final density of 160 individuals/m². The microcosms were irrigated three times per week (1 L/irrigation) during the first 6 weeks of the experiment to improve seed establishment, and once a week (1 L/irrigation) in July and August 2011, to reduce mortality due to summer drought.

The warming treatment started in December 2011, once all the microcosms had an established population. It consisted on the use of OTCs, which have been employed in climate change experiments conducted in arctic (Marion *et al.*, 1997), alpine (Klanderud and Totland, 2005) and dryland (Escolar *et al.*, 2012; Maphangwa *et al.*, 2012) areas around the world. These chambers were built with six methacrylate plates, using a hexagonal design with sloping sides of 65-52-42 cm (Fig. S1). Methacrylate has high transmittance in the visible spectrum (92%), very low emission of the infrared wavelength (4%) and high energy transmission (85%; data provided by the manufacturer, Decorplax Metacrilatos S.L., Madrid, Spain). The OTCs were elevated 5cm from the soil surface to achieve adequate air flow and avoid excessive overheating. We monitored the air temperature and humidity under natural conditions and in OTCs using automated sensors (HOBO U23 Pro v.2 Temp/RH, Onset Corporation, Bourne, MA, USA). Climatic models predict an increase in annual temperature ranging from 2.6°C (B2 IPCC scenario) to 2.8°C (A2 IPCC scenario) in the study area by the second half of the twenty-first century in central Spain (de Castro *et al.*, 2005). Average

temperature increases provided by our experimental OTCs (~ 2.9 °C; Fig. S2b), as well as the seasonal variation in such increase, were within this range.

Microcosms were buried and leveled to the ground, and kept under ambient light and rainfall (Fig. S1). Weeds were regularly removed during the experiment. The microcosms were arranged in four blocks containing a replicate per combination of treatments, resulting in 80 microcosms in total.

Experimental measurements

Net photosynthetic rate was measured in every microcosm using an infrared gas analyzer (IRGA; Li-6400xt, Li-COR, Lincoln, NE, USA). These measurements were conducted every one to four weeks between 1st March and 15th June 2012, and between 22th April and 30th June 2013 (a total of 11 samplings were conducted). These measurements were taken on eight species during 2012 (Fig. 1). Two extra species (*Dorycnium pentaphyllum* and *Phlomis herba-venti*) were measured in 2013. One of the species (*Hedysarum coronarium*) underwent severe mortality during the summer of 2012, and hence was not measured in 2013. At every sampling, measurements were carried out on green and mid-height fully expanded leaves from a randomly selected individual per microcosm between 10:00 to 14:00 hours (local time). Every sampling took place during two consecutive, cloud-free days to maintain homogeneous environmental conditions. Our IRGA was operated as an open dynamic system, i.e., the chamber air was renewed with a continuous external air flow, which was taken at two meters above the soil surface by a rod, and a 6 L bottle was used as a buffer volume to use an input air with a stable $[\text{CO}_2]$. The external air circulated through the system with a flow of $500 \mu\text{mol s}^{-1}$. The net photosynthesis value of a microcosm was recorded when the CO_2 values of the sample were stable (see Ladrón de Guevara *et al.* 2014 for

detailed information). Photosynthetically active radiation (PAR) and leaf temperature was measured with Li-COR internal sensors placed within the leaf chamber, and were recorded at the same time of photosynthesis measurements.

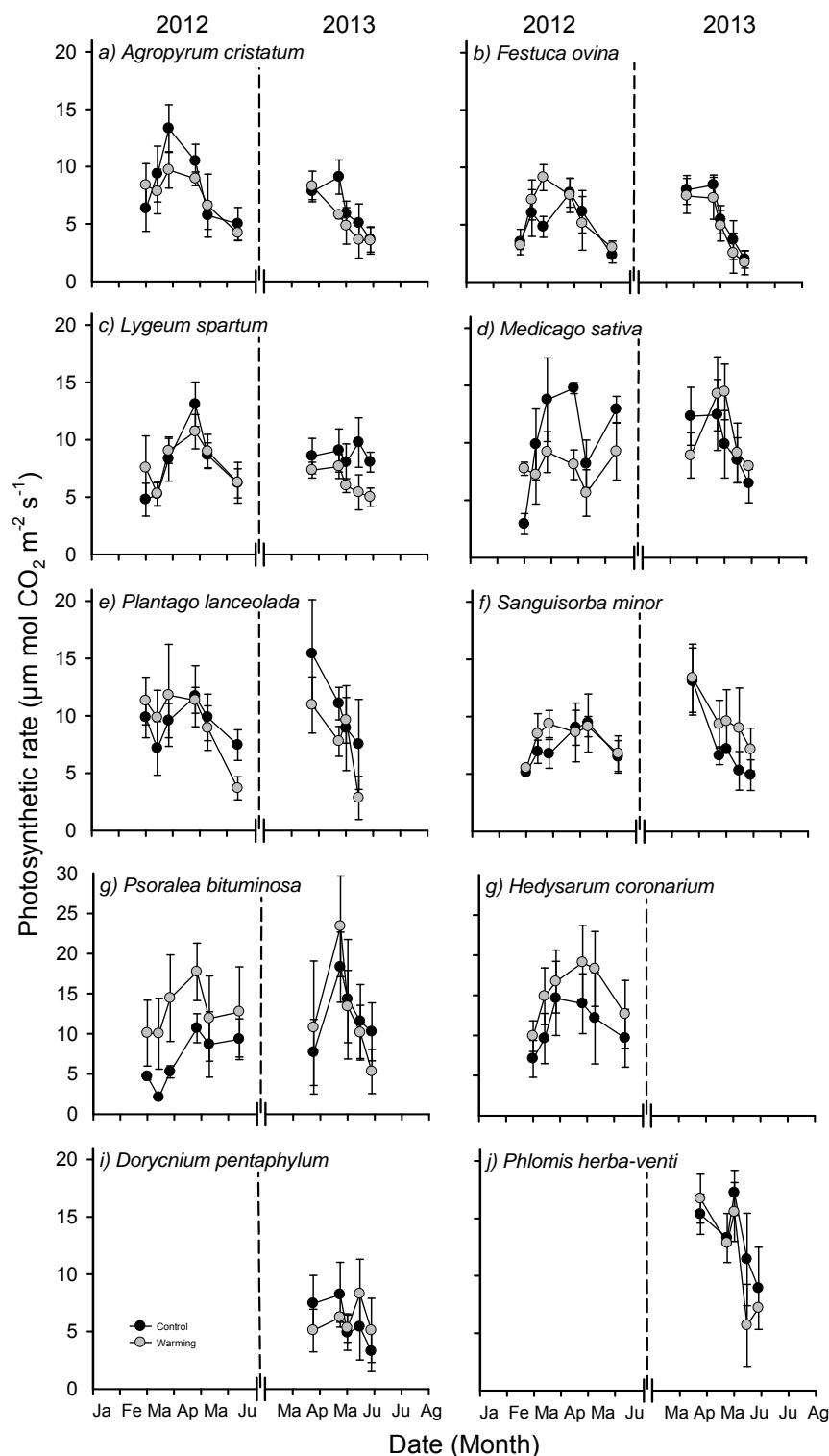


Fig. 1. Temporal dynamics of photosynthetic rate in the different species evaluated. Data represent means \pm SE ($n=4$).

We measured different functional traits in all the species studied after the peak of the growing season (in May 2012 and June 2013). The following traits were quantified following standard protocols (Cornelissen *et al.*, 2003) in one individual per microcosm: (i) vegetative height (cm), which is related to water use efficiency and/or competitive ability (Westoby *et al.*, 2002); (ii) leaf traits such as leaf area (cm²), leaf length (cm), leaf width (cm) and leaf thickness (mm), which reflect light interception and water stress tolerance (Westoby *et al.*, 2002), and specific leaf area (SLA, cm² g⁻¹), and leaf dry matter content (LDMC, g g⁻¹), which correlate with plant relative growth rate and resource capture and utilization (Wright *et al.*, 2004). Additionally, the volume (cm³) occupied by each monoculture was calculated as vegetative height (cm, measured with a rule) \times total cover (cm², visually estimated in each microcosm).

Precipitation, radiation and temperature data were collected using an on-site meteorological station (Fig. S2). We also measured volumetric water content using time-domain reflectometry (TDR; Topp *et al.*, 1984). For these measurements, we installed 10 cm long TDR probes in microcosms with and without plants to measure the effects of both plants and warming on soil moisture. On average, warming reduced soil moisture by 1.5% (Fig. S3).

Statistical analyses

We analyzed net photosynthetic rates separately for each sampling date using a split-plot randomized block design by using Linear and Nonlinear Mixed Effects Models. Warming, species and their interaction were considered as fixed factors in these analyses. The different blocks were considered as a random factor. We also included leaf temperature as a covariate in these models to control for the differences in the net photosynthetic rate promoted by variations in temperature during measurements. When

used as covariates, neither PAR or soil moisture had not a significant effect ($P > 0.05$) on the photosynthetic rate. For the shake of simplicity, we removed these covariates from our models.

To further evaluate how warming modified the photosynthetic response of the species studied, we estimated warming effects with the RII index, calculated as $(X_{\text{amb}} - X_{\text{war}}) / (X_{\text{amb}} + X_{\text{war}})$, where X_{amb} and X_{war} are the photosynthetic rate in the control and warming treatments, respectively (Armas *et al.*, 2004). RII values range from -1 to $+1$; negative values indicate that the photosynthetic rate was higher in the warming treatment, while positive values indicate the opposite. In addition, the slope of the relationship between RII values and the sampling date (SLO) was calculated using standardised major axis regression (Warton *et al.*, 2006). This slope indicate potential differences on the effect of warming on photosynthesis throughout the growing season; for example, a positive slope indicate that photosynthesis under warming decreased compared to that observed in the control treatment as this season progresses. As standardised major axis regression considers the variability in the axis X and Y at each point, and its use is recommended when we want to fit the relationship that best describes the bivariate scatter of Y and X (Warton *et al.*, 2006).

Finally, and to assess how functional traits affected the effect of warming on the temporal dynamics of photosynthesis, we evaluated the relationships between the functional traits evaluated and SLO using simple linear regression. We selected the functional traits less correlated between them to evaluate these relationships (Table S1). Trait and photosynthetic rate data were log-transformed as needed prior to analyses to normalize them. All the analyses were carried out with the packages “lm”, “smatr” (Warton *et al.*, 2012) and “lme4” (Bates *et al.*, 2014) for R (R Core Development Team, 2012).

RESULTS

Photosynthetic responses to warming

Environmental conditions during the growing season markedly differed between the two studied years. The mean temperature, precipitation and air relative humidity in the dry year (2012) were 14.5°C, 141 mm and 56%, respectively. In the wet year (2013), they were 13.6°C, 229 mm and 69%, respectively (Fig. S2).

Photosynthetic rates varied substantially among the studied species in most of the sampling periods (Fig. 1; Table 1). The effects of warming and leaf temperature varied depending on the sampling date considered, but we did not find a significant interaction in any case ($P > 0.05$ in all cases, Table 1). In the dry year, warming enhanced net photosynthetic rate at the beginning of spring (Fig. 1, 1st March 2012, $P = 0.005$, Table 1). This effect gradually disappeared after this date, and no significant differences between control and warming treatments were found during the rest of the growing season (Table 1). The exception to this pattern was *Psoralea bituminosa*, which had higher photosynthetic rate under warming during March and April. Warming did not promote any changes in the measured photosynthetic rates during the wet year (Table 1).

Table 1. Summary results of the ANCOVA analysis evaluating the effects of warming, species, their interaction and leaf temperature on the photosynthetic rate for each sampling date in the dry and wet years.

Dry year (2012)														
		March, 1			March, 14		March, 27		April, 24		May, 10		June, 13	
	Df	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	
Warming	1	13.572	< 0.001	2.783	0.102	1.987	0.165	0.000	1.000	0.318	0.576	0.010	0.920	
Species (sp)	7	5.252	< 0.001	2.137	0.059	2.199	0.054	3.210	0.007	2.642	0.022	2.983	0.011	
Leaf Temperature	1	4.101	0.050	0.205	0.654	0.814	0.389	0.470	0.497	8.158	0.007	5.234	0.027	
Warming×sp	7	1.128	0.363	1.448	0.211	1.419	0.222	1.777	0.115	0.433	0.876	0.575	0.773	
Wet year (2013)														
		April, 23			May, 23		May, 31		June, 12		June, 27			
	Df	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	Df	F	<i>P</i>		
Warming	1	0.059	0.809	0.010	0.920	0.065	0.800	1.806	0.185	1	0.411	0.525		
Species (sp)	8	4.355	< 0.001	8.354	< 0.001	5.120	< 0.001	2.352	0.031	7	2.692	0.021		
Leaf Temperature	1	6.096	0.017	9.580	0.016	1.328	0.279	0.424	0.518	1	3.803	0.075		
Warming×sp	8	0.745	0.651	0.615	0.761	0.389	0.921	1.010	0.441	7	0.917	0.503		

Warming effects on the temporal dynamics of photosynthesis

The values of SLO were positive for all the species in dry year (Fig. 2). These results indicate that the positive effect of warming on the photosynthetic rate observed early in spring, as suggested by RII values < 0 , disappeared as the growing season progressed. Indeed, species such as *Plantago lanceolata* showed reduced photosynthesis under warming at the end of the growing season (RII > 0 , Fig. 2e). The temporal dynamics of measured photosynthetic rates during the wet year showed important differences depending on the species considered. Three species (*Medicago sativa*, *Sanguisorba minor* and *Dorycnium pentaphyllum*) had negative SLO values, indicating that the effects of warming on the photosynthetic response shifted from negative to positive/neutral as the growing season progressed (Figs. 2i, 2f and 2d). The rest of species showed positive SLO values, as found in 2012.

Functional traits and the temporal dynamics of photosynthesis in response to warming

We did not find any relationship between SLO and the different functional traits measured in the dry year (Fig. 3). However, a negative SLO - volume relationship and a positive SLO - leaf length relationship were found in the wet year (Fig. 3). Species with high volume, but low leaf length (e.g., *Medicago sativa*, *Sanguisorba minor* and *Dorycnium pentaphyllum*) maintained or increased their photosynthetic rates under warming throughout the growing season. Consistently, species with opposite traits (low volume and high leaf length), such as *Festuca ovina*, *Lygeum spartum* and *Phlomis herba-venti* decreased their photosynthetic response under warming as the growing season progressed.

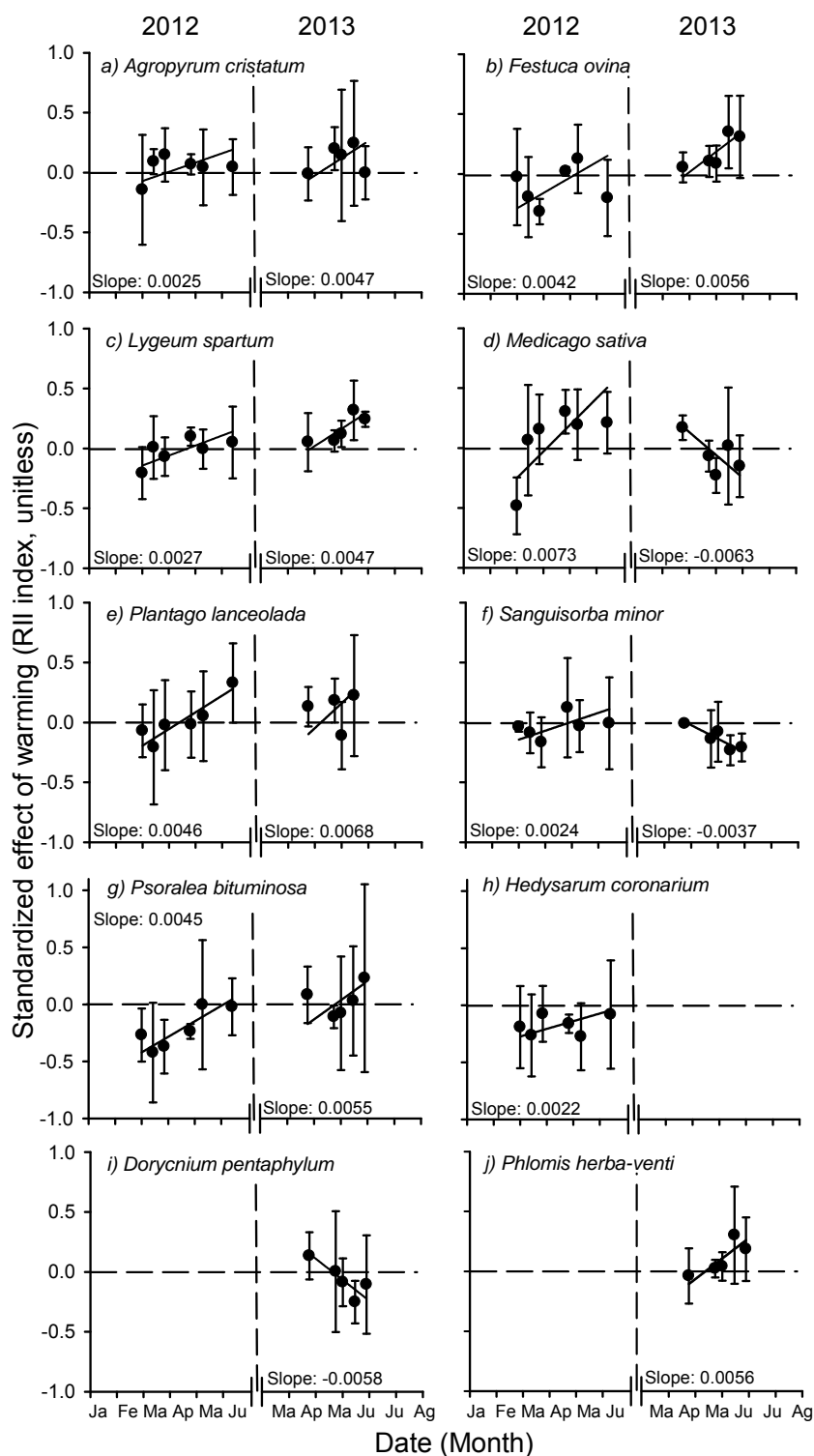


Fig. 2. Temporal dynamics of the effect of warming on the photosynthetic rate. The continuous black line represented the slope per species each year and was obtained with standardised major axis (SMA slope, Falster *et al.*, 2006). Data represent means \pm 95% confidence intervals.

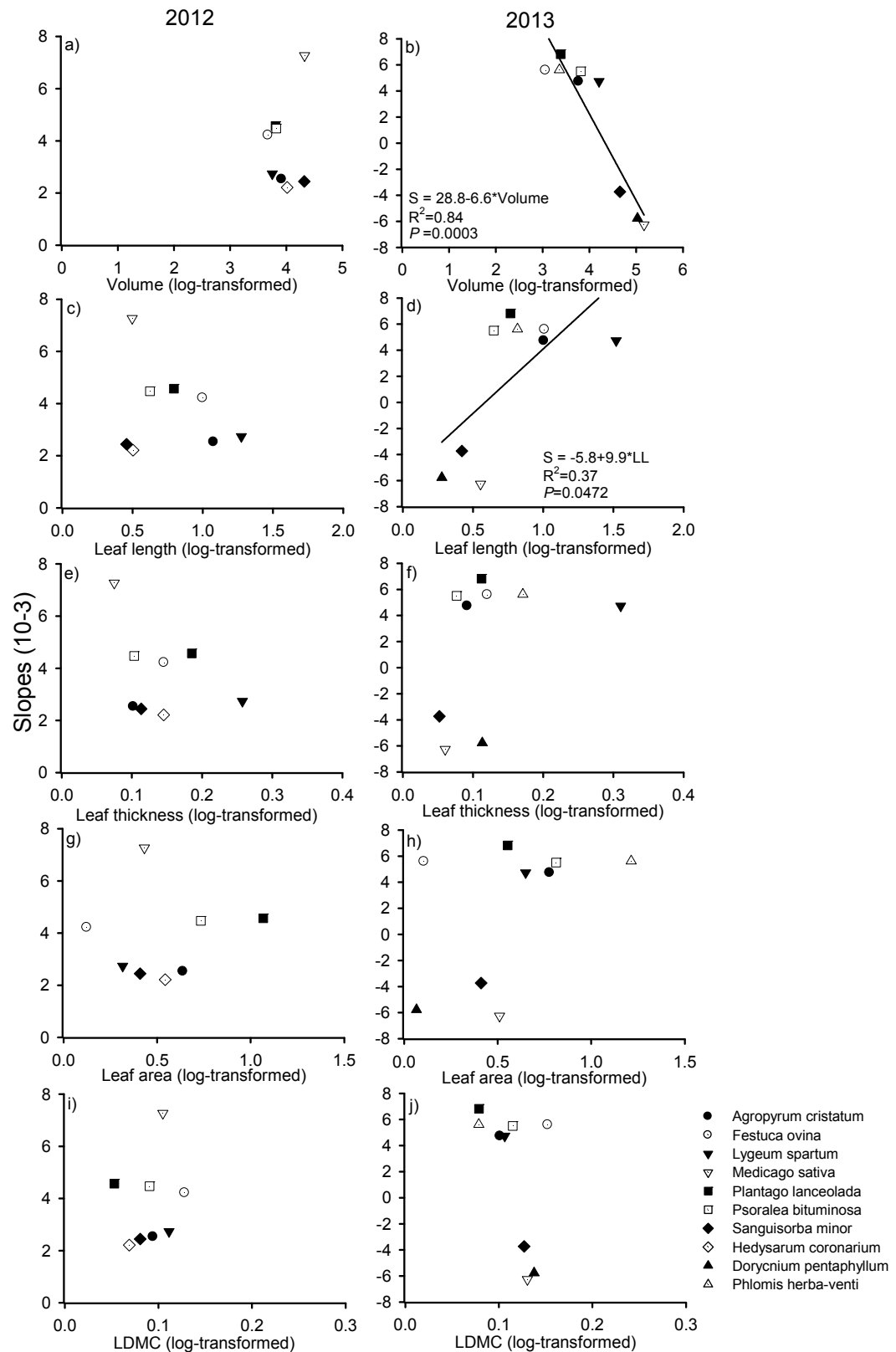


Fig. 3. Relationship between the temporal dynamics of the effect of warming on the photosynthetic rates, represented by the slope of the relationship between the RII values and the sampling date (Fig. 2), and the different traits measured. LDMC = leaf dry matter content. Only linear relationships with P values below 0.05 are shown.

DISCUSSION

All the studied species showed similar responses to warming in the dry year, as this treatment increased their photosynthetic rates at the beginning of spring, an effect that disappeared as the growing season progressed. During the wet year, the temporal dynamics of photosynthesis showed species-specific responses to warming. Such responses were largely modulated by functional traits. Large species with small leaves responded positively to warming, but small species with high leaf length showed the opposite response. As we discuss below, the study of trait-specific differential responses to warming may help us to explain how semiarid species such as those studied may respond to climate change.

Photosynthetic responses to warming

Our results show an increase in photosynthetic rates with warming early during the growing season of the dry year (Table 1), suggesting that our warming treatment simulated an earlier spring and summer. As a consequence, photosynthetic rates increased under warming at the beginning of the growing season, likely due to an advance in the arrival of the optimum temperature (Gunderson *et al.*, 2010). We conclude that warming could modify the growing period, affecting the photosynthetic activity of the species studied. Similar responses have also found by Zhou *et al.* (2007), who found that four species showed higher photosynthetic rate under warming only during the spring, suggesting a simulation of an earlier spring with warming. Other studies, such as Niu *et al.* (2008), found species-specific photosynthetic responses to warming. These authors suggested that differences in the functional traits of the studied species, together with factors such as different growth conditions, could underlie these differences. In our study, species such as *Psoralea bituminosa* had higher

photosynthetic rate under warming throughout the growing season (Figs. 1 and 2). We suggest that this species may be acclimated (*sensu* Smith and Dukes, 2012) to warming. This process has been shown in some studies under cold climates or in areas without water limitations, where species modify the optimal photosynthetic temperatures promoting an increase in the optimal photosynthesis under warming (Gunderson *et al.*, 2010; Peñuelas *et al.*, 2013). There are multiple examples regarding acclimation under laboratory conditions, especially in species from cold ecosystems (Atkin *et al.*, 2000; Bunce, 2000; Xiong *et al.*, 2000; Hikosaka *et al.*, 2006; Way and Sage, 2008), but examples from semiarid species are scarce (Niu *et al.*, 2008).

Temporal variations in photosynthetic responses to warming depend on functional traits

Our hypothesis (i.e. photosynthesis would increase during the first stages of the growing season with warming because there are not water limitations during this period) was supported by our data, but only during the dry year. Similar temporal dynamics have been reported by other studies (Gunderson *et al.* 2000; Zhou *et al.*, 2007). The differences found along the growing season indicate a modification of the growing period (earlier spring and summer) under warming. This process enhanced photosynthetic rate under warming at the beginning of the growing season. As temperature increased and soil moisture decreased during the growing season, the photosynthetic rate was reduced (Figs. S2, S3, S4). Additionally, increased temperatures under warming could exceed the optimum leaf temperature for photosynthesis (Gunderson *et al.*, 2010) or have an effect on enzymatic activities (Smith and Dukes, 2012 and references therein); these mechanisms could also have been responsible for the decline of photosynthetic rates observed in this treatment as the growing season

progressed. In warmer and drier periods, Rubisco could play an important role, since this enzyme could exceed the capacity of being activated at high temperatures (Crafts-Brandner and Salvucci, 2000).

Temporal variations in measured photosynthetic rates under warming during the growing season were similar in all the species during the dry year, but species-specific variations were found during the wet year. These differences were related to key functional traits, as increases of volume and/or decreases of leaf length promoted higher photosynthetic rates under warming than in control as the growing season progressed (negative SLO values; Figs 3a and 3b). Larger species in our study have more above- and below-ground biomass ($r_{\text{volume-aboveground biomass}} = 0.91$, $P < 0.001$; and $r_{\text{volume-belowground biomass}} = 0.53$, $P < 0.001$). These species could overcome the negative effects of reduced soil moisture under warming (Fig. S4) by forming more extensive roots (Lloret *et al.*, 1999; Poorter and Hayashida-Oliver, 2000), which can capture more soil water and therefore increase photosynthesis. This can lead to faster growth and biomass production, which in turn would stimulate the accumulation of a dense litter layer as older leaves senesce. The dense canopy and the litter layer accumulating underneath can reduce soil moisture losses due to evaporation (Holmgren *et al.*, 2012). However, large species could have higher water necessities than smaller ones, although they not necessarily may experience higher water stress, particularly when enough water is available (i.e. during our wet year; Stewart and Bernier, 1995; Lamhamedi *et al.*, 1998). A reason why species-specific differences appeared only in the wet year could be differences in water availability among years. Large plants may avoid water stress (with extensive roots and dense litter layer, as we previously commented), however, in dry years maybe this characteristics are not enough to overcome the water stress, and then even large plants could not stimulate photosynthesis.

Differences in leaf traits also had a significant impact on the temporal dynamics of photosynthetic responses to warming. We found that species with small leaf length, such as *Medicago sativa*, *Sanguisorba minor* or *Dorycnium pentaphyllum*, in comparison with species with high leaf length, such as *Lygeum spartum*, had higher photosynthetic rates under warming at the end of the growing season. It is well known that species with high leaf length increase their photosynthetic rate, and also evapotranspiration (Wright *et al.*, 2004), as temperature increases. Therefore, increasing water limitations are expected to enhance water stress, particularly in plants with high leaf length (Fonseca *et al.*, 2000 and references therein). Consistently, species with smaller, narrower, thicker and denser leaves are adapted to water stress (Shields, 1950; Levitt, 1980). During late spring or summer, warming amplified the effects of low moisture levels typically found during this period (Fig. S3), and this may be particularly detrimental for the photosynthetic performance of species with high leaf length.

Overall, we found that species typical of drylands increased their photosynthetic rate at the beginning of the growing period under warming. Our data also show that differences among species in their functional traits promote different photosynthetic responses to warming, but these differences were modulated by climatic conditions. Species with high size and small leaves would undergo less water limitations and therefore, could overcome detrimental effects of warming on soil moisture. Therefore, these traits could provide competitive advantages during drought periods, which will become more prevalent in drylands worldwide under climate change.

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SUPPORTING INFORMATION

Appendix A.



Fig. S1. General view of the sampling area (a). Partial view of the sampling area with microcosms situated in warming and control treatments (b). Detailed view of a microcosm in an open top chamber (c) and control (d) conditions.

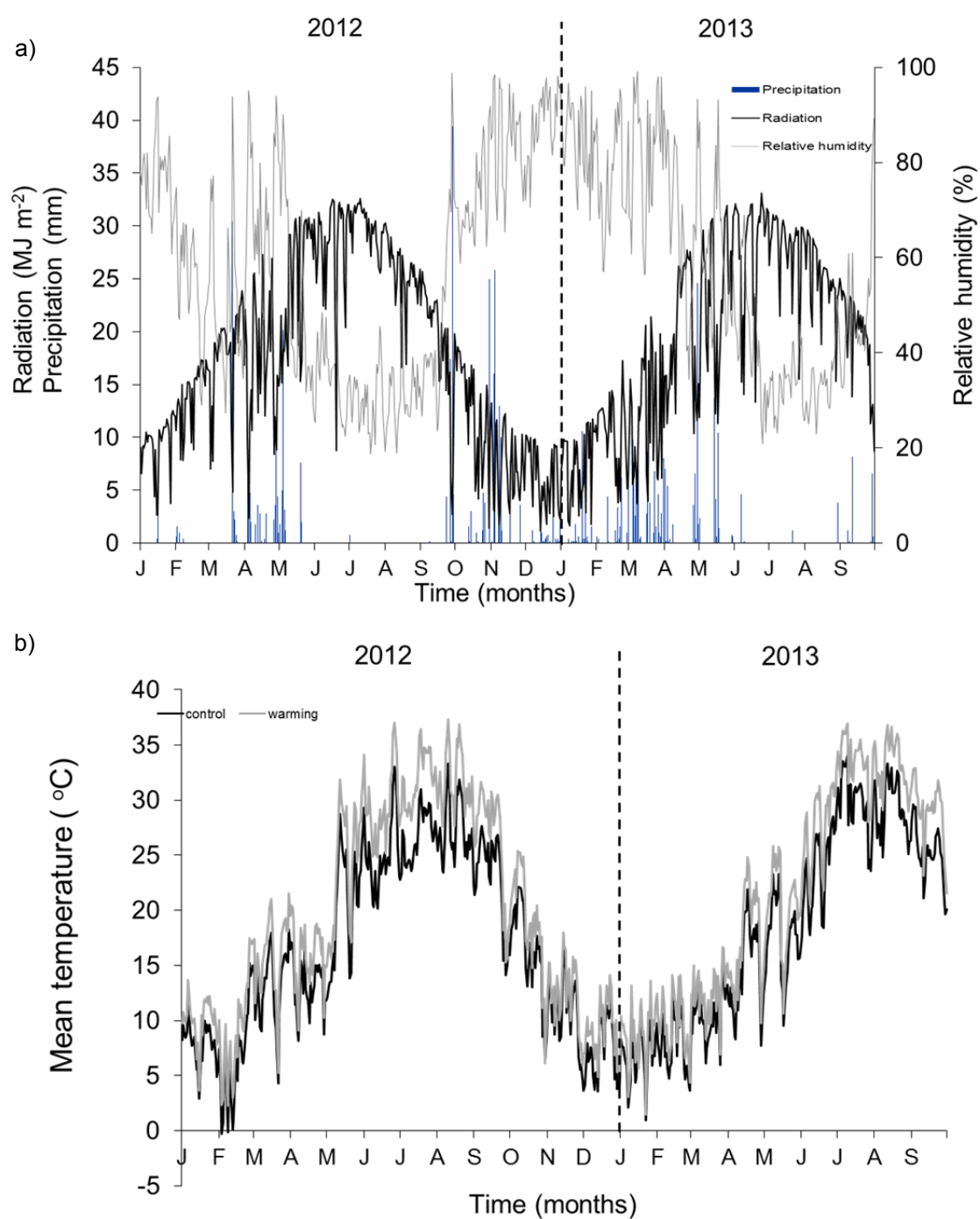


Fig. S2. Temporal variation in radiation, air relative humidity and precipitation (a) and air temperature (b) between January 2012 and October 2013 in the experimental area. The standard errors for air temperature are omitted for clarity (data present the average of $n = 3-4$ sensors for air temperature and relative humidity).

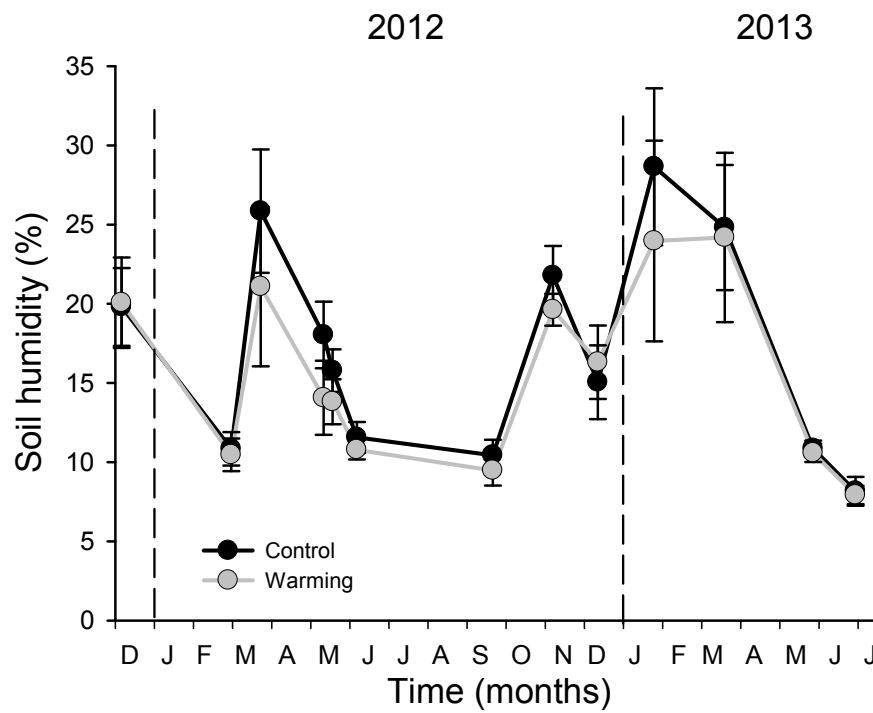


Fig. S3. Temporal variation in soil moisture in microcosms without plants between December 2011 and July 2013 in the experimental area. Data represent means \pm SE ($n = 4$).

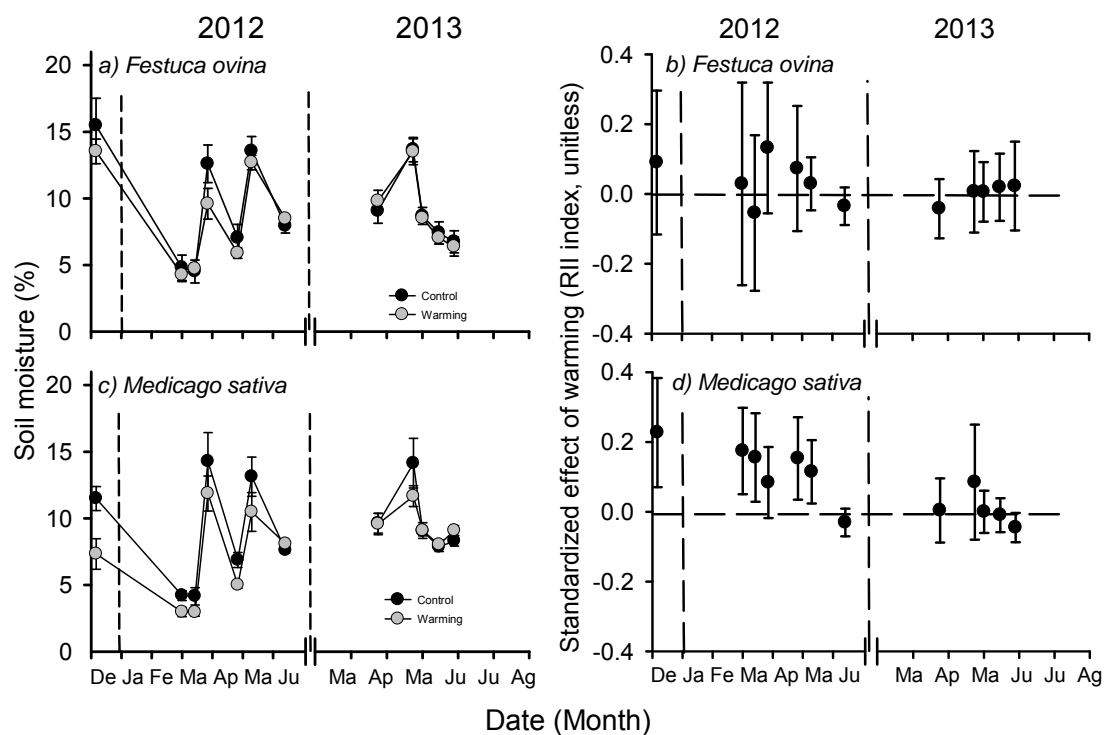


Figure S4. Temporal dynamics of soil moisture (a and c) and of the effect of warming on soil moisture (b and d) in *Festuca ovina* and *Medicago sativa* microcosms during the study (December 2011 and June 2013). Data represent means \pm SE ($n = 4$).

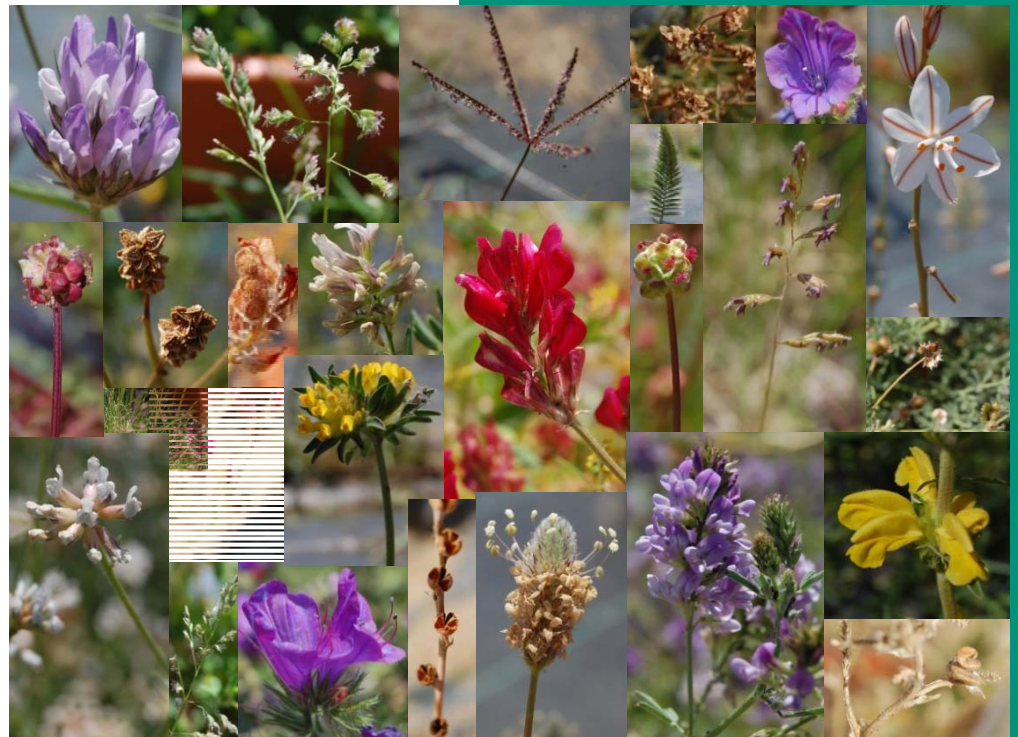
Table S1. Results of Pearson correlation coefficients among trait values in 2012 and 2013. TC: Total cover; VH = vegetative height; LL = leaf length; LT = leaf thickness; LW = leaf width; LA = leaf area; SLA = specific leaf area; LDMC = leaf dry matter content; Volume = Total cover * vegetative height. *, $P < 0.05$; **, $P < 0.01$.

2012	VH	LL	LT	LW	LA	SLA	LDMC	Volume
TC	0.442**	-0.366**	-0.107	0.0784	-0.145	0.209	-0.041	0.763**
VH		-0.243*	-0.450**	-0.100	-0.235*	0.393**	0.222*	0.895**
LL			0.264*	-0.195	0.261*	-0.332**	0.058	-0.367**
LT				0.090	0.169	-0.621**	-0.543**	-0.396**
LW					0.835**	0.498**	-0.665**	-0.015
LA						0.416**	-0.665**	-0.215
SLA							-0.252*	0.411**
LDMC								0.130

2013	VH	LL	LT	LW	LA	SLA	LDMC	Volume
TC	0.805**	-0.513**	-0.261*	0.198	-0.086	0.151	0.222	0.974**
VH		-0.283*	-0.342**	0.125	-0.039	0.231*	0.257*	0.903**
LL			0.508**	-0.267*	0.488**	-0.430**	-0.241*	-0.460**
LT				-0.278*	0.177	-0.827**	-0.423**	-0.320**
LW					0.602**	0.526**	-0.396**	0.167
LA						0.090	-0.545**	-0.083
SLA							-0.112	0.196
LDMC								0.258*

Warming modifies
reproductive phenology in
semiarid Mediterranean
species

CAPÍTULO 4



Enrique Valencia, Marcos
Méndez, Noelia Saavedra,
Fernando T. Maestre

En fase de preparación para su envío a
Basic and Applied Ecology.

SUMMARY

Changes in vegetative and reproductive phenology rank among the most obvious responses of plant species to climate change. Using a manipulative experiment conducted over two growing seasons, we evaluated the responses in reproductive phenology of fourteen Mediterranean semiarid species to a $\sim 3^{\circ}\text{C}$ increase in temperature. We evaluated the responses of three functional groups (grasses, nitrogen-fixing legumes and forbs) to warming, and how leaf and size traits influenced phenological response. Overall, warming advanced the onset of flowering and onset and peak of fructifications, extended the duration of flowering and reduced the production of flowers and fruits. Functional groups differed in their timing of flowering, for instance forbs had an earlier reproductive phenology than legumes and grasses. However, they didn't showed an advanced of phenological response to warming differently among functional groups. We found that species with higher size produced more flowers and fruits, and together with other traits such as small leaf area and specific leaf area, had an earlier flowering phenology and larger flowering duration. The warming effect on flowering and fruiting phenology could affect complementarity among species, but not functional groups, in the community. The observed reduction in flower and fruit production with warming is likely related to the decrease in soil moisture promoted by this treatment. These results could lead to potential ecological asynchronies between plants and insects, but also to changes in the composition, abundance, distribution or even extension of species in the communities. Our results highlight the important and the risk of climate change in phenology of Mediterranean semiarid species, and increase our knowledge of phenological response of plants that could help to accurate predictions in the ongoing climate change context.

Keywords: Climate change; drylands; functional group: functional traits; phenology

INTRODUCTION

Ongoing global warming is altering plant vegetative and reproductive phenology in terrestrial ecosystems worldwide (Parmesan & Yohe 2003; Gordo & Sanz 2010; Körner & Basler 2010). These changes have multiple consequences for ecological processes and services, including potential ecological asynchronies between plants and insects, modification in flower quality and seed mass, or reduced survival of species that cannot adapt to them (Goulden *et al.* 1996; Walther *et al.* 2002; Aerts *et al.* 2004; Williams *et al.* 2007; Inouye 2008; Hegland *et al.* 2009). Vegetative and reproductive phenological responses to warming have been relatively well studied in temperate habitats (Dunne *et al.* 2003; Parmesan & Yohe 2003; Sherry *et al.* 2007; Rollinson & Kaye 2012), where temperature is often a limiting factor for plants (Parmesan 2007; Liang *et al.* 2013). In these areas, both observational and experimental studies have shown that warming causes an earlier and longer flowering season (Dunne *et al.* 2003; Menzel *et al.* 2006; Parmesan 2007; Hovenden *et al.* 2008; Kardol *et al.* 2010).

Phenological responses often show a latitudinal variation, with higher increases of growing season length and a more advanced flower phenology in warmer compared to colder ecosystems (Menzel *et al.* 2006; Gordo & Sanz 2009). Observational and experimental studies in more stress-prone ecosystems like arid, semi-arid and dry-subhumid environments (drylands hereafter, *sensu* UNEP 1992) are scarce (Peñuelas *et al.* 2002; Gordo & Sanz 2010; Liancourt *et al.* 2012). This is a worrying knowledge gap because drylands occupy ~41% of the Earth's surface (Safirel & Adeel 2005), are a key biome for the conservation of global biodiversity (Maestre *et al.* 2012) and for understanding the global carbon budget (Ahlström *et al.* 2015) and are currently threatened by climate change and desertification (Reynolds *et al.* 2007). In addition, the

impacts of warming on plant phenology are likely to differ from those recorded in temperate ecosystems. In water-limited environments, warming could advance flowering, but may decrease the number of flowers, fruits and seed mass (Liancourt *et al.* 2012) due to reductions in soil moisture (Luo *et al.* 2001; Peñuelas *et al.* 2004).

Despite the large body of literature on phenological responses to climate change, there are still large uncertainties about why observed responses differ so widely among different species. To understand this variability, many studies have explored phenological responses to changes in temperature together with factors such as soil moisture, vernalization or photoperiod (Partanen, *et al.* 1998; Boss *et al.* 2004; Turck *et al.* 2008; Korner & Basler 2010). Plant functional groups, i.e. sets of species that encompass a variety of common functional attributes (Díaz & Cabido 1997; Gitay & Noble 1997; Walker *et al.* 1999; McLaren & Turkington 2010), offer a possibility to generalize how species respond to environmental changes (Wilson 1999). Functional groups can be defined *a priori* (based on taxonomy) or *a posteriori* (based on specific functional traits; Díaz & Cabido 1997). Cleland *et al.* (2006) showed that annual grasses flower earlier than forbs, so these groups had different timing of flowering. This study also showed that these groups change in response to different climate change factors (CO₂ and N enrichment). Timing of flowering (i. e. separate plants between early and late flowering) influences the response to warming (Sherry *et al.* 2007); changes in such timing with warming could decrease the coexistence of different species or functional groups within plant communities (i. e. phenological complementarity, Rathcke & Lacey 1985). However, while some studies have found an advance in spring flowering phenology related to timing of flowering and warming (Fitter *et al.* 1995, Peñuelas & Filella 2001, Root *et al.* 2003), others did not found a correlation between them (Liancourt *et al.* 2012). The controversy about this issue must be resolved due to

the risk of the ongoing climate change. Plant functional traits can shed light on the reproductive responses, but their influence has received little attention so far (Bolmgren & Cowan 2008; Miller-Rushing *et al.* 2008a; Richardson *et al.* 2010). For instance, the size of plants should correlate with flowering onset (Vile *et al.* 2006), but its relationship with other phenological variables is unknown. There is a lack of consensus among studies related to reproductive phenological responsiveness when take into account taxonomical functional groups or plant traits (Fitter & Fitter 2002). Furthermore, a better knowledge about the relationship between plant traits and phenological responses is needed to enhance our ability to predict plant responses to warming.

With the aim to fill current gaps in our understanding about the phenological responses of Mediterranean semiarid species to climate change, we experimentally evaluated how warming affects the reproductive phenology of fourteen Mediterranean semi-arid species belonging to different functional groups. Specifically, we asked: 1) which events of reproductive phenology, such as flowering (onset, peak, cessation or range), fruiting (onset, peak), and reproductive output (number of flowers, fruits or seed mass), are influenced by warming, 2) whether results are consistent among the different components of reproductive phenology and between years with contrasting environmental conditions, 3) whether phenological responses differ among functional groups and functional traits, 4) which and how functional traits determine the different phenological responses, and 5) whether warming modifies phenological responses of functional groups. We predict that warmer temperatures would cause an advance of all components of reproductive phenology, an increase in flowering duration and a decrease in the number of flowers, fruits and seed mass (Liancourt *et al.* 2012). This is so because we expect reductions in soil moisture as the growing season progresses from

early to late spring, and this would promote differential phenological responses. We also hypothesize that phenological responses vary among plant functional groups and functional traits such as plant size and leaf area. Differences among functional groups may be modified under warming. For instance, functional groups with earlier flowering dates should advance the flowering variables with warming more than those having later flowering dates (Sherry *et al.* 2007).

MATERIALS AND METHODS

Study species

We studied 14 species belonging to three broad taxonomical functional groups: grasses (*Cynodon dactylon* [L.] Pers., *Poa pratensis* [L.], *Agropyrum cristatum* [L.] Gaertn., *Festuca ovina* [L.]), nitrogen-fixing legumes (*Hedysarum coronarium* [L.], *Dorycnium pentaphyllum* [Scop.], *Anthyllis vulneraria* [L.], *Medicago sativa* [L.], *Psoralea bituminosa* [L.]) and forbs (*Plantago lanceolata* [L.], *Sanguisorba minor* [Scop.], *Echium boissieri* [Steud.], *Echium plantagineum* [L.], *Asphodelus fistulosus* [L.]; Table S1). We selected these species because they typically coexist in abandoned fields and roadside slopes undergoing secondary succession in semi-arid Mediterranean areas (García-Palacios *et al.* 2010).

Experimental design

A microcosm experiment was conducted at the Climate Change Outdoor Laboratory (CCOL), located at the facilities of Rey Juan Carlos University (URJC, Móstoles, Spain: 40°20'37''N, 3°52'00''W, 650 m a.s.l.) between April 1, 2011 and July 31, 2013. Microcosms consisted of plastic pots (depth 38 cm, internal diameter 28 cm, volume 0.023 m³) filled with 32 cm of natural soil (sand content: 73.5 %, silt content: 18.5 %, clay content: 8.0 %) and 3 cm of expanded clay for drainage at the base. All the microcosms were initially watered with 500 mL of a soil microbial inoculum to recreate realistic microbial communities, as described in Maestre *et al.* (2005). All the pots were placed in holes in the ground, and kept under ambient light and rainfall to keep the most natural conditions.

The experiment was designed as a randomized block design, with two

treatments: warming (control vs. ~2.9 °C annual temperature increase), and functional group (three levels: grasses, nitrogen-fixing legumes and forbs). Microcosms were arranged in four blocks containing a replicate of species per combination of treatments (four replicates per functional group and block), resulting in 112 microcosms in total. A minimum distance between microcosms of 1 m was established to minimize the risk of sampling non-independent areas.

Seeds from each species were obtained from commercial sources (Intersemillas Ltd, Valencia, Spain). In April 2011, we randomly sowed seeds of each species within each microcosm. We planted a monoculture in each microcosm at a density of 160 individuals/m² but this density varied among species and microcosms along the experiment. The microcosms were watered with 1L three times per week during the first 6 weeks of the experiment to improve seed establishment, and once a week in July and August 2011, to reduce potential extreme drought conditions. Weeds were regularly removed during the experiment. The warming treatment was set up on December 05, 2011, once all microcosms had an established population, and the experiment terminated in July 2013. We elevated the temperature in the warming treatment by using open top chambers (OTCs), which have been commonly employed in warming experiments (e.g., Marion *et al.* 1997; Hollister & Weber 2000, Escobar *et al.* 2012; Liancourt *et al.* 2012). OTCs were hexagonal chambers built of methacrylate plates, with sloping sides of 65-52-42 cm (Fig. S1). Methacrylate has high transmittance in the visible spectrum (92%), very low emission of the infrared wavelength (4%) and high energy transmission (85%; data provided by the supplier, Decorplax Metacrilatos S.L., Madrid, Spain). Chambers were open on their top to allow rainfall and air flow, and raised 5 cm from the soil surface to achieve adequate air flow and avoid excessive heating. Air temperature and humidity were measured in OTCs and control plots using

automated sensors (HOBO U23 Pro v.2 Temp/RH, Onset Corporation, Bourne, MA, USA). OTCs raised temperature by an average of ~ 2.9 °C compared to control plots during our experiment (Fig. S2b). This temperature increase was consistent with the predictions of climatic models, ranging from 2-3 °C in the study area by the second half of the twenty-first century (de Castro *et al.* 2005).

Phenological and plant sampling

Flowering phenology and flower and fruit production in each microcosm were scored every two-three days throughout the spring flowering period (from March to early July) of 2012 and 2013. We focused on the spring flowering because most species only bloomed in this period. From the raw counts we calculated the following phenological variables: dates of onset, peak and cessation of flowering, flowering duration, maximal number of flowers or inflorescences produced at the flowering peak (flower production hereafter), fructification onset and peak, maximal number of fruits or infructescences produced at fructification peak (fruit production hereafter) and seed mass. Flowering onset, peak and cessation was defined as the number of days elapsed between the 1st of March and the first flower, maximal number of flower and end of blooming, respectively. Range was estimated as the difference between flowering onset and cessation. Accordingly, we measured the onset and peak of fructification as the number of days elapsed between the 1st of March and the date when the first and maximal number of fruits was recorded, respectively. Seed mass was estimated by weighing a sample of twenty seeds per microcosm, when plants had ripe fruits at the end of the fructification period of 2013. The previous year we did not obtained seed mass, because we preferred not alter the natural grow of new plants in the microcosm.

Phenological measurements were gathered on twelve species during 2012 (Fig.

1). *Poa pratensis* and *H. coronarium* suffered from severe mortality under warming during the summer of 2012 and were dropped from the analysis in 2013. *Festuca ovina* and *E. boissieri* did not flower in 2012 but were studied in 2013. At the end of July 2013, when plants had ripe fruits at the end of the fructification period, seed mass was estimated by weighing a sample of twenty seeds per microcosm.

We also measured total cover (TC) and different functional traits in all the microcosms in May 2012 and June 2013. Cover was visually estimated as the proportion of the microcosm occupied (in cm^2). The following functional traits were quantified according to standard protocols (Cornelissen *et al.* 2003) in a randomly chosen individual per microcosm: (i) vegetative height (VH, cm), which is related to water use efficiency and/or competitive ability (Westoby *et al.* 2002); (ii) leaf traits such as leaf area (LA, cm^2), leaf length (LL, cm), leaf width (LW, cm) and leaf thickness (LT, mm), which reflect light interception and water stress tolerance (Westoby *et al.* 2002), and specific leaf area (SLA, $\text{cm}^2 \text{g}^{-1}$), and leaf dry matter content (LDMC, g g^{-1}), which correlate with plant relative growth rate and resource capture and utilization (Wright *et al.* 2004). Additionally, the number of individuals and volume (calculated as $\text{VH} \times \text{TC}$) occupied by each microcosms were measured.

Statistical analyses

Functional trait classification

As many of the functional traits measured are correlated among them (Table S2) we reduced our original list of nine traits by means of a principal component analysis (PCA), which was performed across all species using the mean trait value for each microcosm, to identify the main axes of specialization among species. Trait values of each microcosm were previously normalized using log-transformation. We identified

three independent PCA components, which together explained around 80% of the total variance found in the data (Table S3). These analyses were carried out separately for each sampling year. In both years, the first, second and third PCA components were highly related to plant size (VH, TC and volume), leaf size (LA, LT and LL) and leaf morphology (SLA), respectively (Table S3). To represent each axis of variation, for every year we selected the trait most strongly correlated with each PCA component that did not show a high correlation with the remaining traits (Tables S2 and S3). These traits selected to represent each independent axis in further analyses were volume, LA, and SLA. This procedure has the advantage of considering only independent variables in further analyses, and also allow us to identify important traits that reflect independent functional niche axes (Gross *et al.* 2007; Butterfield & Suding 2013).

Response of flowering and fruiting phenology to warming

We used General Linear Mixed Models (GLMM) to assess the responses to warming of six phenological variables -onset, peak, range and cessation of flowering and onset and peak of fructification- and three reproductive variables -seed mass, flower and fruit production- separately for each year. Seed mass, flower and fruit production were log-transformed to improve normality. In all models, warming, functional group and their interaction were considered as fixed factors, whereas block and species, nested within functional group, were considered as random factors. We included volume, LA, and SLA as covariates in these analyses. In the case of seed mass, flower and fruit production, the number of individuals was also included as a covariate. We used a stepAIC procedure (following Grace 2006) to evaluate the relationships of each factor and covariables with phenological and reproductive variables, and selected the best model based on the Akaike Information Criterion (AIC; Akaike 1973). We used a Type

III sums squares to test the significance of the fixed effects. Additionally, a Tukey post-hoc was conducted to evaluate differences among functional groups in the cases where significant interactions between functional group and warming were found.

All statistical analysis were carried out with R (R Core Development Team 2012), using packages “lme4” (Bates *et al.* 2014) and “multcomp” (Hothorn *et al.* 2008).

RESULTS

The two years studied had contrasting environmental conditions. The first year (2012) was drier (14.5 °C and 141 mm of mean temperature and precipitation during the growing season, respectively) than the second year (2013; 13.6 °C and 229 mm of mean temperature and precipitation during the growing season, respectively; Fig. S2a). Warming treatment increased the air average temperature in 3.3 °C and 2.6 °C in the growing season of drier and wetter years, respectively.

Effects of warming on flowering and fruiting phenology

Trends in phenological responses were consistent across years, except for cessation of flowering where results were highly species-dependent (Fig. 1). In the drier year, warming promoted a significant earlier onset and peak of flowering, earlier onset and peak of fruiting ($P < 0.05$, Table 1). We also found a marginally significant trend towards a larger duration of flowering ($P < 0.10$, Table 1). In the wetter year, warming led to an earlier flowering onset, onset and peak of fruiting, and to a larger flowering duration ($P < 0.05$, Table 1).

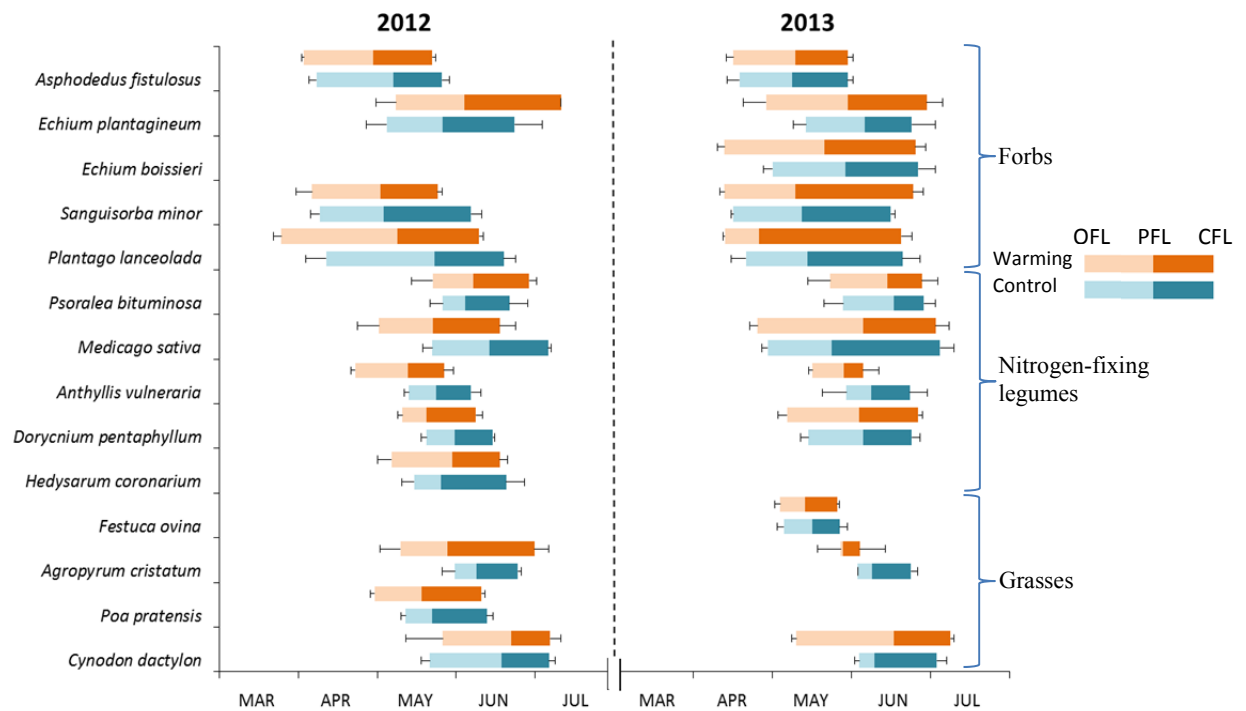


Fig. 1. Flowering phenology of the studied under control and warming in the two years studied. OFL: onset of flowering; PFL: peak of flowering; CFL: cessation of flowering. Data represent means \pm SE at the beginning and end of flowering phenology ($n=4$).

Functional group significantly influenced flowering variables in both years, and fructification variables in the drier year (Table 1, Fig 2). Flowering phenology and duration were significantly earlier and larger in forbs than in grasses and nitrogen-fixing legumes in both years (Fig. 2). The same occurred with fruiting variables, but differences were significant only in the drier year (Fig. 2). There were no significant differences between grasses and nitrogen-fixing legumes in any of the flowering and fruiting variables measured (Fig 2). The interaction between warming and functional group was not significant for any variable (Table 1).

Table 1. Summary results of the General Linear Mixed Models (GLMM) evaluating the effects of warming, functional group (FG), their interaction and functional traits on the phenological events measured in 2012 and 2013. OFL: onset of flowering; CFL: cessation of flowering; Range: flowering duration; PFL: peak of flowering; OFR: onset of fructification; PFR: peak of fructification; volume = total cover x vegetative height, LA = leaf area; SLA = specific leaf area.

2012																			
Treatment	g.l.	est	OFL χ^2	P	est	CFL χ^2	P	est	Range χ^2	P	est	PFL χ^2	P	est	OFR χ^2	P	est	PFR χ^2	P
Warming(W)	1	-8.3	14.762	<0.001	-1.8	0.371	0.543	4.707	3.415	0.065	-4.5	5.657	0.017	-4.2	8.599	0.003	-4.7	5.484	0.019
FG	2		14.225	<0.001		4.936	0.085		11.333	0.003		11.027	0.004		9.370	0.009		2.739	0.254
Volume	1	-4.2	9.210	0.002				4.877	8.173	0.004	-3.0	4.275	0.039	-1.7	3.739	0.053			
LA	1				6.4	15.047	<0.001	5.246	6.001	0.014	8.61	16.083	<0.001				1.6	2.294	0.130
SLA	1																		
W * FG	2					4.697	0.096								4.721	0.094		4.491	0.106
FG:species (random factor)			38.4	<0.001		59.4	<0.001		7.11	0.008		39.3	<0.001		68.851	<0.001		12.361	<0.001
2013																			
Treatment	g.l.	est	OFL χ^2	P	est	CFL χ^2	P	est	Range χ^2	P	est	PFL χ^2	P	est	OFR χ^2	P	est	PFR χ^2	P
Warming(W)	1	-5.7	5.818	0.016				6.1	7.872	0.005				-2.1	5.041	0.025	-3.01	7.595	0.006
FG	2		23.550	<0.001					13.674	0.001		8.098	0.017442						
Volume	1	-	45.602	<0.001				12.0	44.290	<0.001	-6.7	9.970	0.001591				2.93	9.089	0.003
LA	1	3.0	4.610	0.032	4.7	10.236	0.004				3.3	3.123	0.077209	-1.8	6.196	0.013			
SLA	1	4.7	7.701	0.006	3.2	2.452	0.051				5.3	5.543	0.018550						
W * FG	2		4.969	0.083															
FG: species (random factor)			39.8	<0.001		29.7	<0.001		36.387	<0.001		29.5	<0.001		122	<0.001	51.8		<0.001

Leaf and size traits affected flowering variables in both years, but their effects on fructification events were only evident in the wetter year (Table 1). Larger plants had an earlier onset and peak of flowering, and a larger flowering duration, in both years (Table 1), but showed a delayed peak of fructification only in the wetter year (Table 1). Plants with higher LA had a later date of peak and cessation of flowering in both years, and earlier onset of fructification in wetter year. Additionally, these plants maintained their flowers during more time in the drier year (Table 1). A smaller SLA promoted earlier onset and peak of flowering, but only in the wetter year.

Effects of warming on reproductive variables

Warming significantly decreased flower and fruit production only in the wetter year ($P < 0.05$, Table 2). However, both years showed the same trend, that is a decrease of flower and fruit production to warming. Fruit production in forbs was lower than in grasses and legumes in the drier year, but higher in the wetter year (Fig. 2; Table 2). Volume significantly increased flower and fruit production in both years (Table 2). In the wetter year, LA and SLA significantly increased and decreased seed mass, respectively (Table 2).

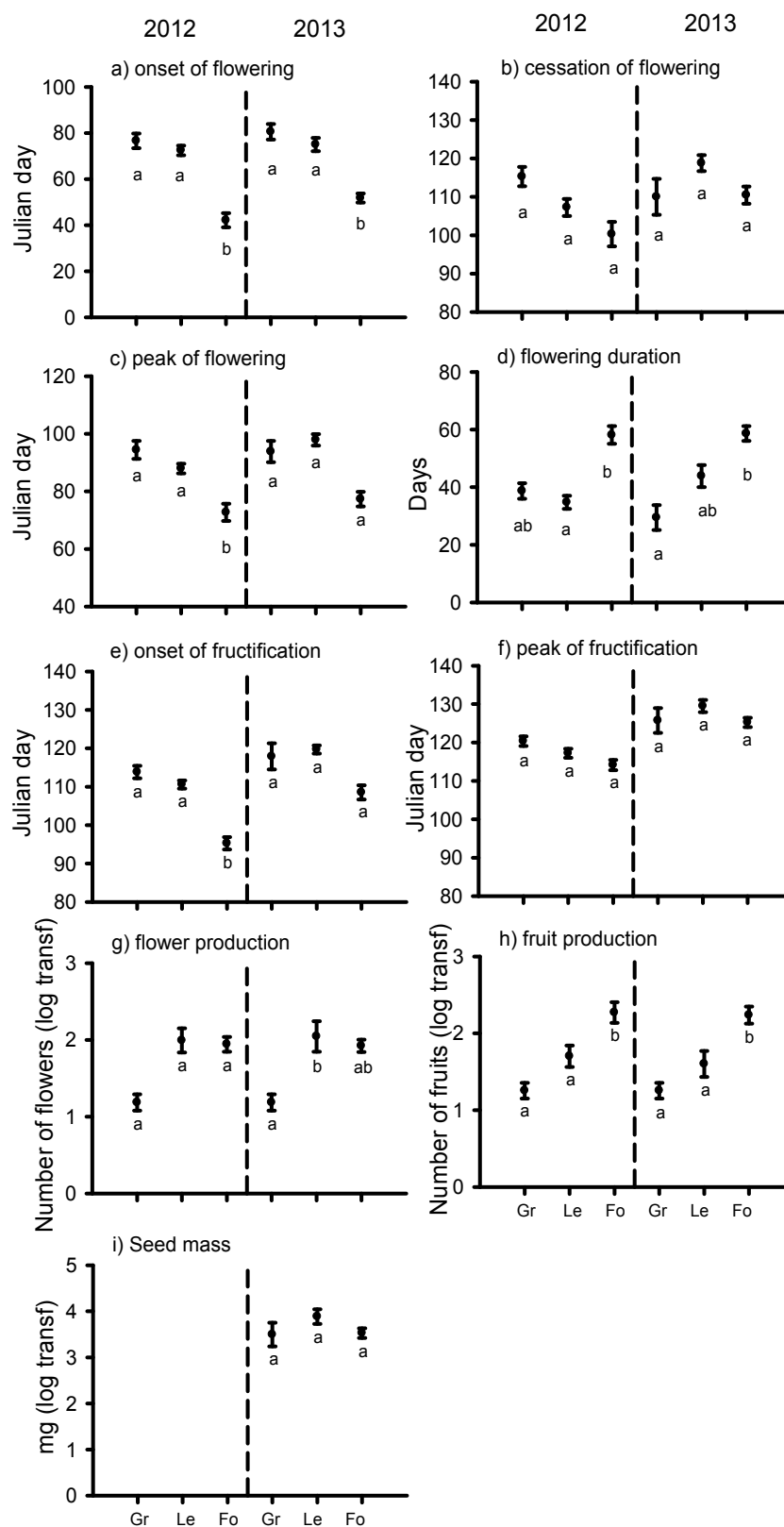


Fig. 2. Differences in the phenological and reproductive variables evaluated are indicated by different letters (Tukey post-hoc, $P < 0.05$). Gr: grasses; Le: nitrogen-fixing legumes; Fo: forbs.

Table 2. Summary results of the Linear Mixed Models evaluating the effects of warming, functional group (FG), their interaction and functional traits on the flower production (FLP), fruit production (FRP) and seed mass in 2012 and 2013. Volume = total cover x vegetative height; LA = leaf area; SLA = specific leaf area; N° ind = Number of individuals per microcosm.

2012										
Treatment	FLP				FRP					
	g.l.	est	χ^2	P	est	χ^2	P			
Warming(W)	1	-0.09	2.720	0.099						
FG	2					14.603	<0.001			
Volume	1	0.34	58.858	<0.001	0.45	32.793	<0.001			
LA	1	0.11	2.723	0.099						
SLA	2									
N° ind	1	-0.01	3.852	0.050						
W * FG	2									
FG:species (random factor)			54.413	<0.001		19.30	<0.001			
2013										
Treatment	FLP				FRP			Seed mass		
	g.l.	est	χ^2	P	est	χ^2	P	est	χ^2	P
Warming(W)	1	-0.17	6.314	0.012	-0.33	7.25	0.007			
FG	2		6.454	0.040		41.02	<0.001			
Volume	1	0.59	101.778	<0.001	0.44	36.61	<0.001			
LA	1							0.09	7.80	0.005
SLA	1							-0.11	4.44	0.035
N° ind	1									
W * FG	2									
FG:species (random factor)			34.16	<0.001		0.04	0.84		167.671	<0.001

DISCUSSION

Flowering and fruiting responses to warming

In agreement with our hypothesis, warming promoted an advance of the onset of flowering, and of the onset and peak of fructifications, and also amplified the duration of flowering in the two years of study (Table 1). These results qualitatively agree with previous observational and manipulative studies conducted around the world (Fitter & Fitter 2002; Peñuelas *et al.* 2002; Matsumoto *et al.* 2003; Gordo & Sanz 2009). The onset of flowering was the variable most affected by warming, as some species advanced their flowering up to ~20 days in this treatment. This advance was, as far as we know, the largest recorded in warming experiments of perennial species, as warming commonly advances the onset of flowering by three days in northeast North America or four days in England (Fitter *et al.* 1995; Miller-Rushing & Primack 2008b). We suggest that the onset flowering would have a higher response to warming compared to the rest of phenological variables because it happens during the first stages of the growing season, when there are less water limitations (Fig. S3). Our results could help to increase our knowledge of flowering and fruiting responses of semiarid Mediterranean species to climate change, and could have important implications. For instance, the huge advances in flowering of some species could promote ecological asynchronies between these species and insects (Williams *et al.* 2007) that impact seriously in the future of some species.

While warming promoted a consistent advance of all phenological variables during the two years studied, its effects were particularly evident during the drier year. Other studies showed different phenological flowering responses to warming between years (Cleland *et al.* 2006; Liancourt *et al.* 2012). Our results and those from available

studies stress the importance of conducting multi-year studies when evaluating phenological responses to climate change. In this manipulative experiment, the 3 °C increase in temperature mimic climatic conditions forecasted for the 21st century (IPCC 2013) and plants responded with an advance in flowering and fruiting phenology. This result could help to increase our knowledge of species phenological sensitivity to climate change across Mediterranean semiarid herbaceous species. Therefore, monitoring changes in plant phenology could be an indicator of the development of ongoing climate change (Glick *et al.* 2011).

Reproductive responses to warming

Warming reduced flower and fruit production in the species studied. A reduction in the number of flowering units with warming is in agreement with our hypothesis. However, there are two contrasting theories about the relationship between leaf or flower phenology and flower and fruit production. Firstly, species that advanced their phenology under warming also increased their vegetative size, and higher vegetative size usually involves higher fitness (Cleland *et al.* 2012; Springate & Kover 2013). In our study, advances in flowering phenology were related with a longer flowering period, and other studies detail the relationship with larger growing season or faster development in growing season (Springate & Kover 2013). Therefore, we expect that in this situation species could accumulate higher amount of biomass (Hollister *et al.* 2005, Cleland *et al.* 2012), a response leading to more flowers and fruits, and to larger seeds. Previous studies have observed this phenomenon in temperate ecosystems, where warming increased flower and fruit production and seed mass (Hollister & Webber 2000; Lemmens *et al.* 2008; Springate & Kover 2013). Secondly, an earlier first flowering of plants could involve a reduced allocation of resources to growth, a smaller

size, and therefore fewer resources available for reproduction (Bolmgren & Cowan 2008). Further research is required to confirm and validate whether the increase in growing season is bigger than the increase in flowering season in temperate system, and by contrast in Mediterranean semiarid the growing season could increase but the flowering season increase more, promoting a reduction in the number of flowers and fruits. Flower and fruit production could be related with photosynthetic activity too; in our experiment, larger species such as *Medicago sativa* and *Dorycnium pentaphyllum* had higher photosynthetic rate than smaller species under warming at the end of the growing season (Enrique Valencia, unpublished data), and consequently had more fruits in this treatment (Table S4). The reduction in the reproductive variables observed with warming could be also explained by the decrease in soil moisture promoted by this treatment (Fig. S3), as speculate Liancourt *et al.* (2012). This effect of warming on soil moisture is particularly important at the end of growing season, where water is scarce and only larger size could face these stress-conditions (Villar-Salvador *et al.* 2013 and references therein).

Phenological responses vary according to the functional group considered

Reproductive phenology differed among functional groups, for instance forbs had an earlier flowering and fruiting phenology than legumes and grasses. This result is consistent among years, and agrees with our third hypothesis; i.e phenological responses vary among plant functional groups. Furthermore, taxonomic functional groups reflect differences among timing of flowering in the species studied. However, the fifth hypothesis, i.e functional groups with earlier flowering dates should advance the flowering variables with warming more than functional groups with later flowering dates, was not supported. Our predictions were based on Sherry *et al.* (2007), who

showed that grassland species flowering before the peak of summer temperatures advanced their flowering, while those flowering after delayed it (Sherry *et al.* 2007). Possibly, our lacks of results are related with the fact that our species flowering in spring and not in autumn, such Sherry experiment. Other studies showed differences in their flowering responses among functional groups to different parameters, such as CO₂, N deposition or grazing (Cleland *et al.* 2006, Spence *et al.* 2014). Manipulative experiments that separated among herbaceous species showed species-specific flowering response without an influence of these functional groups (Hollister & Webber 2000; Fitter & Fitter 2002; Liancourt *et al.* 2012). Overall, flowering and fructifications phenology vary with functional groups, but warming did not change these differences. Furthermore, the lack of differences preclude the possibility of a temporal overlap among functional groups, however could be a species-specific response.

Functional traits affect phenological responses

The traits selected differentiate basic functional properties of plants (Westoby *et al.* 2002), which are likely to be influenced by warming in a distinct manner. Regardless of this, functional size and leaf traits often separate between functional groups (Lavorel & Garnier 2002), but that is not the case in the studied species (Fig. S4). The first axis (volume) is related to plant size, and reflects the competitive plant ability against bioclimatic stress (Cornelissen *et al.* 2003; Pickering & Venn 2003). The observed relationships between increased plant size and an earlier flowering agree with previous studies (Bolmgren & Cowan 2008), and highlight the importance of measuring plant size in phenological studies. Logically, plant size also increased flower and fruit production, as larger plants had more flowering units due to the increased capacity to store resources. Leaf area (second axis) is related to light interception and absorption,

resource allocation, water uptake strategy and water stress tolerance (Westoby *et al.* 2002; Gross *et al.* 2007). We speculate that plants with higher LA could maintain flowers during more time because they could increase their photosynthetic rate, absorbing more resource for plants. However, higher LA also enhances evapotranspiration (Wright *et al.* 2004), and hence increases in temperature and reductions in soil moisture with warming can affect the onset of fructification as the growing season progresses. The third axis (SLA) reflects a trade-off between potential rate of return per leaf mass and duration of return and is a descriptor of dominant plant strategies (Westoby *et al.* 2002); for instance higher SLA is related with fast- growing strategy (Freschet *et al.* 2011). Plants with low SLA had earlier onset and peak of flowering, but only in the wetter year. Both LA and SLA increased and decreased seed mass, respectively. The relationship between seed mass and both traits agree with previous studies (Cornelissen 1999; Reich *et al.* 1998). This is possible a cascading effect between the different traits and leaf and flower phenology, where we must consider each one to explain the other because they evolve together (Oberrath & Böhning-Gaese 2002). In summary, larger species with small LA and SLA had an earlier flowering phenology and larger flowering duration. However, the flower and fruit production only depend on species size. Future investigation could be interesting about trait response to warming and if these changes modify plant vegetative and reproductive phenology responses.

CONCLUDING REMARKS

Mediterranean semiarid species showed an advance on flowering and fruiting phenology, larger flowering duration and a reduction on the number of flower and fruit in response to warming. The large advance in onset of flowering in some species (~20 days) could be related with the high plasticity of dryland species, as resources (water) are more abundant at the beginning of the flowering period. This result could have different implications, for instance potential ecological asynchronies between plants and insects (Williams *et al.*, 2007), or optimization of management agriculture (Ganskopp *et al.*, 2007). However, soil moisture decreases as the flowering period progresses, even more under warming, promoting the observed reductions in flower and fruit production. In a climate change context, these changes could affect phenological complementarity among species, and together with the observed decrease in the number of flowers and fruits due to warming, could lead to changes in the composition, abundance or distribution of species in the communities (Walther *et al.*, 2002). Additionally, functional groups could separate species between timing of flowering in all phenological variables; however, we did not find responses to warming. Furthermore, the changes promoted by warming were species-specific. On the other hand, larger species with small LA and SLA, had an earlier flowering phenology and a longer flowering period. Overall, plant traits must be taken in account when the aim is make accurate predictions of the phenological response of plants.

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SUPPORTING INFORMATION

Appendix A. Supplementary data

a)



b)



Fig. S1. General view of the sampling area, with microcosms situated in warming and control treatments (a), and detailed view of an open top chamber (b).

Table S1. Information about the species studied.

Species	Family	Functional group	Flowering unit
<i>Cynodon dactylon</i> (L.) Pers.	Poaceae	Grasses	Inflorescence
<i>Poa pratensis</i> (L.)	Poaceae	Grasses	Inflorescence
<i>Agropyrum cristatum</i> (L.) Gaertn	Poaceae	Grasses	Inflorescence
<i>Festuca ovina</i> (L.)	Poaceae	Grasses	Inflorescence
<i>Hedysarum coronarium</i> (L.)	Fabaceae	Nitrogen-fixing legumes	Inflorescence
<i>Dorycnium pentaphyllum</i> (Scop.)	Fabaceae	Nitrogen-fixing legumes	Inflorescence
<i>Anthyllis vulneraria</i> (L.)	Fabaceae	Nitrogen-fixing legumes	Inflorescence
<i>Medicago sativa</i> (L.)	Fabaceae	Nitrogen-fixing legumes	Inflorescence
<i>Psoralea bituminosa</i> (L.)	Fabaceae	Nitrogen-fixing legumes	Inflorescence
<i>Plantago lanceolata</i> (L.)	Plantaginaceae	Forbs	Inflorescence
<i>Sanguisorba minor</i> (Scop.)	Rosaceae	Forbs	Inflorescence
<i>Echium boissieri</i> (Steud)	Boraginaceae	Forbs	Flower
<i>Echium plantagineum</i> (L.)	Boraginaceae	Forbs	Flower
<i>Asphodelus fistulosus</i> (L.)	Xanthorrhoeaceae	Forbs	Flower

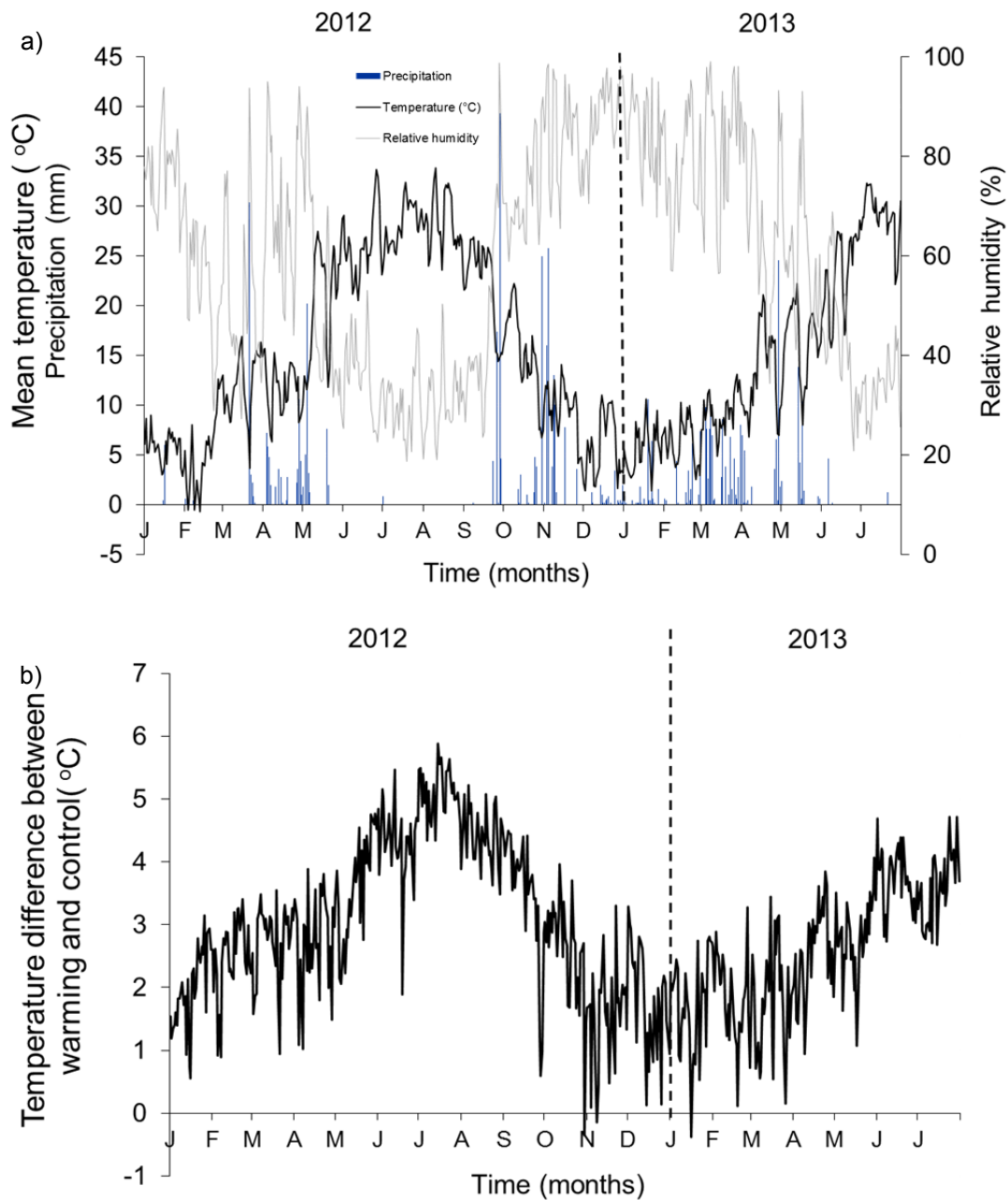


Fig. S2. Temporal variation in air temperature, air relative humidity and precipitation (a), and temperature difference between warming and control treatments (b) from January 2012 to October 2013 in the experimental area. The standard errors for air temperature are omitted for clarity (data present the average of $n = 3-4$ sensors).

Table S2. Pearson correlation coefficients among trait values in 2012 and 2013. TC: Total cover; VH = vegetative height; LL = leaf length; LT = leaf thickness; LA = leaf area; LW = leaf width; SLA = specific leaf area; LDMC = leaf dry matter content; Volume = TC x VH.

Variables		VH	LL	LT	LA	LW	SLA	LDMC	Volume
TC	Correlation	0.401	-0.112	0.099	-0.069	0.022	0.044	-0.082	0.706
	P Value	0.000	0.280	0.342	0.507	0.829	0.669	0.429	0.000
VH	Correlation		-0.333	0.013	0.149	0.348	-0.001	-0.001	0.914
	P Value		0.001	0.898	0.148	0.001	0.993	0.993	0.000
LL	Correlation			0.224	0.574	-0.078	0.018	-0.398	-0.319
	P Value			0.029	0.000	0.455	0.865	0.000	0.002
LT	Correlation				0.328	0.122	-0.682	-0.836	0.057
	P Value				0.001	0.238	0.000	0.000	0.586
LA	Correlation					0.683	-0.009	-0.478	0.076
	P Value					0.000	0.930	0.000	0.466
LW	Correlation						0.135	-0.267	0.286
	P Value						0.193	0.009	0.005
SLA	Correlation							0.225	0.021
	P Value							0.029	0.843
LDMC	Correlation								-0.043
	P Value								0.677

2013

Variables		VH	LL	LT	LA	LW	SLA	LDMC	Volume
TC	Correlation	0.800	-0.307	-0.280	-0.012	0.174	0.131	0.249	0.960
	P Value	0.000	0.002	0.000	0.904	0.084	0.193	0.013	0.000
VH	Correlation		-0.137	-0.260	0.096	0.296	0.084	0.269	0.921
	P Value		0.175	0.010	0.343	0.003	0.404	0.007	0.000
LL	Correlation			0.253	0.519	-0.237	-0.215	-0.233	-0.226
	P Value			0.011	0.000	0.018	0.031	0.020	0.024
LT	Correlation				0.176	-0.136	-0.747	-0.721	-0.284
	P Value				0.079	0.178	0.000	0.000	0.004
LA	Correlation					0.547	0.108	-0.498	0.052
	P Value					0.000	0.285	0.000	0.610
LW	Correlation						0.412	-0.236	0.213
	P Value						0.000	0.018	0.033
SLA	Correlation							0.156	0.108
	P Value							0.120	0.284
LDMC	Correlation								0.265
	P Value								0.008

Table S3. Eigenvectors of the trait variables used in the Principal Component Analysis in 2012 and 2013. TC: Total cover of the microcosm; VH = vegetative height; LL = leaf length; LT = leaf thickness; LA = leaf area; LW = leaf width; SLA = specific leaf area; LDMC = leaf dry matter content; Volume = TC x VH

	2012			2013		
Variables	PC1 (34%)	PC2 (27%)	PC3 (17%)	PC1 (35%)	PC2(27%)	PC3(20%)
TC	0.42	0.19	-0.23	0.46	-0.22	0.24
VH	0.43	0.31	-0.11	0.45	-0.29	0.16
LL	0.04	-0.44	0.17	-0.28	-0.37	0.10
LT	0.24	-0.51	-0.30	-0.30	-0.44	0.26
LA	0.35	-0.26	0.49	-0.04	-0.50	-0.40
LW	0.35	0.06	0.48	0.22	-0.21	-0.56
SLA	-0.08	0.26	0.55	0.25	0.16	-0.53
LDMC	-0.30	0.43	0.02	0.28	0.39	0.20
Volume	0.48	0.29	-0.19	0.48	-0.26	0.22

Table S4. Differences between warming and control plots in phenological events and reproductive variables for each species in 2012 and 2013. oFl: onset of flowering; cFl: cessation of flowering; Range: flowering duration; pFl: peak of flowering; oFr: onset of fructification; pFr: peak of fructification; FIP: flower production; FrP: fruit production. ND: No data.

Species	oFl (days)	cFl (days)	Range (days)	pFl (days)	oFr (days)	pFr (days)	Seed Mass (%)	FIP (%)	FrP (%)
2012									
<i>Cynodon dactylon</i>	-5.1	-0.4	4.9	-3.7	0.9	-0.3	ND	-47.2	184.4
<i>Poa pratensis</i>	12	2	-10	4	0	4	ND	4.5	-24.7
<i>Agropyrum cristatum</i>	21.0	-6.4	-27.4	11.3	1.4	-0.3	ND	80.1	289.5
<i>Festuca ovina</i>	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Hedysarum coronarium</i>	8.8	2.5	-6.3	-4.5	1.3	0	ND	19.1	409.6
<i>Dorycnium pentaphyllum</i>	9.3	6.7	-2.6	11	1.2	-1	ND	-24.5	-42.8
<i>Anthyllis vulneraria</i>	20.8	10.5	-10.3	11	ND	ND	ND	38.7	ND
<i>Medicago sativa</i>	18.8	17.0	-1.8	20.0	2.5	-0.5	ND	-4.4	1.6
<i>Psoralea bituminosa</i>	4	-7.7	-11.7	-3	-1.7	-4	ND	36.1	7.2
<i>Plantago lanceolata</i>	17.3	9.5	-7.8	14.5	4	3.3	ND	-1.6	23.3
<i>Sanguisorba minor</i>	3	13.0	10	1.3	1.8	1.5	ND	-4.9	14.1
<i>Echium boissieri</i>	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Echium plantagineum</i>	-3.6	-18.0	-14.4	-8.2	ND	ND	ND	29.4	ND
<i>Asphododius fistulosus</i>	4.8	3.8	-1	7.8	7.3	10.5	ND	-52.3	-43.7
2013									
<i>Cynodon dactylon</i>	24.3	-5.3	-29.5	-7.3	2.3	1.3	12.2	200.0	5.3
<i>Poa Pratensis</i>	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Agropyrum cristatum</i>	6.7	19.8	13.2	11.2	5.7	10.7	-68.6	-84.6	-87.4
<i>Festuca ovina</i>	1.5	1	-0.5	2.8	2.5	-0.5	-21.3	-56.7	-64.6
<i>Hedysarum cristatum</i>	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Dorycnium pentaphyllum</i>	8.3	-2.5	-10.8	1.5	0.8	5.8	11.5	7.5	77.2
<i>Anthyllis vulneraria</i>	13	18.0	5	10.8	1	-1.3	4.9	-60	-55.6
<i>Medicago sativa</i>	2.3	0	-2.3	-13.8	5.5	2.8	29.1	-15.3	53.5
<i>Psoralea bituminosa</i>	4.9	0.8	-4.2	2.4	0	-0.1	-13.3	18.8	78.6
<i>Plantago lanceolata</i>	8	0.5	-7.5	18.8	0	0	-16.6	60.8	-10.8
<i>Sanguisorba minor</i>	3.5	-8.5	-12	2.5	0.5	4.5	-7.7	-23.3	-15.8
<i>Echium boissieri</i>	18.5	1	-17.5	8.3	-5.1	5.3	-10.6	97.7	-26
<i>Echium plantagineum</i>	15.5	-5.8	-21.3	6.8	7.0	1.7	32.3	227.9	-67.4
<i>Asphododius fistulosus</i>	2.3	0	-2.3	-1	4	0.5	3.8	-73.6	-85.7

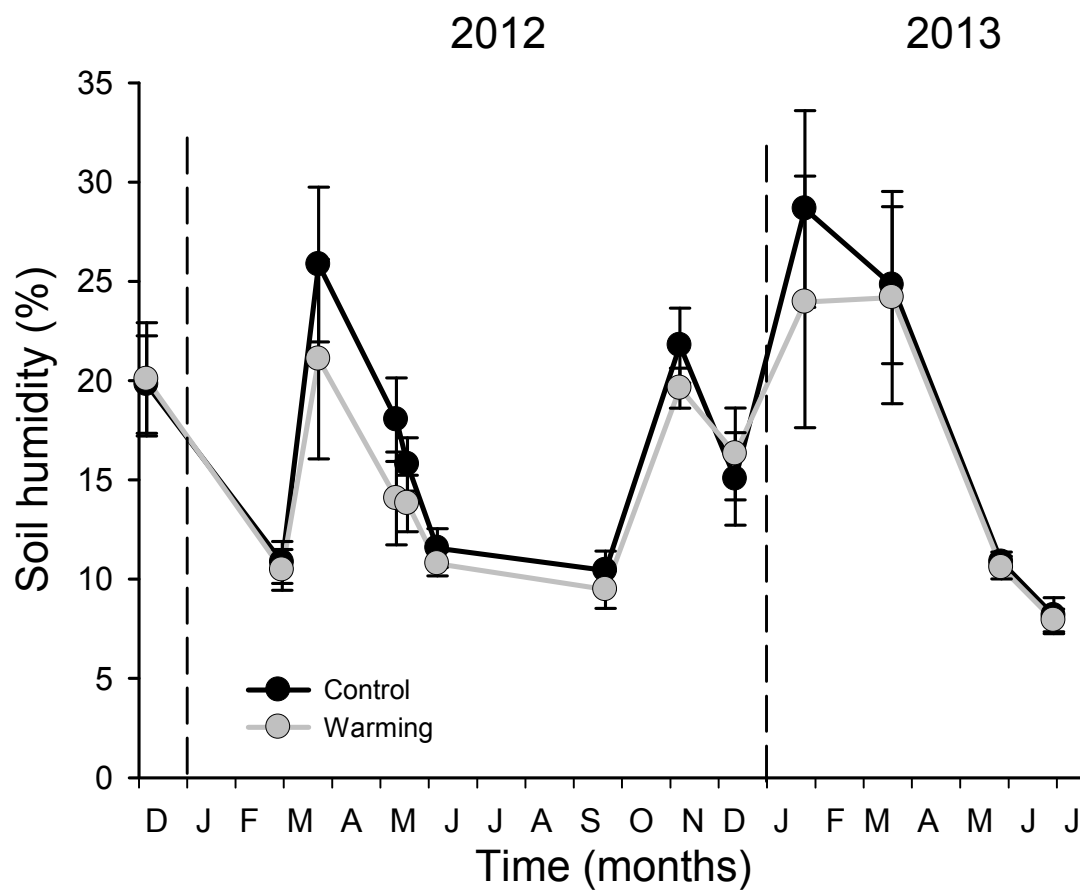


Fig. S3. Temporal variation in soil moisture in microcosms without plants between December 2011 and July 2013. Data represent means \pm SE ($n = 4$).

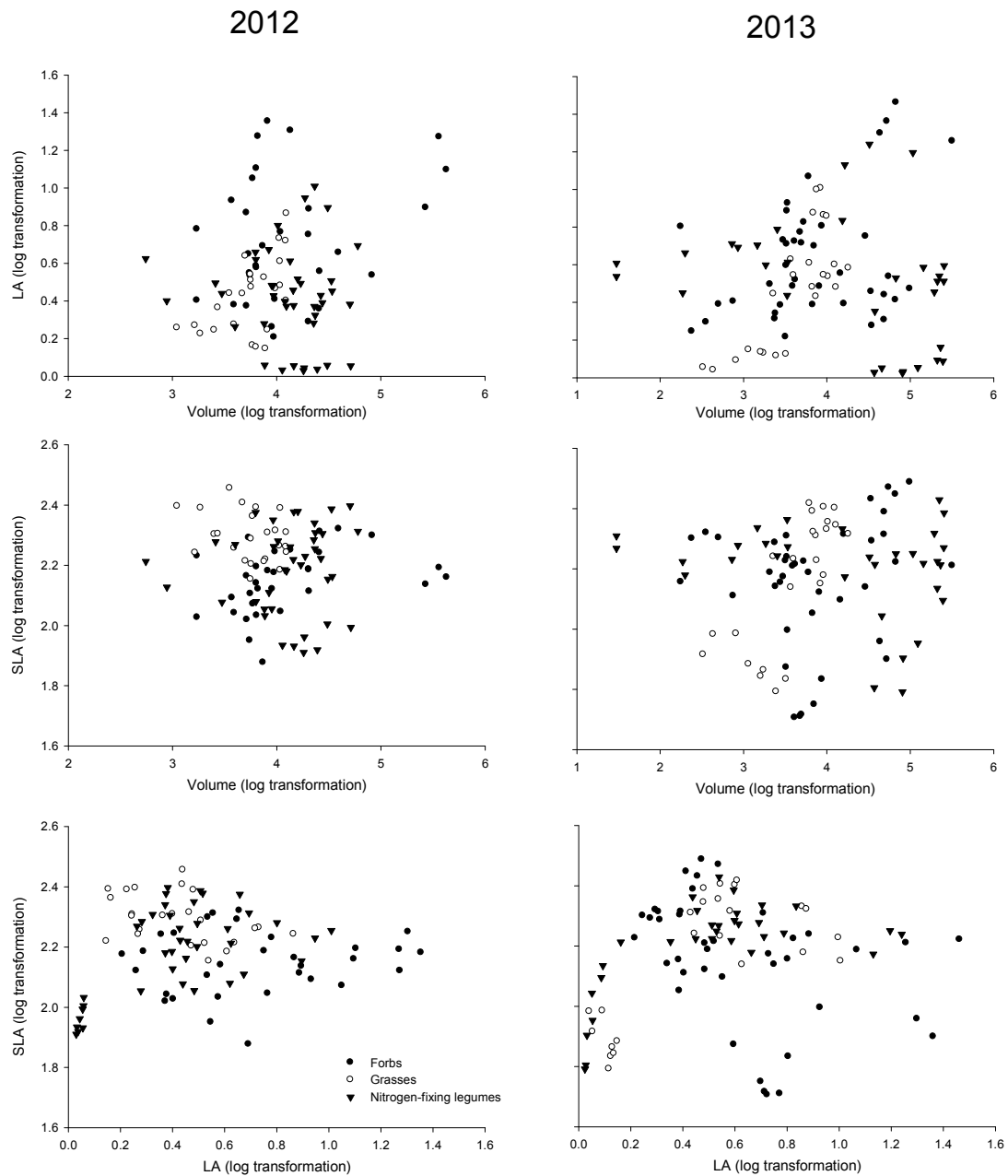


Fig. S4. Relationships among functional traits. LA = leaf area; SLA = specific leaf area; Volume = Total cover of the microcosm x vegetative height.

Effects of plant species richness on multifunctionality are stronger than those of simulated climate change, being mediated by functional traits and soil microorganisms

CAPÍTULO 5



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En fase de preparación para su envío a *Global Change Biology*.

SUMMARY

Changes in biodiversity and climate change significantly affect ecosystem functioning worldwide. However, how these factors interact with plant functional structure and soil microorganisms to determine ecosystem responses to global change are largely unknown. We conducted a common garden experiment over two years to evaluate how changes in plant functional structure and soil microorganisms (bacteria and protozoa) promoted by climate change drivers (a 3°C temperature increase and a 35% rainfall reduction) and species richness impact on multifunctionality (i.e. the provision of multiple ecosystem functions and services simultaneously). The effect of species richness on multifunctionality were largely mediated by changes in the functional identity of plants and by increases in the abundance of microorganisms induced by plant species richness. However, our climate change treatments promoted slight changes on variables related to C and P cycling. Increasing species richness in plant communities could be the major driver of changes in soil processes and ecosystem functioning, helping to improve the quality of the soils, regardless of the climatic condition considered.

Keywords: bacteria, biodiversity, climate change, dryland, ecosystem functioning, nutrient cycles, protist, species richness.

INTRODUCTION

During the last twenty years, the relationship between biodiversity and ecosystem functioning has been a main topic in community and ecosystem ecology (see Naeem *et al.*, 2009; Cardinale *et al.*, 2011 for recent reviews). Our current understanding primarily results from studies that have focused on the role of species richness and, to a lesser degree, of functional group richness in driving above-ground production in addition to other ecosystem functions (e.g. Loreau, Naeem & Inchausti, 2002; Hooper *et al.*, 2005; Cardinale *et al.* 2011). Most of this research has focused on evaluating ecosystem function in isolation (e.g. productivity, Tilman *et al.*, 1997; Montés *et al.*, 2008; Hector *et al.*, 2011). However, ecosystems are valued primarily for the multiple ecosystem functions and services they provide, and thus there is a critical need to examine the effects of biodiversity on multiple ecosystem functions simultaneously (multifunctionality; Stachowicz, Bruno & Duffy, 2007; Reiss *et al.*, 2009; Cardinale *et al.*, 2011). While the use of multiple functions in biodiversity–function research is gaining momentum (Gamfeldt *et al.*, 2008; Pasari *et al.*, 2013; Byrnes *et al.*, 2014), there is a lack of studies evaluating how changes in biodiversity and climate jointly modify multifunctionality. As alterations in climate and biodiversity are key components of ongoing global environmental change, such studies are needed to better understand the ecological consequences of such change.

Drylands (i.e., arid, semi-arid and dry-subhumid ecosystems) are among the most sensitive ecosystems to climate change (Maestre *et al.*, 2012a). These ecosystems cover 40% of the Earth's land surface (Safriel & Adeel, 2005), where live 38% of the total population (Reynolds *et al.*, 2007). Drylands provide goods and services crucial for the sustainability of the global human population, have important effects on global biodiversity (Maestre *et al.*, 2012a) and account for ca. 25% of soil organic carbon

reserves (Safriel & Adeel, 2005). Climate change could enlarge the occupied area by drylands in the future, as climatic models forecast temperature increases ranging from 3.2 °C to 3.7 °C and alterations in precipitation amount and patterns by the second half of the twenty-first century (Solomon *et al.*, 2007). Despite their importance, the effects of climate change drivers on crucial ecosystem processes, such as nutrient cycling or soil respiration, have been much less studied in drylands than in other biomes (Schimel, 2010; Ciais *et al.*, 2011).

Climate change drivers, such as temperature increases or precipitation changes, can directly modulate ecosystem functioning (for instance, by modifying nutrient cycling, Delgado-Baquerizo *et al.*, 2013a). However, ecosystem functional response also could be mediated by ‘indirect effects’ via changes in plant community structure (Suding *et al.*, 2008). The literature highlights the “mass-ratio hypothesis” (Grime, 1998) to explain these changes in community structure. This theory considers that the effects of plant communities on ecosystem functioning are largely driven by the trait of the dominant species (Grime, 1998) captured by community-weighted functional traits (CWT hereafter, Violle *et al.*, 2007; Suding *et al.*, 2008). For instance, Lavorel *et al.* (2011) showed that size and leaf traits responded to land use and abiotic environment, and had important effects on several ecosystem properties. Despite the interest of this hypothesis, it has barely been applied to drylands (de Bello *et al.*, 2010, but see Valencia *et al.*, 2015). As such, an approach based on functional traits could provide important insights into the mechanisms underlying the indirect effects of biodiversity and climate change on multifunctionality.

Plant-soil interactions are central in explaining ecosystem multifunctionality (Zak *et al.*, 2003; Wagg *et al.*, 2014). In addition, climate change drivers may impact the activity of soil microorganisms (Robinson, 2002) directly, via changes in soil

conditions, or indirectly, via alterations in plant growth and in the quantity and quality of litter inputs (Bardgett *et al.*, 2008). For instance, warming will accelerate the activity rates of heterotrophic microorganisms, thereby increasing the outflow of CO₂ into the atmosphere and the leaching of dissolved organic carbon (Jenkinson *et al.*, 1991; Davidson & Janssens, 2006). Also, predicted climate change will likely reduce available soil moisture in drylands (Feng & Fu, 2013), affecting soil moisture conditions that influence the activity of soil microorganisms (Castro *et al.*, 2010). Moreover, the community structure of plant communities may explain the patterns that affect soil microorganisms (de Vries *et al.*, 2012). Changes in plant community structure by climate change can alter plant-soil interactions, with potential consequences for ecosystem nutrient cycling and the exchange of carbon in the soil (Dorrepaal *et al.*, 2005; Fontaine & Barot, 2005). Thus, soil microorganisms are involved in decomposition processes of organic matter and biogeochemical nutrient cycles too (Ingham *et al.*, 1985; de Vries *et al.*, 2013). Overall, soil microorganisms may change with climate change and community species richness, and these changes affect multifunctionality. The literature about these relationships generally focus on bacteria and fungi microorganism, and rarely evaluates higher trophic levels such as protists, regardless they occupy a key position within the soil food web (Bonkowski, 2004). These eukaryotic unicellular organisms feed on soil bacteria and yeasts, linking the flow of energy and the cycling of nutrients to higher trophic levels (Bonkowski, 2004). For instance, they could explain an important part of the nitrogen mineralization process (Ingham *et al.*, 1985 and references therein). In summary, it is important to evaluated their response to climate change and plant diversity, and their effects on ecosystem functioning.

Despite the large number of studies conducted on the ecological consequences of

climate change, the effects of climate change drivers and plant diversity on multifunctionality, mediated by joint changes in different trophic levels, such as plants (community structure), bacterial and protist communities in drylands have never been evaluated. We aimed to do so using a full factorial experiment that involved the use of open top chambers (OTCs) to change temperature, rainfall shelters to alter precipitation, and multiple levels of plant species richness. Using this experimental design, we aimed to evaluate how climate change (warming and rainfall reduction), plant species richness, plant functional structure and soil microorganisms impact multifunctionality (Fig. 1). We tested the following hypotheses: (1) increases in plant species richness will affect plant functional structure and the abundance of soil microorganisms (Steinauer *et al.*, 2015), (2) increases in plant species richness will have direct and indirect, via plant functional community structure and the abundance of and soil microorganisms, effects on multifunctionality (Loreau *et al.*, 2001; Maestre *et al.*, 2012b), (3) increases of temperature and decreases of rainfall could promote a decrease in plant size and small leaves, because plants with these traits are better adapted to arid environments (Westoby *et al.*, 2002) and (4) the main effects of climate change drivers on multifunctionality should be mediated by plant functional structure (Valencia *et al.*, 2015).

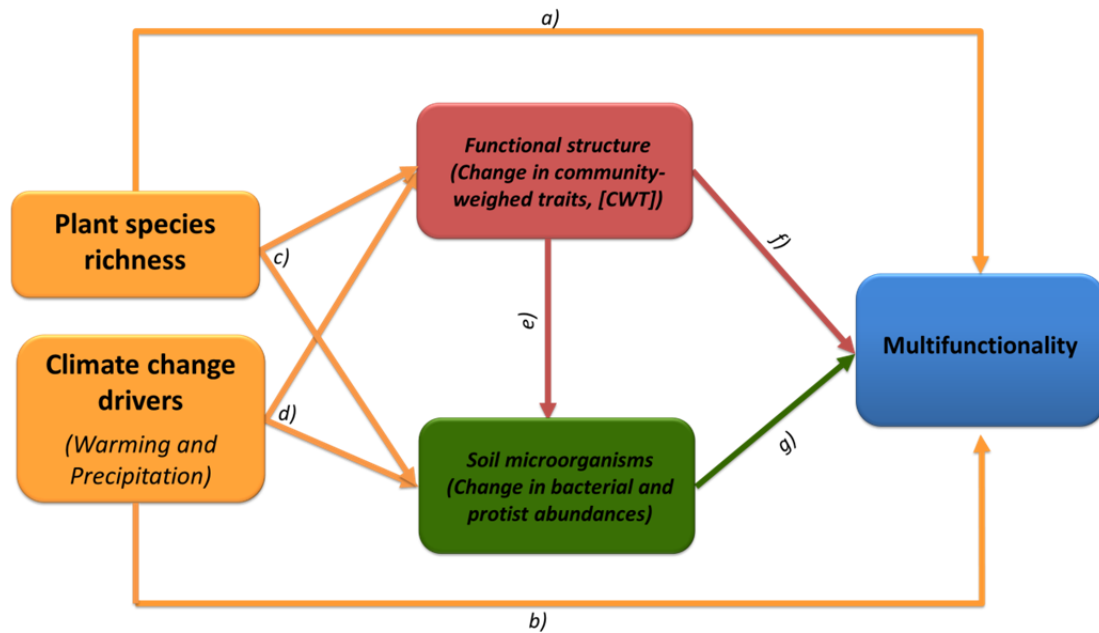


Fig. 1. Hypothetical relationships between climate change drivers, plant species richness, functional structure, soil microorganism community and multifunctionality. Our treatments: warming and rainfall reduction, as climate change drivers, and plant species richness directly impact multifunctionality (“a” and “b”). The left part of the figure represents the response of the functional structure (CWT: mean trait distribution) and soil microorganism community (bacterial and protist abundance) to the treatments (two “c” and two “d” arrows); and co-variation between CWT and microorganism abundance. Changes in community functional structure (mass-ratio process, arrow “f”) and microorganism community (arrow “g”) promote effects on multifunctionality.

MATERIAL AND METHODS

Our microcosm experiment took place in the Climate Change Outdoor Laboratory (CCOL), established at the facilities of Rey Juan Carlos University (URJC, Móstoles, Spain: 40°20'37''N, 3°52'00''W, 650 m a.s.l.; Fig S1), between April 2011 and September 2013. The average environmental variables (mean annual temperature 16.6°C, mean annual precipitation 362 mm) were taken from the university meteorological station. The experiment comprised three treatments: plant richness (one, three and six species), temperature (control vs. +3°C temperature increase) and rainfall exclusion (control vs. ~35% reduction in total annual rainfall).

In April 2011, we randomly sowed seeds (commercial supplier; Intersemillas Ltd, Valencia, Spain) of each species within each microcosm in plastic pots (depth 38 cm, internal diameter 28 cm, volume 0.023 m³). Previously, the base of the pots was filled with 3 cm of expanded clay for drainage and then 32 cm of natural soil (sand content: 73.5 %, silt content: 18.5 %, clay content: 8.0 %). All the pots were placed in holes in the ground, and kept under ambient light and rainfall to keep the most natural conditions (Fig. S1b). All the microcosms were initially irrigated with 500 mL of a soil microbial inoculum to recreate realistic soil microbial communities, as described in Maestre *et al.* (2005). We planted a plant assemblage in each microcosm reaching a final density of 194 individuals/m² per species. We used herbaceous perennial plants species typical of grasslands developing in abandoned fields and restored roadside slopes in semi-arid areas from central Spain (García-Palacios *et al.*, 2010; Mola *et al.*, 2011). The 27 selected species (Table S1) belong to three main functional groups (grasses, nitrogen-fixing legumes and forbs), which reflect differences in traits that are

potentially relevant to the physiological variables of interest for this study, such as biomass production, resource use and N-fixation ability (Reich *et al.*, 2001; Gross *et al.*, 2007; McLaren & Turkington, 2010). Each community was obtained by randomly selecting from the pool of species one, three or six species.

The microcosms were irrigated with 1L three times per week during the first 6 weeks of the experiment to improve seed establishment, and once a week in July and August 2011. We watered during the summer to ensure the survival of the communities because extreme condition of Mediterranean climates could reduce the planted species density in each microcosm before the installation of the warming and rainfall exclusion treatments. Weeds were regularly removed along the experiment.

The warming treatment aimed to simulate the climatic models predictions that stipulate an increase in annual temperature ranging between 2-3 °C in the study area by the end of the 21st century in central and south-eastern Spain (de Castro *et al.*, 2005; IPCC, 2013). To achieve this temperature increase, we used open top chambers (OTCs) similar to those employed in climate change experiments conducted around the world (Hollister & Weber, 2000; Klanderud & Totland, 2005; Escobar *et al.*, 2012; Spence *et al.*, 2014). OTCs were built with six methacrylate plates, using a hexagonal design with sloping sides of 65-52-42 cm (Fig. S1c). Methacrylate material transmits ~92% of visible spectrum, has a 4% emission of the infrared wavelength and passes on 85% of incoming energy (data provided by the manufacturer, Decorplax Metacrilatos S.L., Madrid, Spain). The OTCs were elevated 5 cm from the soil surface to achieve adequate air flow and avoid excessive overheating.

The rainfall exclusion treatment was obtained using passive rainfall shelters, which were based upon the design of Yahdjian & Sala (2002). Each rainfall shelter has an area of 1.68 m² (1.4 m × 1.2 m), and a mean height of 1 m, and is composed of six

methacrylate grooves (Fig. S1d), which cover approximately 35% of the surface, connected to plastic bottles that accumulate the excluded water. These shelters did not modify the frequency of rainfall events, but reduced the size of individual rain events and the total amount of rainfall.

The effects of the OTCs and rainfall shelters on air temperature and humidity were monitored using automated sensors (HOBO U23 Pro v.2 Temp/RH, Onset Corporation, Bourne, MA, USA). The warming and rainfall exclusion treatments were setup in in December 2011, once all the microcosms had an established population. Each treatment combinations were replicated ten times. We eliminated one monoculture of all combinations (four microcosms), because suffered a severe mortality just after the end of irrigation, so the experiment had 116 microcosms in total.

Experimental measurements and harvest

We measured different functional traits at the end of the second growing season (June 2013), just after the peak of vegetation growth to avoid summer drought. The following traits were measured following standard protocols (Cornelissen *et al.*, 2003) on one individual per species and microcosm: vegetative height (VH, cm), lateral spread (LS, cm²), leaf area (LA, cm²), leaf length (LL, cm), leaf width (LW, cm) and leaf thickness (LT, mm), phenology, measured using a phenology index (1 = no reproductive stem; 2 = reproductive stem starting to grow; 3 = flowering; 4 = flower fading; 5 = fruit present; and 6 = fruit absent and senescence of the reproductive stem); and specific leaf area (SLA, cm² g⁻¹), leaf dry matter content (LDMC, g g⁻¹). These traits were selected because they reflect different plant species strategies for acquiring, using and conserving resources (including light, nutrients and water), and for exploiting different

temporal niches (Westoby *et al.*, 2002; Maire *et al.*, 2009). Additionally, we visually estimated total plant cover (cm²) per species in each microcosm.

We cut at the soil surface above-ground biomass of each microcosm at the beginning of September (2013), then dried at 60°C during 72 h and finally weighed. Above-ground plant biomass (APB) is a good proxy of above-ground net primary productivity in experiments such as that we conducted (Scurlock, *et al.*, 2002). Later, three soil cores (0–7.5 cm depth) per microcosm were sampled, which were bulked and homogenized to obtain a unique sample per microcosm. The soil samples were sieved (2 mm mesh) in the laboratory and separated into two fractions. One fraction was air dried for one month for measure soil variables related to nutrient cycles and estimate protist abundance, and the other fraction was frozen at -20° C for quantify the amount of bacterial.

Laboratory analyses

In each air-dried soil fraction, we measured 7 variables related to carbon (organic C, β -glucosidase activity), nitrogen (total N, ammonium, nitrate) and phosphorus (available inorganic P and phosphatase activity) biogeochemical cycles. These variables constitute a good proxy for nutrient cycling, biological productivity, and buildup of nutrient pools, and are important determinants of ecosystem functioning in drylands (Whitford, 2002). Organic C was obtained by colorimetry after oxidation with a mixture of potassium dichromate and sulfuric acid (Anderson & Ingram, 1993). Phosphatase activity was measured by determination of the amount of p-nitrophenol (PNF) released from 0.5 g soil after incubation at 37 °C for 1 h with the substrate p-nitrophenyl phosphate in MUB buffer (pH 6.5; Tabatabai & Bremner, 1969). The activity of β -glucosidase was assayed following the procedure for phosphatase, but using p-nitrophenyl- β -Dglucopyranoside

as substrate and Trishydroxymethyl aminomethane instead of NaOH when preparing the buffer (Tabatabai, 1982). Total N was obtained using a CN analyzer (Leco CHN628 Series, Leco Corporation, St Joseph, MI, USA). Available P was measured following a 0.5M NaHCO₃ (pH: 8.5) extraction (Olsen & Sommers, 1982). Soil extracts in a ratio of 1:5 were shaken in a reciprocal shaker at 200 rpm for 2 h. An aliquot of the centrifuged extract was used to the colorimetric determination of P inorganic available (PO₄⁻³), based on the reaction with ammonium molybdate and development of the “Molybdenum Blue” color (Bray & Kurtz, 1945); the pH of the extracts was adjusted with 0.1N HCl when necessary. The remaining soil variables were measured from K₂SO₄ 0.5 M soil extracts in a ratio 1:5. Soil extracts were shaken in an orbital shaker at 200 rpm for 1 h at 20°C and filtered to pass a 0.45-µm Millipore filter (Jones & Willett, 2006). The filtered extract was kept at 2°C until colorimetric analyses, which were conducted within the 24 h following the extraction. Ammonium (NH₄⁺ -N), nitrate (NO₃⁻ -N) were determined by described Delgado-Baquerizo *et al.* (2010).

The abundance of bacteria was estimated using quantitative PCR. Soil DNA was extracted from defrosted soil using the Powersoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA), according to the instructions provided by the manufacturer. We performed quantitative PCRs on an ABI 7300 Real-Time PCR (Applied Biosystems, Foster City, CA, USA) following Delgado-Baquerizo *et al.* (2013b). The total bacterial 16S was amplified with the Eub 338 – Eub 518, as described in Maestre *et al.* (2013). Results were expressed as number of copies of genes g soil⁻¹.

The abundance of protists was determined using a modified version of the liquid aliquot method (LAM), according to Butler and Rogerson (1995). Additionally 1g of fresh soil was dried at 60°C over 24h. Afterwards the soil was weighed again and the

differences from fresh weight to dry weight were calculated. Finally, results were expressed as total numbers of protists per g dry weight soil.

Assessing multifunctionality

We estimated multifunctionality from all soil variables measured using the quantitative multifunctionality index (M) of Maestre *et al.* (2012b). This index provides an intuitive way to assess changes in multifunctionality, for instance the higher the values for the different ecosystem functions we measured, the higher the overall ecosystem functioning (i.e., M) (Byrnes *et al.*, 2014). Multifunctionality index (M) was obtained at each microcosm and calculated with the 7 soil variables evaluated. These estimates were obtained by using a weighted average of the mean values of each soil variable. Raw data were normalized prior to these calculations; a log10-transformation normalized the variables evaluated. Following this, the Z scores of the 7 variables were averaged to obtain M. This index is being increasingly used when assessing multifunctionality (Quero *et al.*, 2013; Bradford *et al.*, 2014; Pendleton *et al.*, 2014; Wagg *et al.*, 2014; Valencia *et al.*, 2015). Additionally, we calculated functions related carbon, nitrogen and phosphorus cycles by averaging the Z scores of the variables involved in each C, N and P cycle.

Statistical analyses

Functional traits variation among plant microcosms

Community structure response to climate change drivers and species richness was quantified using CWT (Violle *et al.*, 2007). The CWT estimated the “average trait value”, weighted by the relative abundance (total cover) of every species at each microcosm at the end of the experiment. Trait variables were normalized using a log

transformation to reduce the impacts of traits of non-abundant species with unusually high trait values. Functional changes between microcosms were assessed using a principal component analysis (PCA) using the CWT values of the nine traits measured. We used a PCA for these analyses because different traits are associated with different niche axes (Butterfield & Suding, 2013; Ebeling *et al.*, 2014). For each component with an eigenvalue higher than 1, we recorded the PCA coordinates of each microcosm. We used these coordinates to quantify the functional structure of the studied communities. This procedure has the advantage to consider the correlation among traits, and allow us to identify independent axes of specialization for further analyses.

Response of community structure and soil microorganism community to climate change drivers and plant species richness

We first evaluated how community structure, represented by PCA axes of CWT (dependent variables), responded to warming, rainfall reduction and plant species richness (independent variables). To do so, we selected the best model with a stepAIC procedure (following Grace *et al.*, 2006), based on the Akaike information criterion (AIC) (Akaike, 1973). On the other hand, we used the same procedure to evaluate the relationship between soil microbial community (both bacteria and protist; dependent variables), climate change drivers, plant species richness and PCA axes of CWT (independent variables).

Multifunctionality response to climate change drivers and plant species richness

A confirmatory path analysis using a d-sep approach (Shipley, 2009; Laliberté & Tikilianakis, 2012) was carried out to test causal relationships between climate change drivers, plant species richness, community structure, soil microorganisms and multifunctionality. The d-step approach offers a flexible way to test causal relationships

between variables in path analyses by relaxing some important limitations of standard structural equation models, including non-normal data distribution, non-linear relationships between variables and small sample sizes (Shipley, 2000; Grace, 2006). This method is based on an acyclic graph that summarizes the hypothetical relationships between variables to be tested using the C statistic (Shipley, 2013). To simplify our a priori model (Fig. 1), we first conducted a stepAIC procedure to select the most appropriate multifunctionality predictors. Finally, we used standardised path coefficients to measure the direct and indirect effects of predictors (Grace & Bollen, 2005). We also evaluated the effect of all these predictors on C, P and N nutrient cycles and APB, instead of multifunctionality.

All statistical analysis were conducted with R (R Core Development Team, 2012), using packages “lme4” (Bates *et al.*, 2014).

RESULTS

Our warming treatment increased the air average temperature by 2.9 °C and reduced the air relative humidity in 0.9% (Fig. S2). Rainfall shelters promoted a very slightly increase in air temperatures of 0.1 °C compared to the control, and a reduction in the air relative humidity of 1.2% (Fig. S2). Finally, the combination of rainfall shelters and warming treatment promoted an average increase of 3.2 °C in air temperature and an average reduction of 2.2% in air relative humidity (Fig. S2). In the case of rainfall shelters, treatment also decreased the amount of water reaching the soil by an average 35%, varying between a minimum of 9% and maximum of 52% depending on the event (Fig. S2).

Functional structure

CWTs segregated along two PCA components the microcosm community studied and represented 59% of the total variance (Fig. 2). The first PCA component was identified as an axis of plant size (VH, LS), and accounted for 32% of the total variation. The second PCA component (27% of the variance; CW- leaf trait hereafter) contrasted microcosm community according to their leaf traits, where LL, LT and LA correlated negatively with SLA.

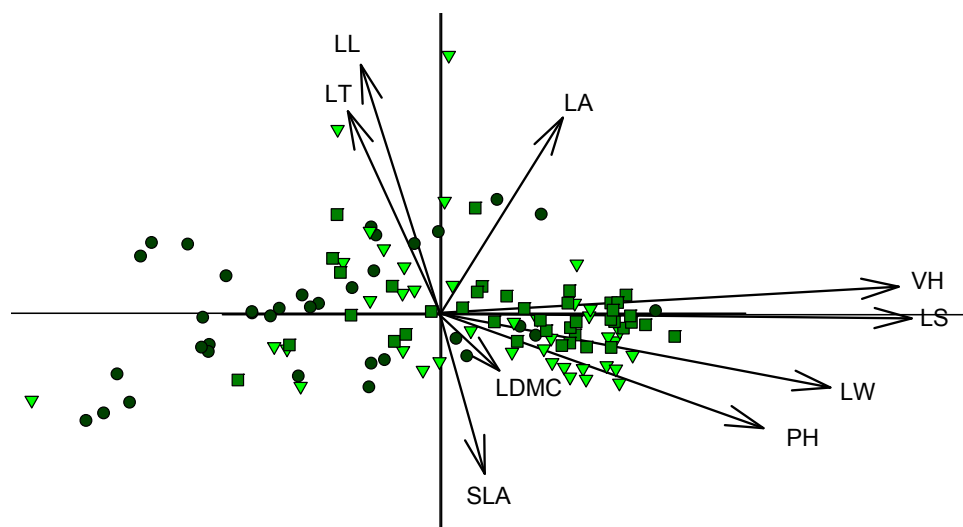


Fig. 2. Principal component analysis of community-weighted mean trait values, extracted from a trait \times species matrix obtained at the end of the experiment. Black dots represent microcosms with 1 species, light green triangles are microcosms with 3 species and dark green squares represent microcosms with 6 species. Trait abbreviations are: PH, index of phenology; LA, leaf area; LDMC, leaf dry matter content; LL, leaf length; LS, lateral spread; LT, leaf thickness; LW, leaf width; SLA, specific leaf area; VH, vegetative height. For each component we indicate the % of variance explained.

Response of community structure and microbial community to climate change drivers and plant species richness

Plant species richness significantly and positively impacted CW-size traits ($\chi^2 = 51.04$; $P < 0.001$), but not CW-leaf traits. Increases in plant species richness promoted larger plant sizes (Fig 1), with increases in VH and LS. Climate change drivers and their interaction with plant species richness did not affect community structure.

Plant species richness also promoted significant and marginally significant changes in bacterial and protist abundance, respectively (bacterial: $\chi^2 = 5.99$; $P = 0.050$; protist: $\chi^2 = 51.04$; $P = 0.088$). Bacterial abundance increased with increases in plant species richness (Fig. 3). Microcosms with three plant species richness increased protist abundance more than microcosms with one or six species (Fig. 3). There were no significant differences between soil microorganism abundance and climate change drivers or interactions between them and plant species richness.

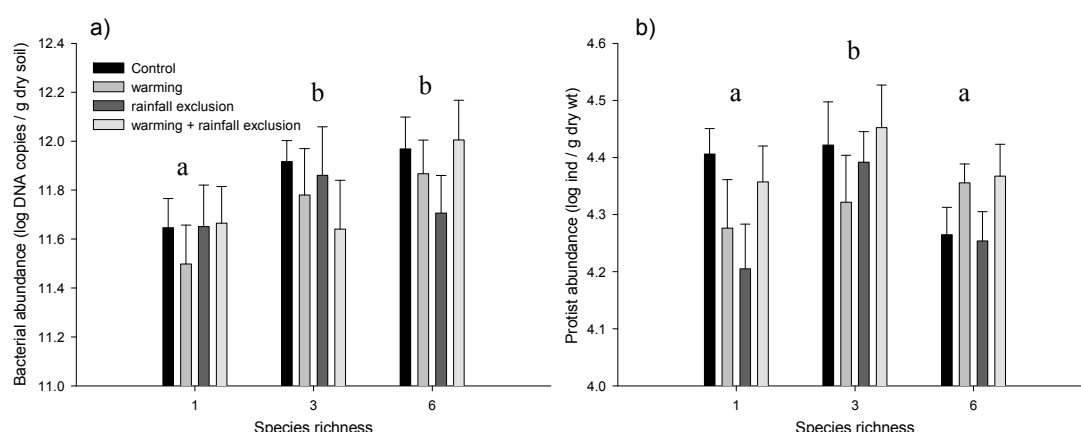


Fig. 3 Variation soil bacterial abundance (a) and soil protist abundance (b) as affected by climate change drivers (warming and rainfall exclusion) and plant species richness (one, three, and six plant species). Differences in the abundance of soil microorganisms and plant species richness are indicated by different letters. Values are means \pm SE ($n = 4$).

Effects of climate change drivers, plant species richness, community structure and soil microorganism abundance on multifunctionality

The model accepted in the d-sep regarding direct and indirect effects on multifunctionality explained 60% of the total variation (Fig. 4). This model revealed that the effects of plant species richness on multifunctionality were driven by indirect effects (Fig. 4). For instance, plant species richness explained CW-size trait values, and increases in CW-size trait values promoted higher multifunctionality. By contrast, CW-leaf trait values were independent of both climate change drivers and plant species richness. Increasing leaf trait values toward higher LL or LA strongly decreased multifunctionality. The effect of plant species richness on bacterial abundance was mediated by CW-size trait values. Increasing plant species richness increased CW-size traits which in turn increased bacterial abundance having a positive effect on multifunctionality. Finally, protist abundance was driven by bacterial abundance and plant species richness, and had a positive impact on multifunctionality. The path analysis showed that climate change drivers did not have either direct or indirect effects

on multifunctionality (Fig. 4). However, warming and rainfall reduction had a positive direct effect on C and P cycles (Fig. 5a and 5c), and rainfall reduction increased APB (Fig. 5d).

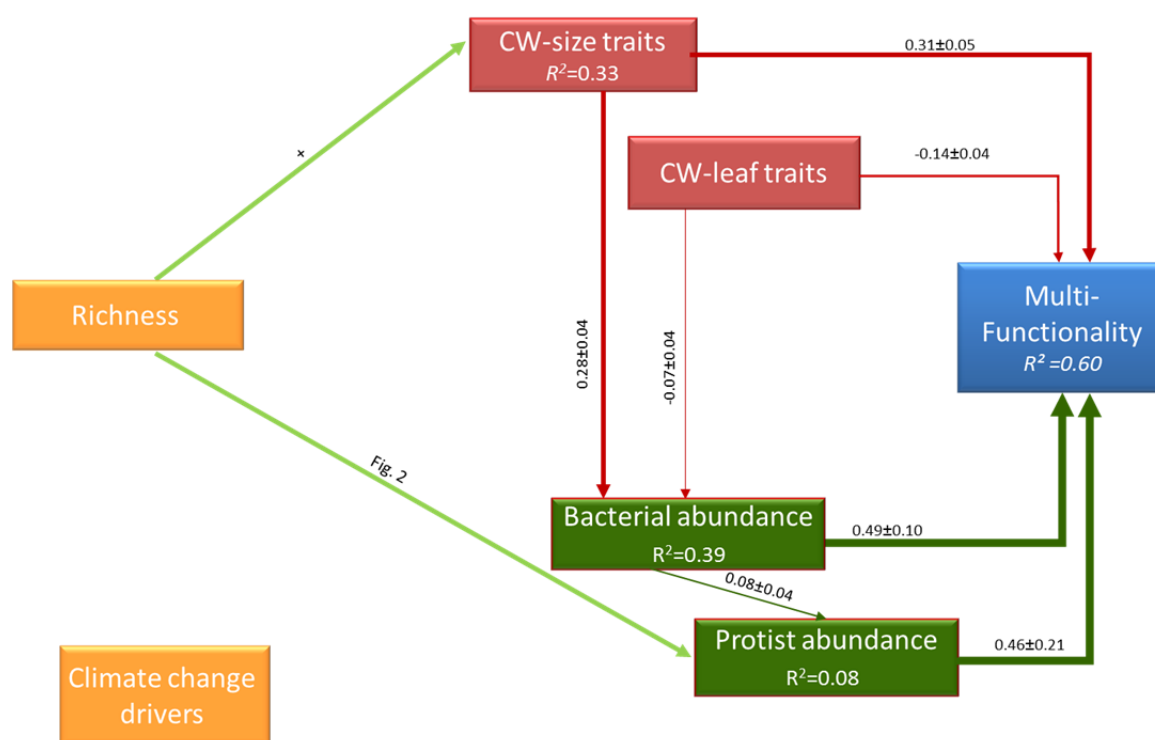


Fig. 4. Relationships between climate change drivers, plant species richness, the functional structure, soil microorganism abundance and multifunctionality. The width of each arrow is proportional to the standardized path coefficients. For graphical simplicity, the influence of species richness and protist abundance are showed in Fig. 2. CW: Community Weighted Mean.

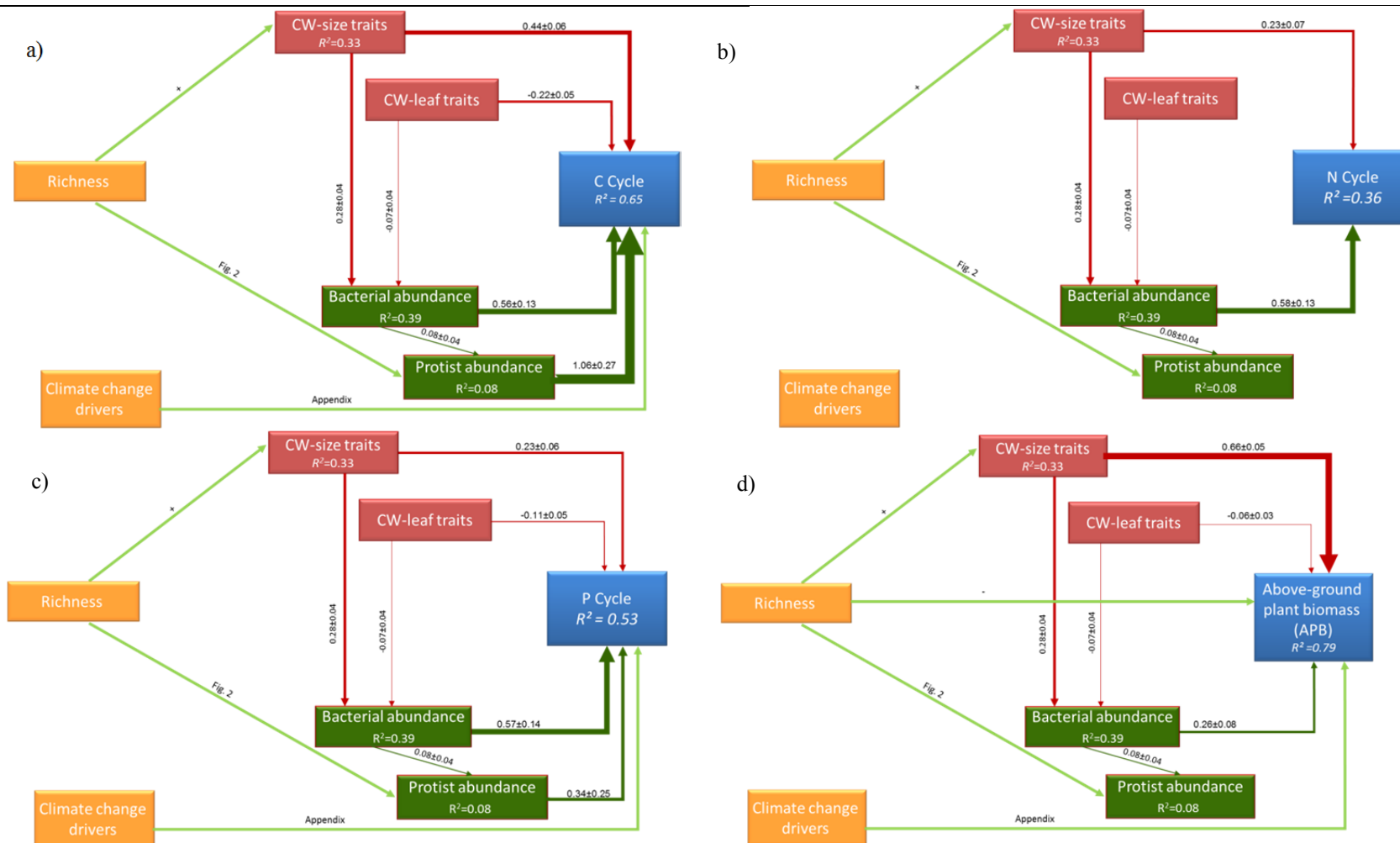


Fig. 5 Relationships between climate change drivers (CCD), species richness, the functional structure, microorganism abundance and C (a), N (b) and P (c) cycles and above-ground plant biomass (APB, d). The width of each arrow is proportional to the standardized path coefficients. For graphical simplicity, the influence of species richness and protist abundance are showed in Fig. 2. Relationships between climate change drivers (warming and rainfall reduction) and different nutrient cycles and APB are showed in Fig. S3. CW: Community Weighted Mean.

DISCUSSION

We found that the effects of plant species richness on multifunctionality were mediated by plant community structure (trait of the dominant species) and soil microorganism abundance, which explained an important part of total variation in multifunctionality. Surprisingly, warming and rainfall reduction had negligible impacts on plant and soil microorganisms, and on multifunctionality. However, warming and rainfall reduction did have a positive direct impact on C and P cycles, and rainfall reduction on APB. These results highlight that the effects of plant species richness are independent of climate change drivers and are the major driver of ecosystem functioning at local scale, via changes in plant and soil microorganism community structures.

Responses of plant functional traits to climate change drivers and plant species richness

Functional structure was explained by two specialization axes. The first axis is the CW-size traits, and reflects water use efficiency and/or competitive ability (Westoby *et al.*, 2002). The second axis is the CW-leaf traits and means that higher CW-leaf trait values lead to higher light interception and lower water stress tolerance (Westoby *et al.*, 2002). In our experiment, CW-size traits increased with higher plant species richness (Fig. 4). This result could be related with different aspects. Firstly, probabilities of large species presence increase with the species number, i.e. sampling effect. Secondly, higher richness may promote resource distribution between species, promoting a feedback for resources and higher sizes for the entire community. Indeed, several studies that showed an increase in nutrient resources with increases in plant species richness (Zavaleta *et al.*, 2010; Cardinale *et al.*, 2011; Maestre *et al.* 2012b). On the other hand, climate change drivers did not affect either CW-size traits or CW-leaf traits. These results are contrary

to our expectations, since we hypothesized that increases of temperature and decreases of rainfall promote a decrease in plant size (Cornwell & Ackerly 2009; Gross *et al.* 2013) and small leaves, because it is assumed that plants with these traits are better adapted to arid environments (Westoby *et al.*, 2002).

Community-structure mediated effects of plant species richness on soil microorganism community

Increases in temperature and reductions of precipitation did not alter soil microorganism community composition and abundance (Fig. 3). The lack of a significant effect is contrary to previous studies that detected that higher temperatures promoted microbial growth of bacterial community (Sardans & Peñuelas, 2004; Geisseler & Horwath, 2009) or decreases in abundance of bacterial and protists with warming and rainfall reduction (Cole *et al.*, 2002; Darby *et al.*, 2006; Allison *et al.*, 2008). Warming and rainfall treatments change soil microorganism abundance, because they modified soil temperature and moisture conditions (Castro *et al.*, 2010). However, according to our results, other studies did not show a response in bacterial abundance with different climate change drivers (Bergner *et al.*, 2004; Bardgett & van der Putten, 2014; Kratz, 2014 and references therein). Thus, soil microorganism community could be acclimated to changes in abiotic conditions (see Crowther & Bradford, 2013 for fungi species). Alternatively, some microorganism species could have high range of temperature for optimal functioning (Tinker & Inson, 1990), and therefore our temperature increase is not enough to affect them. The last possibility could be that changes on temperature and rainfall do not impact in microorganism abundance, but affect microorganism composition. Thus, decreases in abundance of some species of soil microorganisms could be compensated by increases in other species. Soil microorganisms may have

enough mechanisms to support climate change drivers effects. Nevertheless, future studies are needed to evaluate the effect on microorganism community composition and/or the possible acclimation or adaptation to these factors.

As hypothesized, soil bacterial and protist abundance responded to plant species richness. These results agree with previous studies that showed increases in soil microbial biomass with increases in plant diversity (Naeem *et al.*, 2002; Zak *et al.*, 2003; Eisenhauer *et al.*, 2010; Zhang *et al.*, 2010). The increase in plant species richness is linked to a higher number of soil microorganisms associated with plants (Carney & Matson, 2005; Scherber *et al.*, 2010; Hendriks *et al.*, 2013). Increasing in plant species richness are correlated with increases in APB (Tilman *et al.*, 1996; Hector *et al.*, 1999) and, in consequence, litter material and soil carbon content also (Fornara & Tilman, 2009) increases microorganism abundance. Accordingly, we observed that increases in plant species richness augmented size traits of the community and that promoted an increase in bacterial abundance. It is important to remark plant species richness affected bacterial community via community structure (CWT). Larger sizes promote changes in soil shading that improve temperature and moisture conditions for microorganism (Rustad *et al.*, 2001). It seems reasonable that increases in bacterial abundance are related with increases in protist abundance, as the latter grazes bacterial community (Bonkowski, 2004). Overall, plant species richness is the major driver of microorganism community, however, indirectly via functional traits.

Direct and indirect effects of plant species richness and climate change on multifunctionality

Our results did not show either direct or indirect effects of climate change drivers on multifunctionality (Fig. 3), in spite of that effects of climate change drivers in

ecosystem functioning of dryland ecosystems has been largely observed at global and regional scale (Maestre *et al.*, 2010; Maestre *et al.*, 2012b; Bowker *et al.*, 2013). The lack of relationship between climate change drivers and multifunctionality disagrees with our hypothesis; however we detected a positive direct impact on C and P cycles (Fig. S4). This direct impact of climate change drivers on these nutrient cycles agree with other warming studies that show an increase of rates of soil C cycle with the treatment (Rustad *et al.*, 2001). Rainfall reduction increased the APB of the microcosm (Fig. S3d), affecting positively to nutrient cycles.

Our results highlight the importance of evaluated indirect impact of plant species richness (i.e., plant community structure and microorganism abundance) on multifunctionality (Fig. 3). Despite the large body of literature that highlights the positive effects of plant species richness on ecosystem functioning (review in Schl  pfer & Schmid, 1999), there are an increasing attention on the importance of functional traits in this relationship (review of de Bello *et al.*, 2010). As expected (hypothesis 2), multifunctionality was driven by CW-size and -leaf traits in our study, since microcosm plant communities with larger species and small leaf size showed higher multifunctionality (Fig. 3). Larger size traits are related with higher APB (Fig. 5d), that would stimulate a dense litter layer, reducing the soil moisture losses due to evaporation (Holmgren *et al.*, 2012). Additionally, larger species promote shading to other species, facilitating the survival of other species, providing opportunities for more efficient resource use (D  az & Cabildo, 2001). Plant communities with greater leaves species can increase water losses by evapotranspiration (Wright *et al.*, 2004), which worsens their performance, negatively affecting multifunctionality.

Our results provide evidence of the impact of both bacterial and protist abundance on multifunctionality. Increasing both type of microorganism increased

multifunctionality. Previous studies showed how microorganism communities alter decomposition of litter materials and nutrient cycles (Angers & Caron, 1998; Hooper *et al.*, 2000; Carney & Matson, 2005). The magnitude of the effect of both bacterial and protist abundance on multifunctionality highlight the importance of measure different trophic levels, and the interactions between them, to increase our ability to explain complex ecosystem responses to climate change.

CONCLUDING REMARKS

Our results highlight the importance of evaluating and understanding the direct and indirect effects of multiple global change drivers on multifunctionality. Grasslands with larger species and species with smaller leaves could improve multifunctionality. Higher bacterial and protist abundance had also positive impacts on multifunctionality, accelerating the decomposition of litter materials and increasing nutrient contents. Warming and rainfall reduction did not have a major impact on plant and soil communities nor on multifunctionality.

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SUPPORTING INFORMATION

Appendix A.

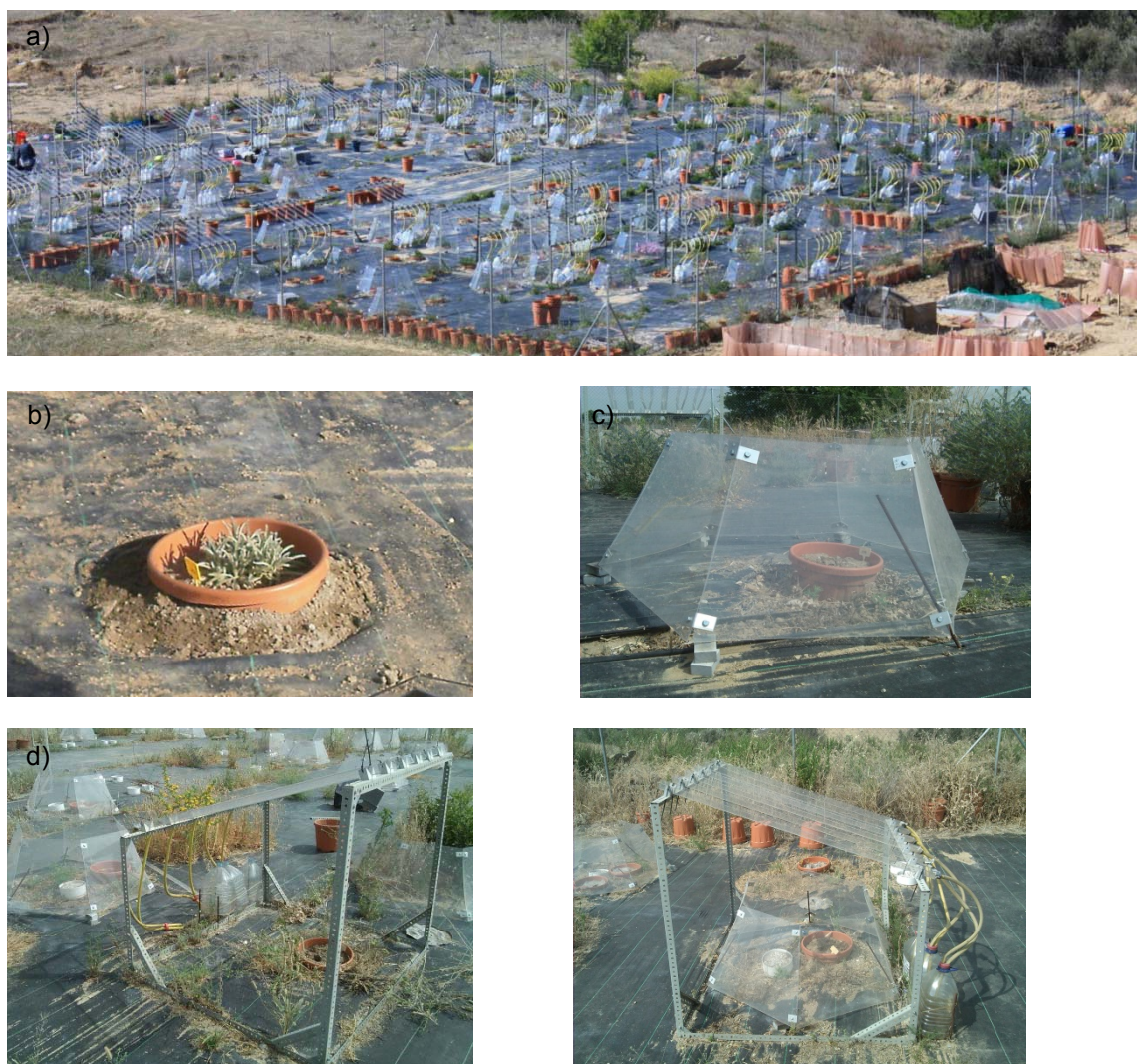


Fig. S1. General view of the sampling area (a). Detailed view of a microcosm in each climate change driver: control (b), open top chamber (c), rainfall shelter (d), and combination of open top chamber and rainfall shelter (e).

Table S1. Information about the species poll used in the study.

Species		Family
<i>Agropyron cristatum</i>	(L.) Gaertn.	Poaceae
<i>Brachypodium phoenicoides</i>	(L.) Roem. & Schult.	Poaceae
<i>Briza media</i>	L.	Poaceae
<i>Bromus inermis</i>	Leyss.	Poaceae
<i>Cynodon dactylon</i>	L.) Pers.	Poaceae
<i>Festuca ovina</i>	L.	Poaceae
<i>Festuca rubra</i>	L.	Poaceae
<i>Lolium perenne</i>	L.	Poaceae
<i>Lygeum spartum</i>	Loefl. ex L.	Poaceae
<i>Astragalus lusitanicus</i>	Lam.	Fabaceae
<i>Dorycnium pentaphyllum</i>	Scop.	Fabaceae
<i>Lotus corniculatus</i>	L.	Fabaceae
<i>Medicago lupulina</i>	L.	Fabaceae
<i>Medicago sativa</i>	L.	Fabaceae
<i>Melilotus officinalis</i>	(L.) Pall.	Fabaceae
<i>Onobrychis viciifolia</i>	Scop.	Fabaceae
<i>Psoralea bituminosa</i>	L.	Fabaceae
<i>Trifolium pratense</i>	L.	Fabaceae
<i>Trifolium repens</i>	L.	Fabaceae
<i>Achillea millefolium</i>	L.	Asteraceae
<i>Dianthus barbatus</i>	L.	Caryophyllaceae
<i>Echium boissieri</i>	Steud	Boraginaceae
<i>Echium plantagineum</i>	L.	Boraginaceae
<i>Eryngium campestre</i>	L.	Apiaceae
<i>Plantago lanceolata</i>	L.	Plantaginaceae
<i>Sanguisorba minor</i>	Scop.	Rosaceae
<i>Saponaria Ocymoides</i>	L.	Caryophyllaceae

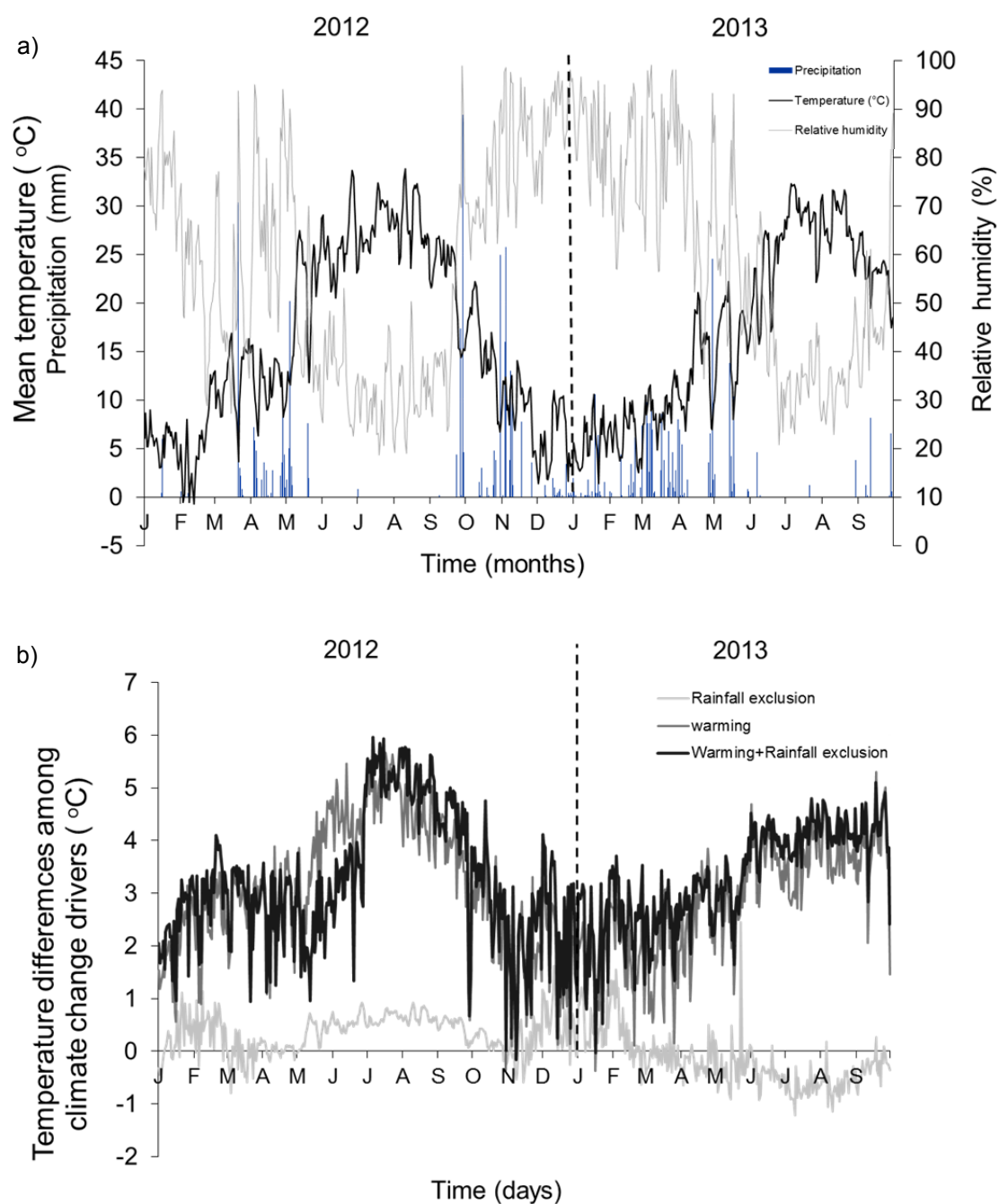


Fig S2. Temporal variation in air temperature, air relative humidity and precipitation (a) and temperature difference among climate change drivers (b) from January 2012 to October 2013 in the experimental area. The standard errors for air temperature are omitted for clarity (data present the average of $n = 3$ sensors for air temperature).

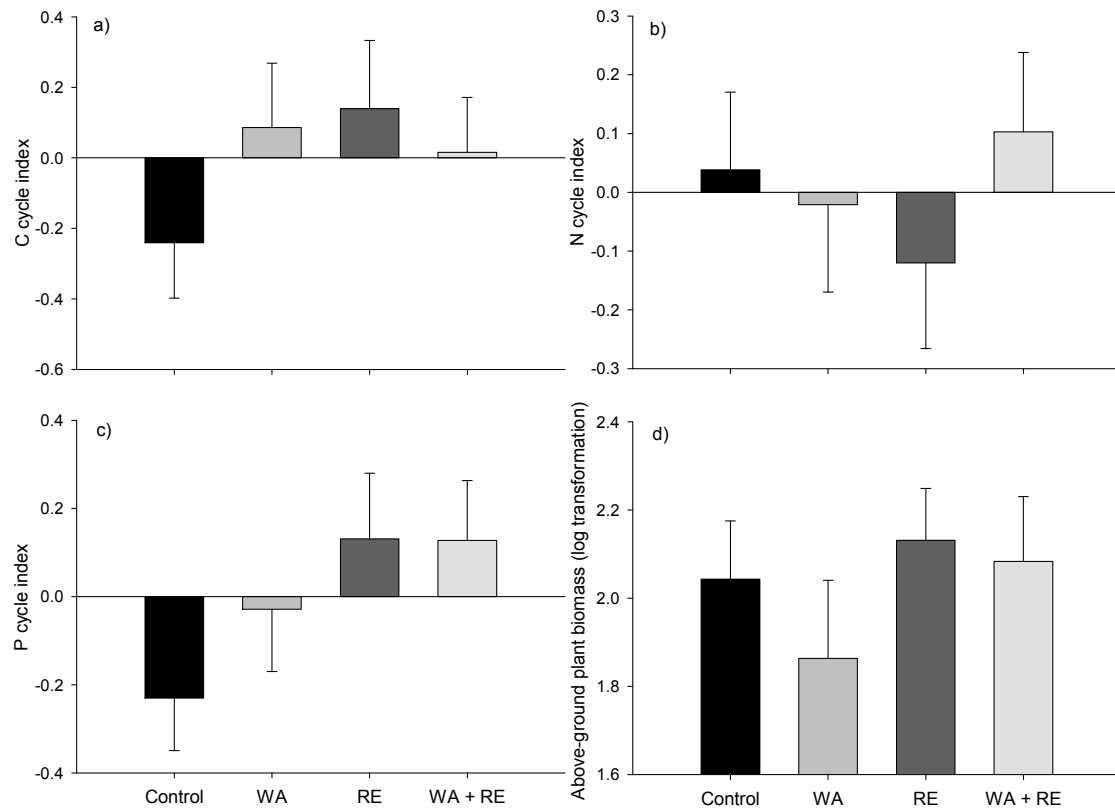


Fig S3. Differences in the in the indices of carbon (a), nitrogen (b), phosphorus (c) cycles and above-ground plant biomass among the climate change tratments. Values are means \pm SE (n = 29). WA, warming; and RE, rainfall exclusion.

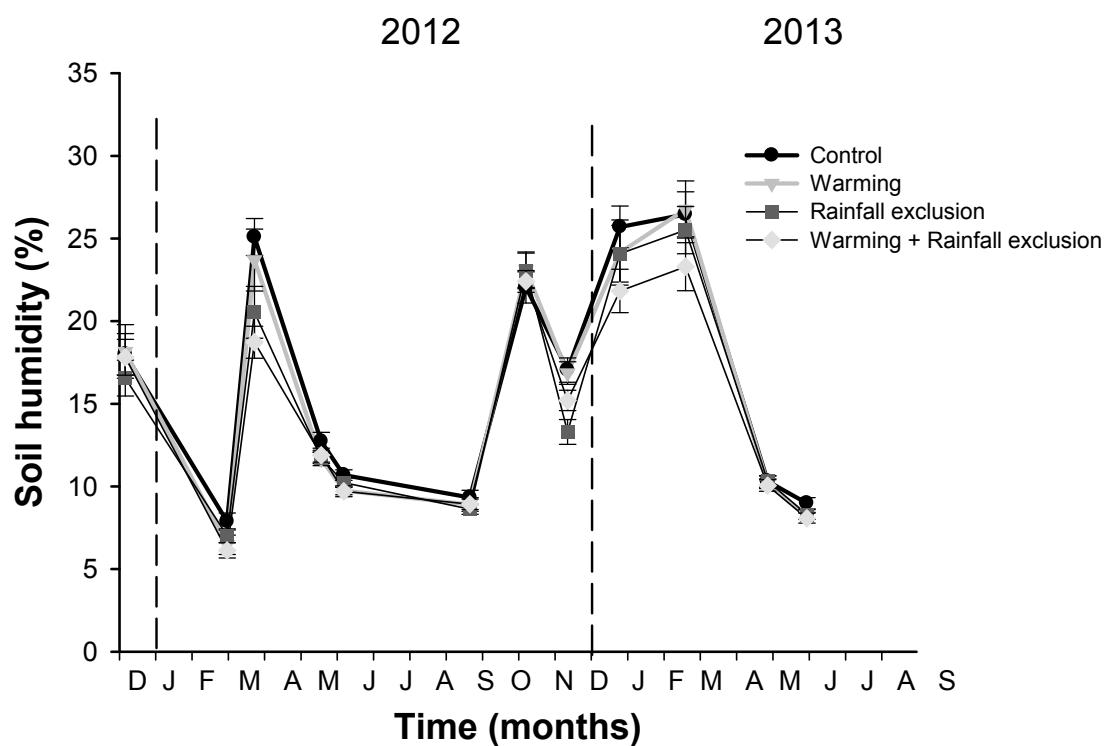


Fig. S4. Temporal dynamics of soil moisture in the different climate change treatment (control, warming, rainfall exclusion, and warming +rainfall exclusion) during the study (December 2011 and September 2013). Data represent means \pm SE ($n = 19$).

CONCLUSIONS

1. Plant community structure of semi-arid communities is determined by both habitat filtering and niche differentiation processes. Moreover, biotic interactions, such as competition and facilitation, have an important role on community structure. Competition explains higher functional trait diversity, and facilitation promotes subordinate and/or rare species that may enhance biodiversity in semi-arid shrublands.
2. Size traits (e.g., vegetative height or lateral spread) and leaf traits (e.g., specific leaf area and leaf dry matter content) captured the effect of shrub encroachment on multifunctionality with a relative high accuracy in semi-arid Mediterranean ecosystems.
3. Functional structure of Mediterranean semi-arid ecosystems is determined by aridity and land use changes, such as shrub encroachment. Functional traits of dominant species and their functional diversity are the main drivers of multifunctionality, more than direct effects of aridity and shrub encroachment.
4. The maintaining and/or enhancing functional diversity (by increasing sprouting shrubs) may help to buffer negative effects of ongoing climate change on dryland multifunctionality.
5. Warming, predicted by current climate change models for the second half of this century in semi-arid Mediterranean regions, increases photosynthetic rates at the

beginning of the growing season, affecting ultimately plant flowering. Functional traits affect photosynthetic response, having larger species with small leaves higher photosynthetic rates under warming.

6. Warming advanced flowering and fruiting phenology and reduced flowers and fruits productions. These individual plant species responses could affect complementarity among plant species or promote asynchronies between plant species and pollinators. The observed reduction in flowers and fruits could reduce the future abundance of some species, affecting community assembly.
7. Rainfall exclusion and warming promote neither plant functional structure nor abundance of soil microorganism. However, functional traits of dominant species, such as plant architecture or leaf structure, modulate the abundance of soil microorganisms and multifunctionality. Moreover, microorganism abundance must be take into account because may explain a large part of the multifunctionality variation, highlighting its interest in future studies.
8. The response-effect framework based on plant functional traits of Mediterranean semi-arid ecosystems provides important insights on patterns underlying plant functional structure response to global change drivers, and how changes in the community impact on multifunctionality. Thus, this framework should be used in the evaluation of semi-arid ecosystems to improve restoration programs.

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