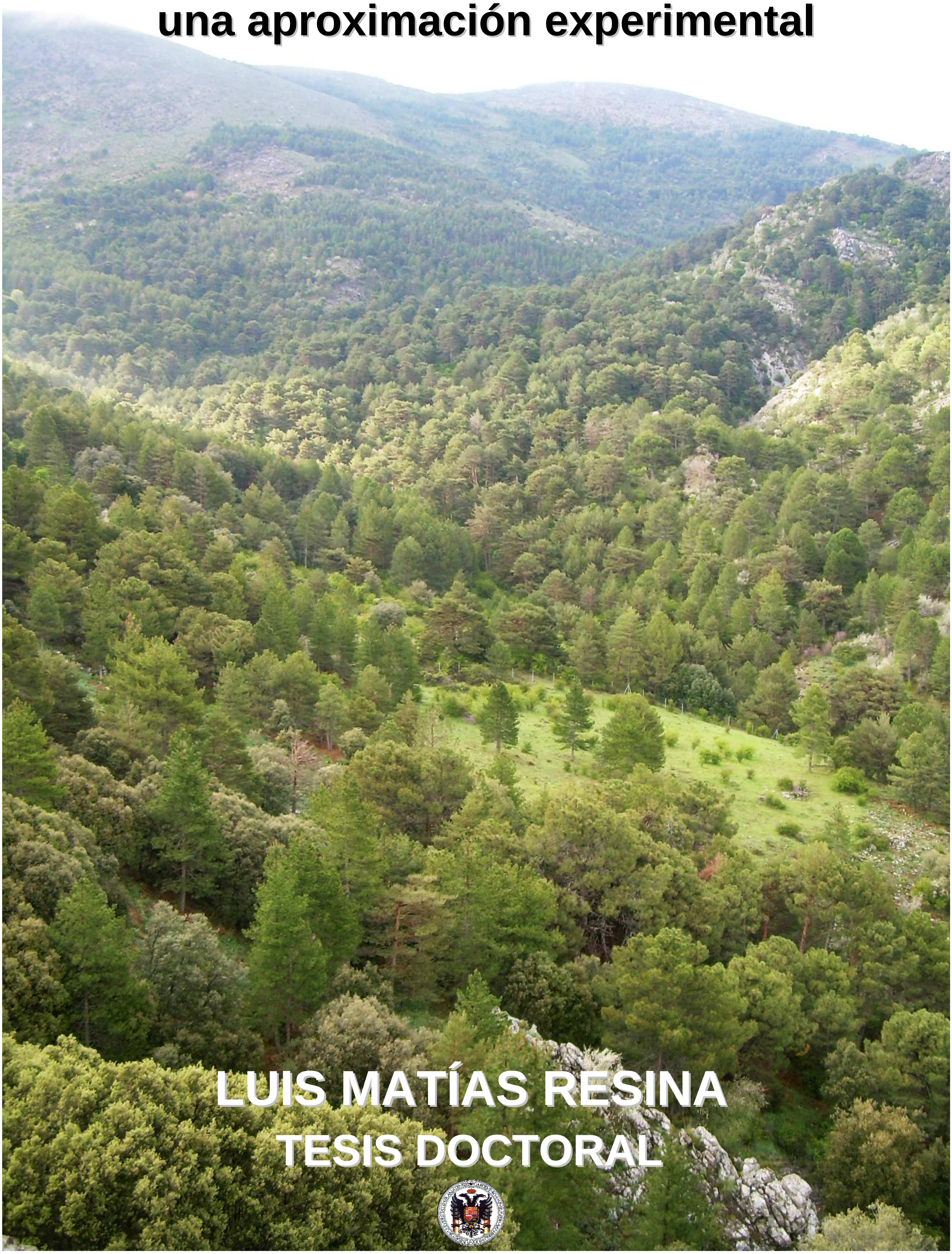
Efectos del Cambio Climático sobre la regeneración del bosque mediterráneo: una aproximación experimental



LUIS MATÍAS RESINA

TESIS DOCTORAL

UNIVERSIDAD DE GRANADA
DEPARTAMENTO DE ECOLOGÍA

EFECTOS DEL CAMBIO CLIMÁTICO SOBRE LA REGENERACIÓN DEL BOSQUE MEDITERRÁNEO:
UNA APROXIMACIÓN EXPERIMETAL

TESIS DOCTORAL

Luis Matías Resina
Granada 2010

Editor: Editorial de la Universidad de Granada
Autor: Luis Matías Resina
D.L.: GR 4248-2010
ISBN: 978-84-693-5970-9

EFECTOS DEL CAMBIO CLIMÁTICO SOBRE LA REGENERACIÓN DEL BOSQUE MEDITERRÁNEO: UNA APROXIMACIÓN EXPERIMENTAL

Memoria que el Licenciado Luis Matías Resina presenta para aspirar al Grado de Doctor por la Universidad de Granada

Esta memoria ha sido realizada bajo la dirección de:
Dr. Regino Zamora Rodríguez y Dr. Jorge Castro Gutiérrez

Ldo. Luis Matías Resina

Aspirante al Grado de Doctor

Granada, julio de 2010

Dr. Regino Zamora Rodríguez, Catedrático de Ecología de la Universidad de Granada **y Dr. Jorge Castro Gutiérrez**, Profesor Titular de Ecología de la Universidad de Granada

CERTIFICAN

Que los trabajos de investigación desarrollados en la Memoria de Tesis Doctoral: ″Efectos del cambio climático sobre la regeneración del bosque mediterráneo: una aproximación experimental**″, son aptos para ser presentados por el Ldo. Luis Matías Resina ante el Tribunal que en su día se designe, para aspirar al Grado de Doctor por la Universidad de Granada.**

Y para que así conste, en cumplimiento de las disposiciones vigentes, extendemos el presente certificado a 12 julio de 2010

Dr. Regino Zamora Rodríguez **Dr. Jorge Castro Gutiérrez**

Durante el tiempo de realización de esta Tesis Doctoral he disfrutado de una Beca del Programa Nacional de Formación de Personal Investigador del Ministerio de Educación y Ciencia Ref. (BES–2006–13562).

Este trabajo estuvo financiado por los proyectos, DINAMED (CGL2005–05830-C03-03) del MEC y GESBOME (P06-RNM-1890) de la Junta de Andalucía.

La investigación presentada en esta Tesis Doctoral se ha realizado en el Departamento de Ecología de la Universidad de Granada.

A Belén,
porque sin ti nada de esto habría sido posible.

La frase mas excitante que se puede oír en ciencia, la que anuncia nuevos descubrimientos, no es "¡Eureka!" (¡Lo encontré!) sino "Es extraño ..."
Isaac Asimov (1920-1992)

AGRADECIMIENTOS

Sólo cuando se llega al final de un camino se puede echar la vista atrás y se comprueba la larga lista de personas que, de una u otra forma, han ayudado a recorrerlo. Me considero incapaz de poder expresar sólo en unas pocas páginas todo lo que las personas con las que he compartido este trayecto han significado para mí, y estoy seguro de que estas palabras se quedarán cortas. A través de estas líneas sólo espero conseguir que todos los que de una forma u otra han colaborado a completar este trabajo sientan como suya al menos una parte de esta tesis.

Este trabajo no se podría haber realizado sin la colaboración directa con la Consejería de Medio Ambiente y la dirección del Parque Nacional de Sierra Nevada. Nos han permitido llevar a cabo este estudio y nos han cedido todas las instalaciones y equipos necesarios para ello. En especial, debo agradecer todo su esfuerzo y apoyo al personal del Jardín Botánico *La Cortijuela*, Francis, Ángel y Joaquín, que sabiamente nos han aconsejado poniendo de manifiesto su amplio conocimiento de Sierra Nevada.

Ante todo, debo agradecer todo su gran trabajo a mis directores de tesis: Regino, gracias por dar la oportunidad a aquel recién licenciado sin ningún tipo de experiencia de meter el primer pie en el mundo de la Ciencia, hacer crecer en mí la ilusión por este mundillo, y después darme la oportunidad de hacer esta tesis. Ha sido un verdadero placer poder aprender tantas cosas a tu lado a lo largo de este camino, y no solo científicas. Gracias por estar ahí siempre que he necesitado un buen consejo. Y a Jorge, la única persona que cumple el Principio de Máxima Incertidumbre de Heisenberg: "*Hay momentos y lugares donde es más fácil encontrarlo, pero nadie puede asegurar que realmente vaya a estar ahí*". Gracias por todo tu apoyo y tu gran trabajo, especialmente importante en la recta final de esta tesis. Gracias a los dos por todo vuestro tiempo y dedicación.

Como suele decirse, "*ningún taburete de tres patas puede cojear*". Pues esta tesis se ha apoyado no sobre tres patas, sino sobre tres grandes pilares: Nacho estuvo en la parte inicial y gracias a él toda la infraestructura necesaria estuvo finalizada a tiempo. Susana puso a punto todas las técnicas de análisis de suelos y sin su trabajo el primer capítulo de esta tesis no hubiese sido posible. Ramón se incorporó al equipo en la recta final, pero gracias a él la gran cosecha final del experimento y su procesamiento se llevó a cabo con éxito. Grandes compañeros, amigos y mejores personas. A vuestro lado, este recorrido ha sido enormemente más fácil. Los tres habéis sido una parte esencial en esta tesis. De todo corazón, infinitas gracias a los tres.

Si por algo se caracteriza el RNM 220, es por la buena relación que siempre ha existido entre los investigadores en formación. Yo he tenido la suerte de formar parte de este grupo y de aprender de las experiencias de todos mis compañeros. Una parte muy importante de esta tesis es debida a las personas con las que empecé en este camino: Irene, José Luis y Asier. Junto a Irene di mis primeros pasos en este largo camino: el primer congreso y el primer artículo, primeros pasos que me han hecho avanzar con más firmeza en los sucesivos. Con José Luis no sólo he aprendido cómo ser un mejor científico y no morir en el intento, sino que han sido infinitos los momentos compartidos que, sin duda, me han hecho crecer como persona. Además de esto, ha sido parte activa del capítulo 3 de esta tesis. Te lo repito de nuevo Jose: yo de joven quiero ser como tú. Y junto a Asier he recorrido todo este camino como compañeros de viaje. Han sido muchos los momentos compartidos, tanto dentro del trabajo como fuera de él. Gracias por estar ahí en las ocasiones clave, a menudo acompañadas de un buen patxarán. Las "*becarias senior*" del grupo me han enriquecido gracias a su vasta experiencia: con Lorena he comprendido el gran esfuerzo necesario para hacerse un hueco en este competitivo mundo y he aprendido mucho mientras trabajábamos en el capítulo 5 de esta tesis; de Elena me llevo su forma de ver la vida y su enorme corazón. Y qué decir de José Antonio, el "*becario honorífico*", gracias por soportar las continuas dudas que aparecían a cada paso y por todos los buenos ratos y consejos que me llevo conmigo.

Desde luego, esto no hubiese sido lo mismo sin el tropel de gente que habita la 3ª planta y sus alrededores. Junto a Javi, Moja y Jesús Q. he compartido infinidad de vivencias irrepetibles desde mucho antes de comenzar esta tesis. He aprendido muchísimo de vosotros, pero sin duda es aún más lo que me queda por aprender. Quiero agradecer también al resto de la gran familia de ecólogos y genéticos, siempre dispuestos a echar una mano cuando ha sido necesaria, y a escuchar y dar buenos consejos en los momentos de flaqueza. Pero sobre todo, por todos los buenos momentos compartidos y por hacer que el ambiente de trabajo sea tan ameno y entretenido: Ángela, Sara, Ana, Karim, Modesto, Eulogio, Juande, Ayub, Eli, María, Jesús C., Lucía, Jorge, Oti, Caro, Jose... y los "ceameros" Curro, Currete, Antonio, Irene, Luis, Pepa... ¡GRACIAS!

Junto a Milena Holmgren disfruté enormemente y aprendí (aún más) preparando un bonito, aunque fallido, experimento durante mi estancia breve en la Universidad de Wageningen (Holanda). Ese experimento aún no está abandonado. Algún día lo conseguiremos. Gracias por todo Milena.

Este trabajo es uno de los resultados finales del proyecto coordinado DINAMED, el cuál me ha dado la oportunidad de conocer, colaborar y aprender de un grupo de personas enormemente enriquecedor. Gracias a los Sevillanos Teo, Luis, Cristina, Edu, Nacho... y a los Cordobeses Rafa, Vico, David,..

Especialmente les agradezco todo (y aún más) a mi gran familia: padres, hermanos, sobrinos y resto de la tropa, porque ellos me han hecho ser la persona que soy. Me lo han dado todo, y me han apoyado siempre a lo largo de este camino. Gracias por estar ahí cuando os he necesitado.

Y por supuesto, a Belén, por la ayuda en el campo, por la ayuda en el laboratorio, por compartir los buenos momentos y por soportar los malos. Por hacer que me levante con ilusión cada mañana. Por hacer de los obstáculos insalvables un pequeño grano de arena. Por estar siempre ahí.

GRACIAS

ÍNDICE

RESUMEN

RESUMEN

En la presente tesis doctoral tratamos de analizar los efectos producidos por los posibles escenarios climáticos previstos para las próximas décadas en el funcionamiento de un ecosistema de montaña mediterránea. Para ello, hacemos una aproximación a diferentes niveles, pero partiendo en todos ellos de un diseño experimental común. En este diseño experimental simulamos bajo condiciones naturales tres escenarios climáticos que varían en el patrón de precipitación durante los meses de verano: veranos más secos de acuerdo a la reducción del 30% prevista por los principales modelos climáticos, veranos excepcionalmente húmedos mediante la simulación de tormentas de verano hasta alcanzar los máximos históricos en el área de estudio, y condiciones climáticas actuales durante el desarrollo del experimento. Estos diferentes escenarios climáticos se simularon en los tres principales hábitats que componen las montañas mediterráneas, es decir, zonas de bosque, bajo cobertura de matorral y en claros. En el **capítulo 1** analizamos los efectos sobre la diversidad del banco de reclutas que se establecen de forma natural bajo las diferentes combinaciones de hábitats y escenarios climáticos. A pesar de las diferencias que existen en el establecimiento entre los distintos hábitats estudiados, encontramos que los veranos excepcionalmente húmedos son capaces de incrementar tanto el número de individuos como el número de especies establecidas, mientras que bajo el escenario más seco se reduce la densidad y diversidad del banco de reclutas. En el **capítulo 2** estudiamos el crecimiento y la probabilidad de reclutamiento de ocho especies leñosas representativas de los principales grupos funcionales (matorrales pioneros, matorrales caducos y arbóreas) bajo la combinación de escenarios climáticos y estructura de hábitat. Un incremento en la precipitación es capaz de aumentar el crecimiento y la supervivencia de estas especies, mientras que la reducción sólo los disminuye en unos determinados casos. Estos resultados estuvieron además

modulados por el tipo de hábitat. Las especies de matorral presentaron un mayor crecimiento y supervivencia, mientras que algunas de las especies arbóreas presentaron una elevada dependencia de los veranos más húmedos. En el **capítulo 3** determinamos la resistencia a la sequía de estas mismas ocho especies leñosas, así como las relaciones entre la morfología y resistencia a la sequía, y entre crecimiento y supervivencia. De forma general, la resistencia a la sequía estuvo modulada por el hábitat, siendo mayor bajo cobertura de matorral y para las especies de matorral pionero, aunque una de las especies arbóreas (*Quercus ilex*) presentó también una elevada resistencia en todos los hábitats. Un mayor crecimiento o una mayor biomasa foliar por peso de planta (LMR) pueden incrementar la resistencia a la sequía, mientras que las especies con una mayor inversión en los órganos de captación de luz respecto a las estructuras para la captación de agua y nutrientes (LARMR) están menos preparadas para soportar la sequía. Además, no encontramos ningún indicio de compromiso entre crecimiento y supervivencia. En el **capítulo 4** analizamos las consecuencias sobre la disponibilidad de nutrientes en suelo y su captación por microorganismos. Bajo un escenario más húmedo, la comunidad microbiana aumenta la captación de nutrientes, acelerando de esta forma su reciclado y disponibilidad para las plantas. Al contrario, al incrementarse la aridez se liberan al suelo una gran cantidad de nutrientes, que no pueden ser asimilados por las plantas debido a la falta de humedad para tomarlos en disolución, aumentando de este modo el riesgo de pérdida por erosión o escorrentía. Por último, en el **capítulo 5** analizamos de una forma integrada mediante modelos de ecuaciones estructurales cuáles son los principales recursos que afectan al reclutamiento de las especies leñosas a través de las sucesivas fases demográficas. La emergencia de plántulas está principalmente determinada por las condiciones lumínicas, el crecimiento por la luz y la humedad durante los meses de verano, y la supervivencia especialmente por la disponibilidad hídrica estival. Sin embargo, las respuestas a la disponibilidad de estos recursos varían entre las diferentes especies y grupos funcionales de la comunidad. Una respuesta diferencial en el reclutamiento de las diferentes especies que conforman la comunidad bajo los distintos escenarios climáticos posibles puede alterar la dinámica, composición y estructura del ecosistema durante las próximas décadas.

INTRODUCCIÓN GENERAL

INTRODUCCIÓN GENERAL

CAMBIO CLIMÁTICO Y CAMBIO GLOBAL

El clima es un sistema complejo altamente heterogéneo debido a variaciones latitudinales, topográficas, estacionales, o en la cobertura vegetal entre otras. Durante todo el siglo XX, pero de forma especial desde la década de los 70, la temperatura media global ha ido subiendo en buena parte del planeta a un ritmo mucho más elevado de lo observado hasta la fecha, incrementándose 0.6 ± 0.2 ºC desde finales del s. XIX (Houghton *et al*. 2001). Además, se ha observado también un cambio en los patrones de precipitación globales, de forma que se ha incrementado de un 0.2 a 0.3% por década en las regiones tropicales (10º N a 10º S) y de un 0.5 a 1% en las latitudes altas del hemisferio N (Houghton *et al*. 2001). Sin embargo, en las zonas subtropicales (10º N a 30º N) se ha detectado una reducción en el total de precipitación de un 0.3% por década durante el s. XX. Estos cambios en la temperatura media del planeta y en los patrones de precipitación han sido atribuidos tanto a causas naturales (Crowley & North 1988) como antropogénicas (Oreskes 2004), entre las que destaca el aumento en las emisiones de gases de efecto invernadero. Convencionalmente, se ha establecido que el término *cambio climático* se use sólo para aquellas variaciones del clima respecto al historial climático a escala global o regional cuyas causas son de origen humano (Convenio Marco de las Naciones Unidas sobre el Cambio Climático).

Las predicciones de los principales modelos climáticos para finales del presente siglo prevén un incremento aún mayor de las temperaturas a nivel global, así como un aumento en la variabilidad de las precipitaciones, encontrando tanto aumentos como disminuciones, que variarán a escala regional dependiendo de las características específicas de cada zona. Concretamente, la región mediterránea es una zona de transición entre el clima del norte de África y el clima templado y húmedo de centroeuropa, viéndose afectado por las interacciones entre las latitudes medias y los procesos tropicales (Giorgi & Lionello 2008). Debido a estas características, incluso pequeños cambios en los modelos generales de circulación (como por ejemplo cambios en la localización de células de alta presión subtropicales o de las tormentas centroeuropeas) pueden conllevar cambios sustanciales en el clima del Mediterráneo, lo que ha llevado a identificarlo como uno de los "puntos calientes[*]" en las predicciones de cambio climático (Giorgi 2006). Aunque se prevén alteraciones climáticas durante todo el año, los cambios más importantes están previstos durante los meses de verano, tanto en temperatura como en precipitación. De forma general, se espera tanto un aumento paulatino de la temperatura media durante los meses de verano (desde +1.2 ºC en el período 2001-2020 a +4.6 ºC en 2081-2100) como una reducción de la precipitación (desde -7% en 2001-2020 a -28% en 2081-2100) con respecto al promedio de 1961-1990 (Giorgi & Lionello 2008). Aunque esta es la predicción generalizada para toda la Región Mediterránea, en las zonas montañosas del SE de la Península Ibérica podemos encontrar unas pequeñas variaciones (Moreno *et al*. 2005), encontrándose esta reducción en la precipitación en torno a un 30% (Fig. 1). Otra importante variación climática prevista bajo un escenario de cambio climático es el cambio en la variabilidad interanual, más clara en el caso de la precipitación, y que tenderá a aumentar con el avance del s. XXI (IPCC 2007). Este aumento de la variabilidad nos puede hacer pensar que no tienen por qué cancelarse los veranos excepcionalmente lluviosos que aparecen en las montañas mediterráneas (Fig. 2) que suelen aparecer con una frecuencia de 30-40 años (Rodó & Comín 2001) y que son de suma importancia para el funcionamiento de los ecosistemas.

---

[*] Traducción libre del inglés "hot-spots"

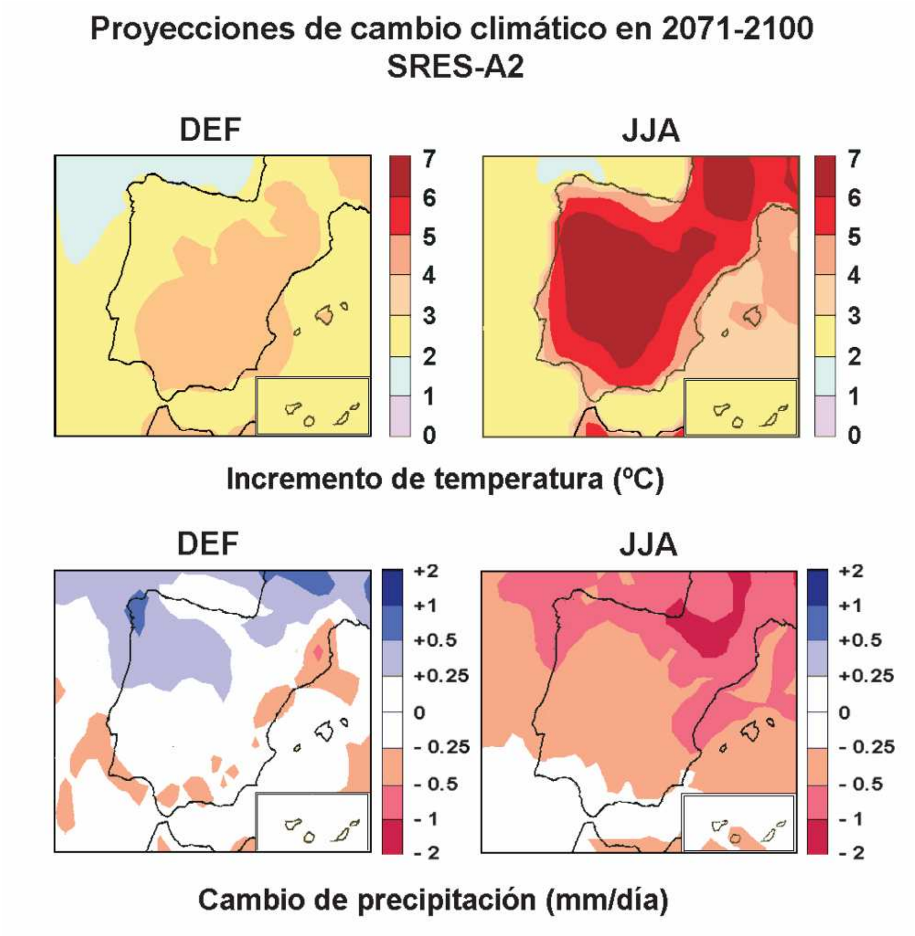


Figura 1: *Proyecciones de cambio de temperatura media diaria del aire superficial (ºC) y de cambio de precipitación (en mm/día), promediadas para dos estaciones del año (DEF invierno y JJA verano) en la Península Ibérica, Baleares y Canarias (esquina inferior derecha en cada mapa), correspondientes al escenario SRES de emisiones A2. Los valores corresponden a diferencias entre la simulación del periodo 2071-2100 y la de control (1961-1990). Extraído de Moreno* et al. *(2005).*

Sin embargo, el *cambio global* es un concepto mucho más amplio que engloba tanto al cambio climático, como los cambios en el uso del suelo, las invasiones biológicas o los cambios socioeconómicos. Estos otros motores de cambio pueden, no solo ser tan importantes o más que el cambio climático, sino que además pueden actuar de forma sinérgica (Sala *et al*. 2000; Matesanz *et al*. 2009). En la región mediterránea, la milenaria presencia humana y el importante manejo que ésta ha ejercido sobre los sistemas naturales ha dado como resultado un paisaje en el que se mezclan hábitats con diferente grado de manejo (Terradas 2001; Valladares *et al.* 2004), que resalta la importancia histórica de este motor de cambio.

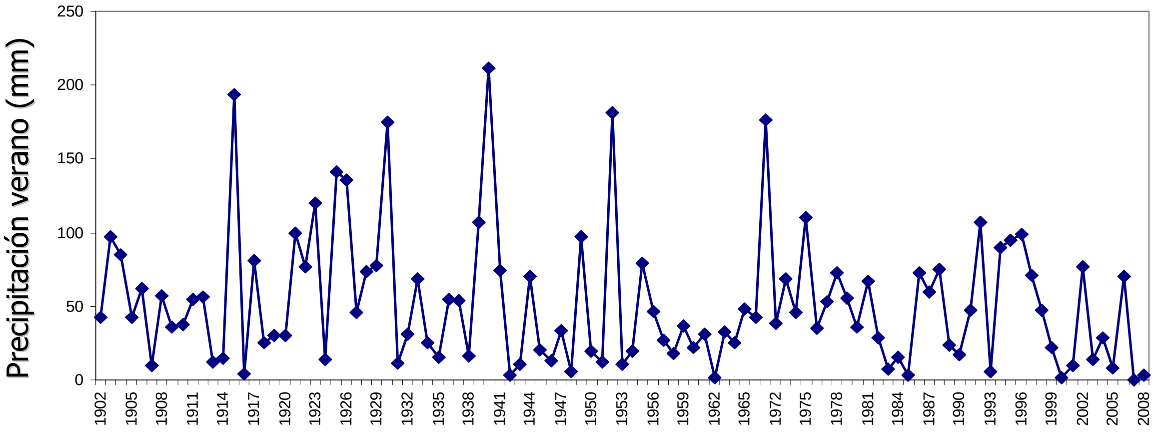


Figura 2: *Precipitación durante los meses de verano (Junio, Julio y Agosto) para la zona de estudio durante la serie climática 1902-2008. Los datos de 1902-1990 han sido extrapolados de la estación meteorológica de La Cartuja (Granada;* $R^2 = 0.85$; $P < 0.0001$*).*

EFECTOS DEL CAMBIO CLIMÁTICO SOBRE LOS ECOSISTEMAS

Los efectos del cambio climático sobre diferentes aspectos relacionados con la estructura y el funcionamiento de los ecosistemas terrestres ya son patentes a escala global. Entre estos efectos podemos destacar los cambios fenológicos (Peñuelas y Filella 2001; Walther *et al.* 2002; Parmesan y Yohe 2003; Root *et al.* 2003), decaimiento forestal (Allen & Breshears 1998; Breshears *et al.* 2005; Allen *et al.* 2010), pérdida de especies (Thomas *et al.* 2004; Thuiller *et al.* 2005), cambios en la estructura y diversidad de las comunidades (Tilman 1998; Chapin *et al.* 2001), o en la disponibilidad de nutrientes en el suelo (Nadelhoffer *et al.* 1991; Jonasson *et al.* 1999; Jensen *et al.* 2003; Rinnan *et al.* 2007).

En el caso de los ecosistemas de la región mediterránea, y en concreto de los de la Península Ibérica, la marcada sequía estival hace que las comunidades vegetales estén ya al límite se sus posibilidades (Castro *et al*. 2005; Valladares *et al*. 2005). Esta característica, unida a la elevada variabilidad climática, topográfica y edáfica le confiere a estos ecosistemas una alta vulnerabilidad bajo un escenario de cambio global. El aumento en la temperatura suele llevar asociado tanto un aumento en la demanda de agua por las plantas (ya que se eleva la tasa de transpiración foliar), como un incremento en la evapotranspiración potencial del suelo, lo que hace que la disponibilidad hídrica sea el factor crítico para evaluar los efectos del cambio climático en los ecosistemas terrestres. Así, en los ecosistemas con déficit hídrico que representan la mayoría de la Península, se pueden esperar cambios importantes que van desde la reducción en la densidad arbórea hasta cambios en la distribución de especies (Gracia *et al*. 2002). En casos extremos, áreas actualmente ocupadas por bosque pueden ser sustituidas por matorral, y áreas ocupadas por matorral pueden sufrir serios problemas de erosión (Peñuelas *et al*. 2008).

Los primeros estadíos de los ciclos vitales son los más dependientes de las condiciones ambientales (Houle 1994; Hampe & Arroyo 2002), por lo que cabe esperar que el reclutamiento de nuevos individuos a la población se vea seriamente afectado por las variaciones climáticas. Además, las consecuencias demográficas en el reclutamiento pueden ser fácilmente extrapoladas a la estructura de las fases adultas en un plazo de tiempo no demasiado elevado, lo que hace un buen sistema de estudio a la hora de detectar los efectos del cambio climático. Los efectos producidos por las variaciones climáticas a este nivel se pueden expresar de diferentes formas, desde cambios en la densidad y/o diversidad de plántulas emergidas y establecidas (Lloret *et al*. 2004) a cambios en la distribución de biomasa y morfología (Walters y Reich 1996; Quero *et al*. 2006). Además estas primeras fases del reclutamiento pueden ser más dependientes de los cambios producidos en la dinámica de disponibilidad de nutrientes en suelo como consecuencia de las alteraciones climáticas (Cox *et al*. 2000), especialmente de los cambios en la humedad del suelo.

IMPORTANCIA DE LOS EXPERIMENTOS MANUPULATIVOS

Para poder comprender y predecir cuáles van a ser las consecuencias reales del cambio climático sobre determinadas especies o comunidades son necesarios una serie de estudios que se pueden abordar desde diversas perspectivas. En primer lugar encontramos los estudios de modelización (Huntley *et al*. 1995; Erasmus *et al*. 2002; Peterson *et al*. 2002; Thuiller *et al*. 2004; Nogués-Bravo *et al*. 2007), en los que se simulan diferentes tipos de respuesta por parte de los organismos estudiados ante una serie de condiciones preestablecidas. Este tipo de aproximación nos permite ganar en generalidad en nuestros estudios y predecir las respuestas a lo largo de amplios gradientes geográficos o de condiciones ambientales. Sin embargo, estos estudios son poco predictivos a la hora de reflejar las respuestas microclimáticas o microtopográficas a escalas espaciales más reducidas, así como a la hora de reflejar las complejas interacciones bióticas y abióticas que aparecen en la naturaleza. El segundo tipo de aproximación es la experimentación bajo condiciones totalmente controladas (King *et al*. 1996; Perkins y Owens 2003; Maestre y Reynolds 2007), donde se analiza el efecto aislado producido por los factores estudiados de forma independiente a todos los demás, que permanecen constantes. Con este tipo de estudios obtenemos una información muy detallada de las respuestas ante el factor de estudio, pero se pierde en realidad biológica. Aunque estos dos tipos de aproximación son muy importantes y aportan una información muy valiosa, los experimentos en campo nos ofrecen una visión más integrada del funcionamiento de nuestro sistema de estudio. Dentro de estos, los experimentos de manipulación de precipitación permiten obtener una visión realista y sin producir demasiados artificios, a la vez que nos permiten simular una gran variedad de escenarios climáticos diferentes.

ÁREA DE ESTUDIO Y DISEÑO EXPERIMENTAL GENERAL

La zona de estudio en la que se han desarrollado todos los experimentos de esta tesis ha sido el Parque Nacional de Sierra Nevada, concretamente dentro de las instalaciones del Jardín Botánico de La Cortijuela (Granada; 37º05' N, 3º28' O; Fig. 3), a una altitud de unos 1650 m s.n.m. El paisaje de la zona está compuesto por una mezcla de parches de vegetación con diferente grado de manejo, en la que predominan tres diferentes unidades de paisaje o hábitats:

a) Pinares de repoblación de *Pinus sylvestris* L. y *P. nigra* Arnold. en la que podemos encontrar dispersos algunos ejemplares de otras especies arbóreas, entre las que cabe destacar *Quercus ilex* L. y *P. sylvestris* L. ssp. *nevadensis* Christ. Estas dos especies forman la vegetación potencial de la zona (Rivas-Godoy & Rivas-Martínez 1971), diferenciándose sus poblaciones naturales en cuanto a altitud y orientación. Además, podemos encontrar otras especies arbóreas acompañantes como *Acer opalus* L. ssp. *granatense* Boiss., *Sorbus aria* L., y en las zonas más umbrías *Taxus baccata* L.

b) Zonas dominadas por matorral, en las que predominan tanto especies pioneras como *Cytisus scoparius* L., *Salvia lavandulifolia* Vahl., *Ononis aragonensis* Asso. o *Adenocarpus decorticans* Boiss., como otras especies de mayor porte y hoja caduca (matorrales sucesionales), entre los que destacan por abundancia *Crataegus monogyna* Jacq., *Berberis vulgaris* L., *Prunus ramburii* Boiss. o *Amelanchier ovalis* Medik.

c) Claros entre la vegetación cubiertos por suelo desnudo o por algunas especies herbáceas dispersas.

Una descripción más detallada de la flora y vegetación de la zona puede ser encontrada en Martínez-Parras *et al*. (1987) y en Molero-Mesa *et al*. (1992).

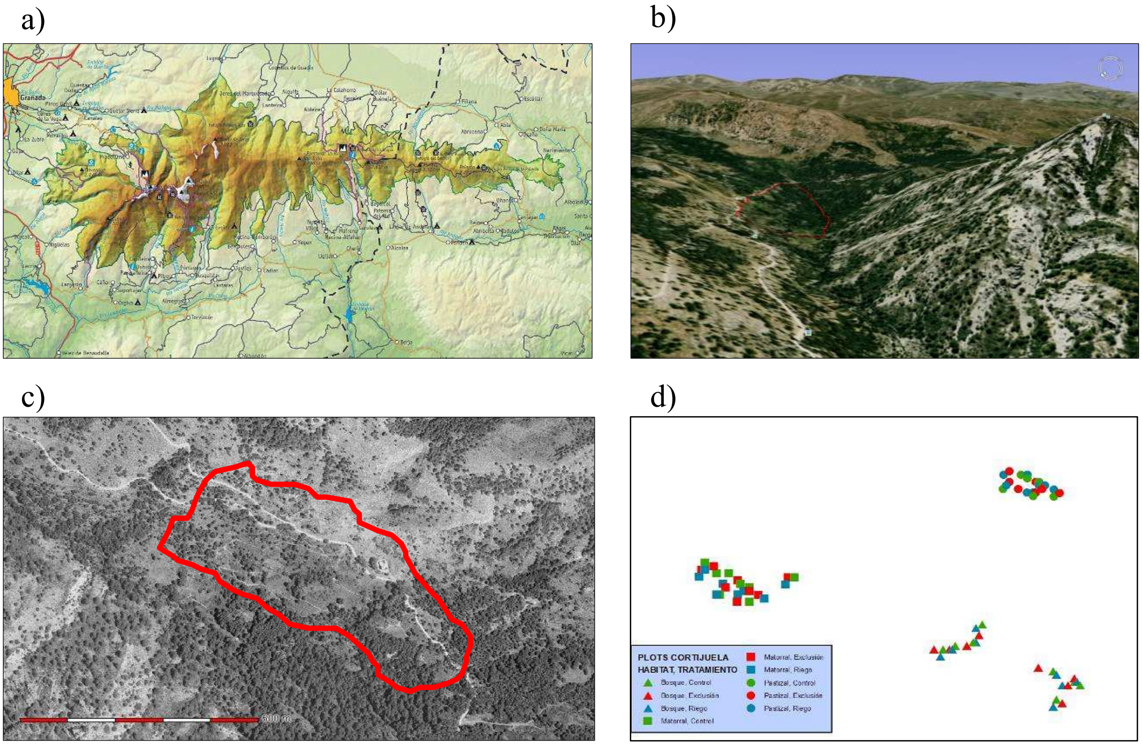


Figura 3: *Área de estudio. a) Límites del Parque Nacional de Sierra Nevada; b) Valle del Arroyo de Huenes, donde se sitúa el Jardín Botánico de La Cortijuela; c) Límites y estructura del paisaje en el Jardín Botánico La Cortijuela y Alrededores; d): Distribución espacial de los plots experimentales.*

Todos los experimentos de esta tesis se desarrollaron siguiendo el mismo diseño experimental que a continuación se detalla. En esta zona, se seleccionaron durante la primavera de 2006 tres parcelas de estudio representativas de cada uno de los tres principales hábitats de la zona, y se establecieron 24 plots de estudio de 2 × 2 m en cada una de ellas (72 en total; Fig. 3d). Durante la primavera de 2007, aleatoriamente se asignaron 24 plots (8 en cada hábitat) a cada uno de los siguientes escenarios climáticos:

a) *Verano seco*. En este escenario simulamos las condiciones climáticas durante los meses de verano esperadas para la última parte del presente siglo bajo el escenario climático SRES A-2 del IPCC, donde se prevé una reducción del total de precipitación durante los meses de verano del 30% (Fig. 1). Para ello, construimos unas casetas de exclusión de lluvia basadas en el diseño original de Yahdjian y Sala (2002). Estas casetas de exclusión están formadas por una estructura metálica que sostiene unas bandas de metacrilato transparentes sin filtros ultravioletas (Barlocast®; Faberplast S.L., Madrid) plegadas en forma de V a 90º (Fig. 4). Estas bandas de metacrilato cubren el 35% de la superficie de la caseta, interceptando la misma proporción del total de precipitación recibido de forma natural. El agua retirada, se recoge por medio de unos canalones y se conduce fuera del plot. Además, para evitar la entrada de agua por escorrentía, cavamos una zanja de unos 20 cm de profundidad y 10 de ancho alrededor de todo el plot, dejando una salida para que el agua pudiese fluir fuera. La altura de las casetas fue de 120 cm en la zona más alta y 80 en la más baja, dejando una pendiente de 11.3º. Estas estructuras permiten la libre circulación de aire por su interior, por lo que el efecto microclimático inducido sobre la parcela estudiada es mínimo. Para simular veranos más secos y más largos como los previstos por los modelos climáticos, las bandas de metacrilato se colocaron durante los veranos de 2007, 2008 y 2009 desde mediados de abril hasta mediados de septiembre, dejando los plots al descubierto durante el resto del año.

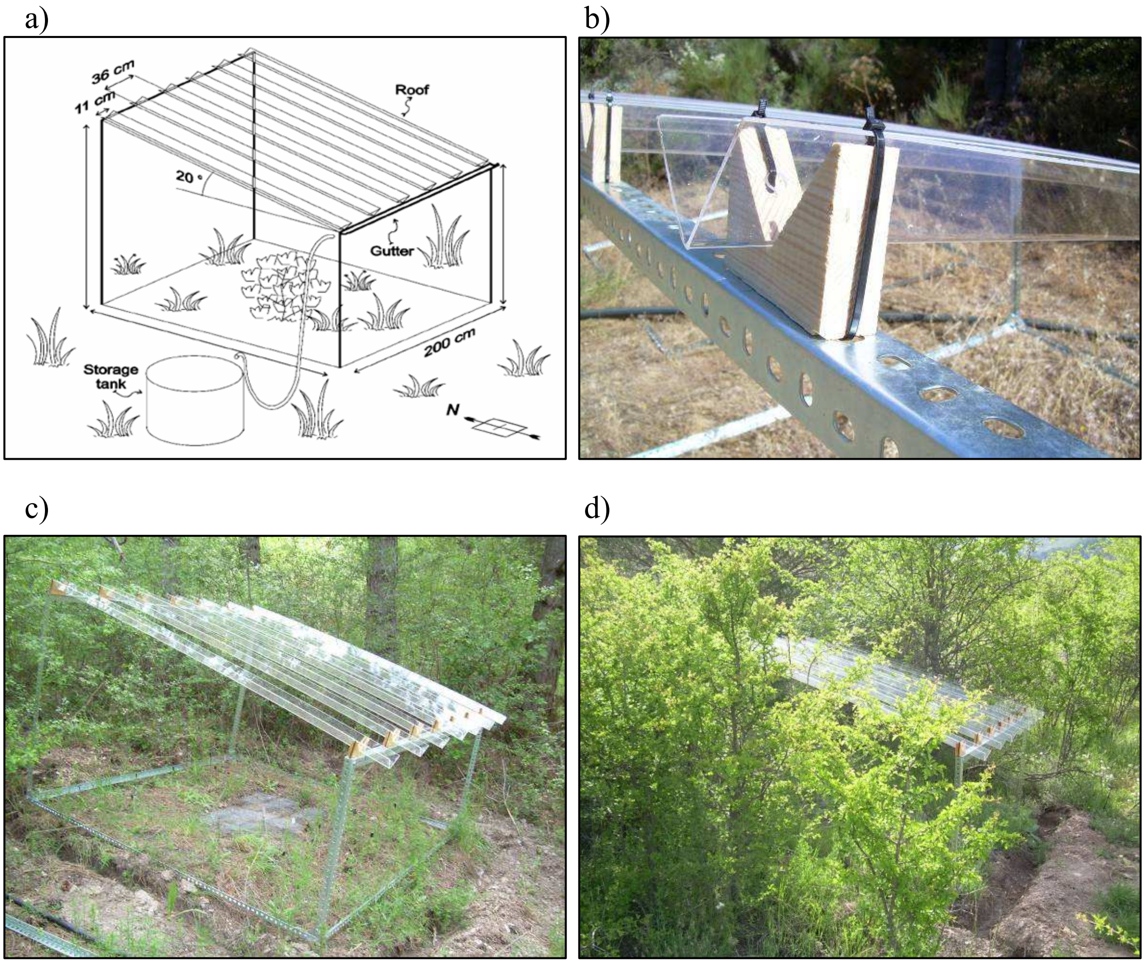


Figura 4: *a) Diseño original de las casetas de exclusión de lluvia (extraído de Yahdjian y Sala 2002); b) detalle de sujeción de las bandas de metacrilato a la estructura metálica; c) y d) casetas de exclusión de lluvia montadas en las parcelas de bosque y matorral respectivamente.*

b) *Verano húmedo*. Para la simulación de los veranos excepcionalmente húmedos que aparecen en las montañas mediterráneas nos basamos en las precipitaciones calculadas para zona de estudio de la serie de 1902 a 2006 (Fig. 2). De esta serie temporal, seleccionamos los cinco años con los veranos más húmedos (1915, 1930, 1940, 1952 y 1967), en los que el promedio de precipitación fue de 187 mm. Este promedio de precipitación fue el nivel elegido para simular este escenario climático. Para ello, colocamos un sistema de riego en los plots de 2 × 2 m formado por 4 microaspersores colocados en cada una de las esquinas con un ángulo de apertura de 90º (Fig. 5). Este sistema simulaba una tormenta de verano, de corta duración (unos 10 minutos) y alta intensidad (12 l m^{-2}) de forma semanal desde el fin de las lluvias de primavera (principios de junio) hasta el comienzo de las lluvias otoñales (mediados-finales de septiembre). Si durante una semana se producía una tormenta natural, esa semana no se aplicaba el riego, de forma que el total de lluvia recibido durante todo el verano coincide con el promedio de la serie temporal antes mencionado.

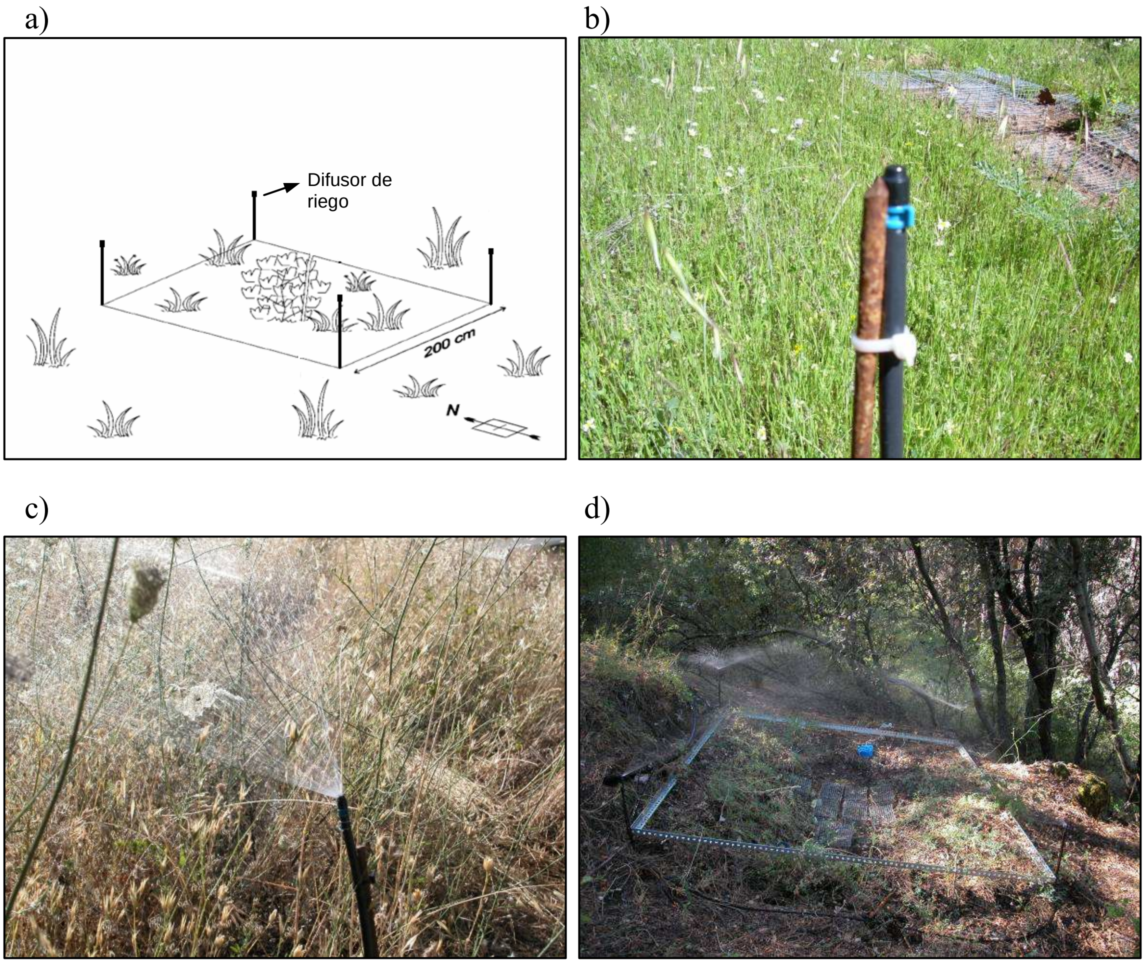


Figura 5: *a) Esquema de un plot experimental con los cuatro difusores de riego abiertos a 90º para simular una tormenta puntual; b) y c) detalles de un difusor de riego; d) plot con simulación de lluvia en funcionamiento.*

c) *Condiciones climáticas actuales*. El último de los tres escenarios climáticos contemplados consiste en las condiciones climáticas durante el desarrollo del experimento, de forma que en este caso no alteramos el patrón natural de precipitación. Este último escenario climático nos servirá además como control experimental.

OBJETIVOS DE LA TESIS Y ESTRUCTURA EN CAPÍTULOS

Como se ha indicado, el cambio climático puede tener importantes consecuencias en el funcionamiento de los ecosistemas a varios niveles, y tanto las diferentes especies de forma individual, como el total de la comunidad vegetal se pueden ver afectadas en distinto grado bajo diferentes escenarios climáticos. El objetivo general de la presente tesis es analizar el funcionamiento del ecosistema de montaña mediterránea bajo diferentes escenarios climáticos esperables para las próximas décadas. Concretamente, abordaremos los siguientes objetivos específicos:

1. Analizar los efectos sobre la diversidad del banco de reclutas.
2. Estudiar las consecuencias demográficas sobre la comunidad de plantas leñosas.
3. Estudiar qué variables morfológicas de las plántulas confieren una mejor adaptación para las condiciones de sequía.
4. Analizar los efectos de los diferentes escenarios climáticos sobre la dinámica de nutrientes en suelo.
5. Conocer los principales factores que afectan a la regeneración de la comunidad de plantas leñosas.

Para abordar estos cinco objetivos específicos, la tesis se ha dividido en cinco capítulos, dedicando uno de ellos a cada objetivo (Fig. 6). Como resultado de las diferencias en humedad del suelo la actividad microbiana del suelo se ve afectada, y por tanto también las tasas de descomposición, captación y liberación de nutrientes por parte de microorganismos, lo que puede implicar importantes consecuencias para el desarrollo vegetal. En el **capítulo 4** estudiamos la dinámica de nutrientes a través de la interacción suelo-planta-microorganismo y cómo se ve afectada por los diferentes factores, tanto climáticos como propios de cada uno de los hábitats estudiados.

En el **capítulo 2** estudiamos las consecuencias demográficas sobre ocho especies vegetales representativas de toda la comunidad de leñosas de una montaña mediterránea: árboles perennes y caducos, y matorrales pioneros y sucesionales. Analizando las respuestas de cada una de estas especies en términos de crecimiento y supervivencia comprobaremos qué grupos funcionales son capaces de adaptarse mejor a cada uno de los diferentes escenarios climáticos, y así predecir la dinámica sucesional de la comunidad para las próximas décadas.

En el **capítulo 3** se analiza la resistencia a la sequía en términos de supervivencia de diferentes especies mediterráneas y se relaciona con los rasgos morfológicos y de distribución de biomasa de cada una de ellas. Además, estudiamos las variaciones entre los diferentes hábitats que conforman el paisaje y las relaciones entre crecimiento y supervivencia. Conocer los rasgos implicados en una mejor adaptación a la sequía nos puede ayudar a determinar las especies más ventajosas en cualquier comunidad sometida a un elevado estrés hídrico.

En el **capítulo 4** se estudian los efectos de la variabilidad climática a nivel de comunidad en el banco de reclutas que se establece de forma natural. Para ello, analizamos las diferencias tanto en número de individuos como de especies de plántulas de leñosas que emergen y son capaces de establecerse bajo los diferentes escenarios climáticos estudiados. Esto nos permitirá evaluar los efectos del cambio climático en dos parámetros sumamente importantes en la estructura de la comunidad: la densidad y la diversidad de especies.

Por último, en el **capítulo 5** hacemos una síntesis de cuáles son los factores abióticos más importantes en cada uno de los diferentes estadíos demográficos implicados en el reclutamiento vegetal: emergencia, crecimiento y supervivencia. Para ello, construimos un modelo de ecuaciones estructurales que validamos con los datos demográficos y abióticos obtenidos en los capítulos anteriores. Esta aproximación nos permitirá conocer cuáles son los factores que pueden estar limitando el reclutamiento de los diferentes grupos funcionales que conforman la comunidad vegetal.

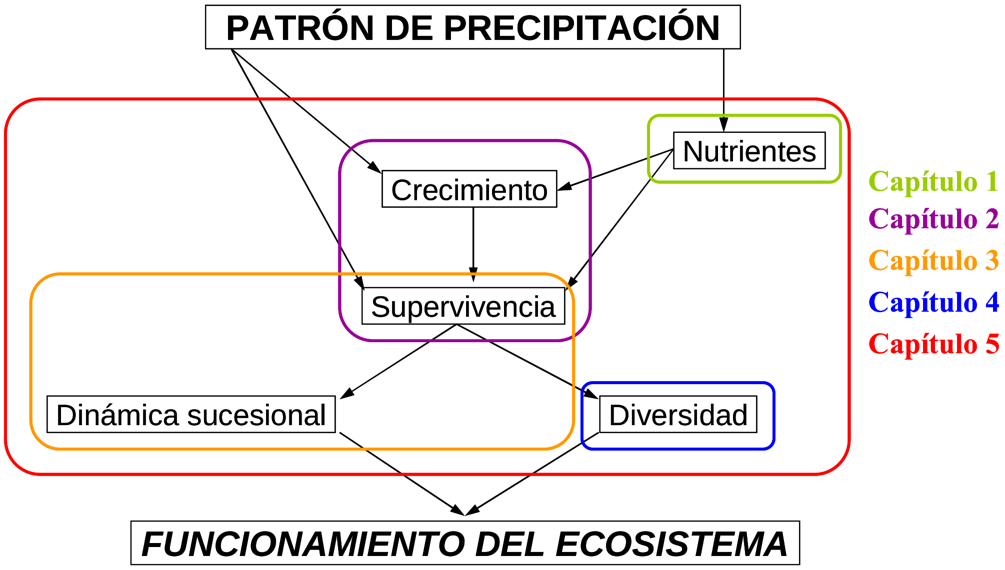


Figura 6: *Esquema conceptual de los efectos inducidos por los cambios en el patrón de precipitación esperados bajo un escenario de cambio climático, que en última instancia afectan al funcionamiento del ecosistema. En cada uno de los capítulos de esta tesis se abordan uno o varios de los procesos afectados, de forma que nos podamos aproximar a determinar el efecto global sobre el ecosistema.*

Los resultados obtenidos de este trabajo permitirán una mejor comprensión de los mecanismos y procesos implicados en la regeneración de los bosques y matorrales de las montañas mediterráneas. Estos conocimientos serán de gran valor a la hora de gestionar los espacios naturales y prever las consecuencias de los escenarios climáticos previstos para las próximas décadas, de forma que podrá ser una importante herramienta a la hora de planificar una gestión adaptativa de estos espacios, de especial interés para las montañas mediterráneas, como es el caso de los Parques Natural y Nacional de Sierra Nevada.

REFERENCIAS

Allen, C.D., Breshears, D.P. 1998. Drought-induced shift of a forest-woodland ecotone: Rapid landscape response to climate variation. *Proceedings of the National Academy of Sciences* 95: 14839-14842.

Breshears, D.P., Cobbs, N.S., Rich, P.M., Priece, K.P., Allen, C.D., Balice, R.G., Romme, W.H., Kastens, J.H., Floyd, M.L., Belnap, J., Anderson, J.J., Myers, O.B., Meyer, C.W. 2005. Regional vegetation die-off in response to global-change-type drought. *Proceedings of the National Academy of Sciences* 102: 15144-15148.

Castro, J., Zamora, R., Hódar, J.A., Gómez, J.M. 2005. Alleviation of summer drought boosts establishment success of *Pinus sylvestris* in a Mediterranean mountain: an experimental approach. *Plant Ecology* 181: 191-202.

Chapin III, F.S., Sala, O.E., Huber-Sannwald, E. 2001. *Global Biodiversity in a Changing Environment*. Springer, New York.

Cox, P.M., Betts, R.A., Jones, C.D. 2000. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. *Nature* 408: 186-187.

Crowley, T.J., North, G.R. 1988. Abrupt Climate Change and Extinction Events in Earth History. *Science* 240: 996-1002.

Erasmus, B.F.N., Van Jaarsweld, A.S., Chown, S.L., Kshatriya, M., Wessels, K.J. 2002. Vulnerability of South African animal taxa to climate change. *Global Change Biology* 8: 679-693.

Giorgi, F. 2006. Climate change Hot-spots. *Geophysical Research Letters* 33: L08707.

Giorgi, F., Lionello, P. 2008. Climate change projections for the Mediterranean region. *Global and Planetary Change* 63: 90-104.

Gracia, C.A., Sabaté, S., Sánchez, A. 2002. El cambio climático y la reducción de la reserva de agua en el bosque mediterráneo. *Ecosistemas* 11(2). http://www.revistaecosistemas.net/articulo.asp?Id=300

Hampe, A., Arroyo, J. 2002. Recruitment and regeneration in populations of an endangered South Iberian Tertiary relict tree. *Biological Conservation* 107: 236-271.

Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Xiaosu, D. 2001. Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change (IPCC), Cambridge, England

Houle, G. 1994. Spatiotemporal patterns in the components of regeneration of four sympatric tree spacies, *Acer rubrum*, *A. saccharum*, *Betula alleghaniensis* and *Fagus grandifolia*. *Jounal of Ecology* 82: 39-53.

Huntley, B., Berry, P.M., Cramer, W., McDonald, A.P. 1995. Modelling present and potential future ranges of some European higher plants using climate response surfaces. *Journal of Biogeography* 22: 967-1001.

Jensen, K.D., Beier, C., Michelsen, A., Eemmett, B.A. 2003. Effects of experimental drought on microbial processes in two temperate heathlands at contrasting water conditions. *Applied Soil Ecology* 24**:**165-176.

Jonasson, S., Michelsen, A., Schmidt, I.K., Nielsen, E.V. 1999. Responses in microbes and plants to changed temperature, nutrient, and light regimes in the arctic. Ecology 80: 1828-1843.

IPCC. 2007. Climate Change, 2007. The Physical Science Basis: Working Group I Contribution to the Fourth Assessment Report of the IPCC. Cambridge University Press, Cambridge.

King, J.S., Thomas, R.B., Strain, B.R. 1996. Growth and carbon accumulation in root system of *Pinus tadea* and *Pinus ponderosa* seedlings as affected by varying CO_2, temperature and nitrogen. *Tree Physiology* 16: 635-642.

Lloret, F., Peñuelas, J., Estiarte, M. 2004. Experimental evidence of reduced diversity of seedlings due to climate modification in a Mediterranean-type community. G*lobal Change Biology* 10: 248-258.

Maestre, F.T., Reynolds, J.F. 2007. Amount or pattern? Grassland responses to the heterogeneity and availability of two key resources. *Ecology* 88: 501-511.

Martínez-Parras, J.M., Peinado-Lorca, M., Alcaraz-Ariza, F. 1987. Comunidades vegetales de Sierra Nevada (España). Universidad de Alcalá de Henares, Alcalá de Henares.

Matesanz, S., Escudero, A., Valladares, F. 2008. Additive effects of a potentially invasive grass and water stress on the performance of seedlings of gypsum specialists. *Applied Vegetation Science* 11: 287-296

Molero-Mesa, J., Pérez-Raya, F., Valle, F. 1992. Parque Natural de Sierra Nevada. Rueda, Madrid.

Moreno, J.M. (ed.). 2005. Principales conclusiones de la evaluación preliminar de los impactos en España por efecto del cambio climático. Ministerio de Medio Ambiente, Madrid.

Nadelhoffer, K.J., Giblin, A.E., Shaver, G.R., Laundre, J.A. 1991. Effects of temperature and substrate quality on element mineralization in six arctic soils. *Ecology* 72: 242-253.

Nogués-Bravo, D., Araújo, M.B., Errea, M.P., Martínez-Rica, J.P. 2007. Exposure of global mountain systems to climate warming during the 21st Century. *Global Environmental Change* 17: 420-428.

Ogaya, R., Peñuelas, J., Martínez-Vilalta, J., Manguirón, M. 2003. Effect of drought on diameter increment of *Quercus ilex*, *Phylirea latifoia* and *Arbutus unedo* in a holm oak forest of NE Spain. *Forest Ecology and Management* 180: 175-184.

Oreskes, N. 2004. Beyond the Ivory Tower. The Scientific Consensus on Climate Change. *Science* 306: 1686.

Parmesan, C., Yohe, G. 2003. A globally coherent fingerprint of climate change impact across natural systems. *Nature* 421: 37-42.

Peñuelas, J., Filella, I. 2001. Phenology: Responses to a warming world. *Science* 294: 793-795.

Peñuelas, J., Sabaté, S., Filella, I., Gracia, C. 2008. Efectos del cambio climático sobre los ecosistemas terrestres: observación, experimentación y simulación. En: Valladares, F. (Ed.). 2008. Ecología del bosque mediterráneo en un mundo cambiante (Segunda edición). Páginas 427-462. Ministerio de Medio Ambiente, EGRAF, S.A., Madrid.

Perkins, S.R., Owens, M.K. 2003. Growth and biomass allocation of shrub and grass seedlings in response to predicted changes in precipitation seasonality. *Plant Ecology* 168: 107-120.

Peterson, A.T., Ortega-Huerta, M.A., Bartley, J., Sánchez-Cordero, V., Soberon, J., Buddemeier, R.H., Stockwell, D.R.B. 2002. Future projections for Mexican faunas under global climate change scenarios. *Nature* 416: 626-629.

Quero, J.L., Villar, R., Marañón, T., Zamora, R. 2006.Interactions of drought and shade effects on seedlings of four *Quercus* species: physiological and structural leaf responses. *New Phytologist* 170: 819-834.

Rinnan, R., Michelsen, A., Bååth, E., Jonasson, S. 2007. Fifteen years of climate change manipulations alter soil microbial communities in a subartic heath ecosystem. *Global Change Biology*, 13: 28-39.

Rivas-Godoy, S., Rivas-Martínez, S. 1971. Vegetación potencial de la provincia de Granada. *Trabajos del Departamento de Botánica y F. Vegetal,* 4: 3-85.

Rodó, X., Comín, F. 2001. Fluctuaciones del clima mediterráneo: conexiones globales y consecuencias regionales. En: Zamora, R. y Pugnaire, F. (Eds.) Ecosistemas mediterráneos. Análisis funcional. CSIC-AEET, España.

Root, T.L., Price, J.T., Hall, K.R., Scheneider, S.H., Rosenzweig, C., Pounds, J.A. 2003. Fingerprints of global warming on wild animals and plants. *Nature* 421: 57-60.

Sala, O.E., Chapin III, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M., Wall, D.H. 2000. Global Biodiversity Scenarios for the Year 2100. Science 287: 1770 – 1774.

Tilman, D. 1998. Species composition, species diversity, and ecosystem processes: understanding the impacts of global change. In: *Success, Limitations, and Frontiers in Ecosystems Science* (eds. Pace, M.L., Groffman, P.M.) pp. 452-472. Springer, New York.

Terradas, J. 2001. Ecología de la vegetación: de la ecofisiología de las plantas a la dinámica de comunidades y paisajes. Ediciones Omega, S.A. Barcelona.

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., Erasmus, B.F.N., Ferreira de Siquiera, M., Grainger, A., Hannah, L., Huges, L., Huntley, B., van Jaarsveld, A.S., Midgley, G.F., Miles, L., Ortega-Huerta, M.A., Peterson, A.T., Phillips, O.L., Williamns, S.E. 2004. Extinction risk from climate change. *Nature* 427: 145-148.

Thuiller, W. 2004. Patterns and uncertainties of species' range shifts under climate change. *Global Change Biology* 10: 2020-2027.

Thuiller, W., Lavorel, S., Araújo, M., Sykes, M.T., Prentice, C. 2005. Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Science* 102: 8245-8250.

Valladares, F., Camarero, J.J., Pulido, F. & Gil-Pelegrín, E. 2004. El bosque mediterráneo, un sistema humanizado y dinámico. En: Valladares, F. (Ed.). 2004. Ecología del bosque mediterráneo en un mundo cambiante. Pag. 13-25. Ministerio de Medio Ambiente, EGRAF, S.A., Madrid.

Valladares, F., Dobarro, I., Sánchez-Gómez, D., Pearcy, R.W. 2005. Photoinhibition and drought in Mediterranean woody saplings: Scaling effects and interactions in sun and shade phenotypes. *Journal of Experimental Botany* 56: 483-494.

Walter, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.M., Hoegh-Guldbergi, O., Bairlein, F. 2002. Ecological responses to recent climate change. *Nature* 426: 389-395.

Walters, M.B., Reich, P.B. 1996. Are shade tolerance, survival, and growth linked? Low light and nitrogen effects on harwood seedlings. *Ecology* 77: 841-853.

Yahdjian, L., Sala, O. 2002. A rainout shelter design for intercepting different amounts of rainfall. *Oecologia* 133: 95-101.

CHAPTER 1:

SOIL-NUTRIENT AVAILABILITY UNDER A GLOBAL-CHANGE SCENARIO IN A MEDITERRANEAN MOUNTAIN ECOSYSTEM

Luis Matías, Jorge Castro, and Regino Zamora

Under review in: Global Change Biology

ABSTRACT

Changes in rainfall availability will alter soil-nutrient availability under a climate-change scenario. However, studies have usually analyzed the effect of either drier or wetter soil conditions, despite the fact that both possibilities will coexist in many climatic regions of the world. Furthermore, its effect may vary across the different habitats of the ecosystem. We experimentally investigated the effect of three contrasting climatic scenarios on different carbon (C), nitrogen (N), and phosphorus (P) fractions in soil and microbial compartments among three characteristic habitats in a Mediterranean-type ecosystem: forest, shrubland, and open areas. The climatic scenarios were dry summers, according to the 30% summer rainfall reduction projected for the coming decades in the Mediterranean; wet summer, simulating summer storms to reach the maximum historical records in the study area; and current climatic conditions (control). Sampling was replicated during two seasons (spring and summer) and two years. The climatic scenario did not affect the nutrient content in the litter layer. However, soil and microbial nutrients varied among seasons, habitats, and climatic scenarios. Soil-nutrient fractions increased with lower soil-moisture conditions —that is, under a dry scenario and summer—, whereas microbial nutrients increased under the wet summer scenario and spring. This pattern was consistent both studied years, although it was modulated by habitat, differences being lower with denser plant cover. Holm oak seedlings, used as live control of the experiment, tended to increase their N and P content (although not significantly) with water availability. Thus, the results support the idea that higher rainfall boosts microbial and plant-nutrient uptake, and hence nutrient cycling. By contrast, a rainfall reduction leads to an accumulation of nutrients in the soil, increasing the risk of nutrient loss by leaching or erosion. These results show that the climate change projected for the coming decades will have significant effects on C, N, and P cycles, and therefore will have important implications on the ecosystem functioning.

Keywords: Carbon, climate change, drought, irrigation, litter, microbial, nutrients, nitrogen, phosphorus.

INTRODUCTION

Soil-nutrient availability is one of the most important limiting factors affecting plant growth (Killham 1994, Lambers *et al.* 1998). However, this availability is highly heterogeneous, showing a strong spatial and temporal variation (Gallardo and Schlesinger 1994, Ettema and Wardle 2002), frequently associated with seasonal and climatic conditions, habitat structure, or root and microbial activity (Díaz-Raviña *et al.* 1993, Gallardo and Schlesinger 1995, Criquet *et al.* 2004, Monokrousos *et al.* 2004). Under a global-change scenario, where habitat as well as climatic conditions will be altered (Houghton *et al.* 2001), it is not clear how these alterations will affect the dynamics of soil nutrients and its interaction with the plant community (Jensen *et al.* 2003, Andersen *et al.* 2010).

Global-circulation models forecast a generalized reduction in precipitation at a 30-40º of latitude for the coming decades (Houghton *et al.* 2001), which together with the increase in temperature will increase drought, especially during summers. However, climate change is also augmenting variability of precipitation in many areas (Rodrigo 2002, Beniston *et al.* 2007). Although this is less well documented, it is very likely that the greater aridity in some regions will not cancel the possibility of eventual rainy years (Beniston *et al.* 2007). These sporadic rainy years have a strong impact for regeneration (Castro *et al.* 2005, Holmgren *et al.* 2006, Mendoza *et al.* 2009). However, their possible role under a climate-change scenario has been scarcely explored, despite that they could be a key in the maintenance of the ecosystem structure (Castro *et al.* 2005, Holmgren *et al.* 2006).

Climate change is not the only global-change driver affecting ecosystems. Soil processes can be affected simultaneously by diverse human-driven factors (Sala *et al.* 2000). Interactions among these drivers frequently generate non-additive responses that cannot be predicted based on single-factor studies (Sala *et al.* 2000). Land-use change is another major driver with important consequences not only for ecosystem functioning itself (Sala *et al.* 2000, Lindenmayer and Fischer 2006) but also interacts with climate change, exacerbating or ameliorating it effects (Matesanz *et al.* 2009). This becomes especially relevant for

Mediterranean ecosystems, in which the profound human alterations undergone over centuries have produced several phases of habitat degradation (Kosmas *et al*. 2002, Matesanz *et al*. 2009).

It is increasingly clear that changes in temperature or precipitation provoked by climate change will alter nutrient cycles (Nadelhoffer *et al*. 1991, Jonasson *et al*. 2006, Riinnan *et al*. 2007, Sardans and Peñuelas 2007), and therefore nutrient availability for plants (Michelsen *et al*. 1999). Differences in carbon (C), nitrogen (N), and phosphorus (P) availability have severe effects for plant communities, since these are fundamental nutrients for plant growth (Killham 1994, Lambers *et al*. 1998), and because P has strong implications in the water-use efficiency (Graciano *et al*. 2005), modulating plant vulnerability to drought stress. These changes induced by the different climatic conditions, together with the high spatial heterogeneity of soil nutrients and processes associated with changes in habitat quality (Gallardo and Schlesinger 1995, Criquet *et al*. 2004, Monokrousos *et al*. 2004) would result in a complex situation affecting soil microbial activity (Jensen *et al*. 2003, Cookson *et al*. 2007), and therefore nutrient availability for plants. However, plant-nutrient uptake also depends on soil-water content (Kozlowski and Pallardy 2002), so it is not clear the way in which differences in soil-nutrient availability will affect plant nutritional status.

The consequences of alterations in rainfall upon soil-nutrient availability due to climate change has been widely addressed for some ecosystem, (Jensen *et al*. 2003, Allison and Treseder 2008, Sardans and Peñuelas 2007, Andersen *et al*. 2010, Illeris *et al*. 2003, Johnson *et al*. 2008), but usually within the same habitat. Only in a few cases has habitat heterogeneity or land use been considered (Cookson *et al*. 2007, Casals *et al*. 2009). However, to our knowledge, there are no field studies that analyze simultaneously the effect of habitat type and climatic change on soil-nutrient availability. This is a key point for understanding nutrient cycling and plant-soil-microbial interactions in a heterogeneous environment at the community level. Studies integrating different climatic scenarios and habitat types are necessary to properly assess the effects of climate change on plant communities for the coming decades.

In this study, we performed a field experiment to test the effect on soil C, N and P of three contrasting climatic scenarios differing in water availability and its consequences for plant-soil-microbial interaction. The scenarios were 1) current conditions (no manipulation of rainfall availability), 2) more severe summer drought according to a widely accepted IPCC scenario for the area, and 3) heavier summer rainfall simulating eventual rainy years (following maximum average records for the study area). In addition, we performed the study in the main successional habitats in the area: forest, mid-successional shrubland, and open habitat. The experiment was repeated in two consecutive summers, and samplings were performed in spring and summer in order to explore temporal variability and the possibility for lasting consequences of rainfall manipulation. Four specific questions were posed: 1) What is the effect of different climatic scenarios on the soil and microbial C, N, and P fractions? 2) Is the effect of climatic scenario interacting with habitat type? 3) Are the effects consistent through time, both at seasonal and inter-annual level? and 4) What might be the consequences for nutrient cycling?

METHODS

STUDY SITE

The study was conducted in La Cortijuela, a mountain area at 1650 m a.s.l. within the limits of Sierra Nevada National Park (37º05' N, 3º28' W, Granada, SE Spain). The area has a continental Mediterranean climate, with cold winters and hot dry summers. Mean minimum temperature in the coldest month (January) is -1.1ºC, and mean maximum of the hottest month (July) is 29.2ºC. Rainfall is 811 mm/yr, accumulated mostly during spring and autumn (means 1990-2008). Total rainfall during the experiment was 641.5 mm in 2007 and 874.8 mm in 2008. The experiment was conducted inside a natural 12.4-ha fenced area with ungulate exclosure since 1986, covered by trees (mainly *Pinus sylvestris*, *Pinus nigra*, and *Quercus ilex*), shrubs (mainly *Crataegus monogyna*, *Berberis vulgaris*, *Salvia lavandulifolia* or *Cytisus scoparius*) and open areas without woody cover (bare soil or with a sparse herbaceous cover). The bedrock is calcareous, with regosols and cambisols as predominant soil types (Delgado *et al*. 1989). Across-habitat soil texture at 0-20 cm depth is 32% sand, 48% silt, and 20% clay, with pH from 6.8 to 8.5 (mean 7.9 ± 0.2; values from Laboratorio Agroalimentario de Atarfe, Junta de Andalucía, Granada; *unpublished data*).

EXPERIMENTAL DESIGN

We performed a fully-factorial field experiment crossing habitat type and climatic scenario, each with three levels. For the habitat factor, we selected the three main successional habitats in terms of plant cover in the study area: *open*, open areas with bare soil or sparse grass cover; *shrubland*, covered by the main mid-successional shrubby species in the area (mainly *Crataegus monogyna* and *Berberis vulgaris*); and *forest*, covered by tree species, mainly *Pinus sylvestris* and *P. nigra* with scattered individuals of *Quercus ilex*. The climatic scenario factor was represented by three levels differing in water availability during summer, 1) dry summer, 2) wet summer, and 3) current climatic conditions:

1) The dry-summer scenario was based on the SRES A-2 model by Intergovernmental Panel on Climate Change (IPCC 2001), where a reduction in summer rainfall of 30% was predicted for Mediterranean areas. For this treatment, we built rain-exclusion shelters (Yahdjian and Sala 2002) formed by a 2 × 2 m metal frame supporting V-shaped clear methacrylate bands without UV filter (Barlocast®; Faberplast S.L., Madrid), covering 35% of the surface, and intercepting the same percentage of natural water supply by rain. A 20-cm deep ditch was excavated along the entire shelter to intercept runoff water. Rainout shelters were placed from April to September, simulating drier and longer summers.

2) The wet-summer scenario was simulated by placing 2 × 2 m squares on the soil with a water addition system composed of 4 sprinklers at the corners. Each week, from mid June to end September (years 2007 and 2008), we added 12 L/m^2 of water, simulating a summer storm. If a natural storm occurred one week, the irrigation pulse was not added. Thus the total water added during the summer was 180 mm, the equivalent to the mean summer rainfall of the five milder summers of the 1902-2006 series in the study area (Appendix 1).

3) Current climatic conditions during experiment development. We placed 2 × 2 m squares without water addition or exclusion, acting as a control for the experiment.

These three climatic levels will be referred to hereafter as *dry*, *wet*, and *control* scenarios, respectively. Eight replicated plots of each climatic scenario were placed in each of the three habitats, for a total amount of 72 study plots (8 replicates × 3 climatic scenarios × 3 habitats). Soil-water content was monitored monthly from May to September in all the plots by the time domain reflectometry method (TDR-100, Spectrum Technologies Inc., USA). Each plot was sampled by two perpendicular transects recording the volumetric water content every 0.5 m.

SOIL SAMPLING

Soil samples were taken three times during the experiment performance, coinciding with the moments of maximum water stress in soil (end of summer, August of 2007 and 2008), and the maximum soil biological activity (mid spring, May of 2008). Soil cores were extracted using a gouge auger (2.5 cm diameter) at two depths, 0-8 cm and 8-16 cm. Previous studies determined that this was the maximum depth that could be reached in all the habitats (Gómez-Aparicio *et al*. 2008), and we split the soil profile in half. From each study plot, we took at least four cores, which were homogenized within the same depth. Samples were immediately sieved at 2 mm removing stones, roots, and visible plant remains, and stored at 4ºC for extraction. For gravimetric determination of the water content by the difference between fresh and dry weight, a 30-g subsample was oven-dried at 105ºC for 48 h and stored for further analyses. In the same sampling dates, the litter contained in a square 10 × 10 cm was collected in all plots. Litter samples were oven-dried at 60ºC for 72 h, weighted, and ground for analysis.

Similar soil sampling was also performed in the previous summer (August of 2006) and spring (May of 2007) until the start of the experiment in order to quantify any possible variation among plots where the climatic scenarios were later simulated. All parameters measured differed among habitats, as expected, but plots where climatic scenarios were later simulated did not differ within each habitat (Appendix 2). We may thus consider that differences detected in the following years were due to the treatment.

PLANT-NUTRIENT UPTAKE

Plant-soil-microbial interactions determine nutrient availability and immobilization on a continuous time scale, making it difficult to interpret the interactions of soil and microbes with plant-nutrient acquisition at a particular sampling date (Jonasson *et al*. 2006). To determine the treatment effect on plant nutrient uptake, we sowed five Holm oak (*Quercus ilex* L.) acorns in each of the 72 experimental plots. *Q. ilex* is the most abundant tree species in these mountains, and constitutes the natural potential vegetation in the area (Rivas-Godoy and Rivas-Martínez 1971). Acorns were sowed inside 25 × 25 cm quadrats within each plot in December 2006, emergence occurred in May-June 2007, and the plants were grown during two complete growing seasons until September 2008, thus coinciding with the time of rainfall manipulation. At the end of the experiment, all surviving seedlings were harvested, (extracting roots completely, with the help of a pneumatic hammer), and oven-dried at 60ºC for 72 h, weighed, and ground for analysis of N and P pooling all seedlings (leaves, shoots, and roots together) growing in the same plot.

CHEMICAL ANALYSES

Within 24 h from soil sampling, three subsamples of 15, 15, and 7.5 g of soil were extracted for 1 h in agitation with 75ml of 2M KCl, 0.5M K_2SO_4, and 0.5M $NaHCO_3$ respectively, and filtered through Whatman GF-D filter. Another subsample was fumigated with $CHCl_3$ for 24 h in vacuum to release the nutrients in the microbial biomass (fumigation-extraction method, Jenkinson and Powlson 1976), after which the soil was extracted with 0.5 M K_2SO_4 and 0.5 M $NaHCO_3$ and filtered as above. Fumigated and non-fumigated extracts were frozen at -20ºC until analyzed (Schinner *et al*. 1995).

From the dried subsample, soil organic-matter content (SOM) was determined by the incineration at 550ºC with a thermobalance (Leco TGA 701) to constant weight (Sparks 1996), while total C (C_{tot}) and N (N_{tot}) were determined by combustion at 850ºC (Leco TruSpec autoanalyzer), and total inorganic C (TIC) was measured by acidification with $HClO_4$ in a TIC analyzer (UIC CM-5014). The difference between C_{tot} and TIC gave the total organic C (C_{org}). Ammonium (NH_4^+) and nitrate (NO_3^-) were determined from KCl extracts by the Kjeldhal method (Bremner and Keenely 1965) with a Buchi distillation unit B-324 and a Metrohm SM Titrino 702 titrator. These two elements were combined into inorganic N (N_{inorg}). From K_2SO_4 extracts (fumigated and non-fumigated), we determined the dissolved organic C (DOC) and dissolved organic N (DON) with a Shimadzu TOC-V CSH analyzer. Microbial C and N (C_{micro} and N_{micro}, respectively) were determined by the difference in DOC and DON between fumigated and non-fumigated subsamples. Inorganic P (P_{inorg}) was determined in non-fumigated $NaHCO_3$ extracts by the Olsen method (Watanabe and Olsen 1965) with a Perkin Elmer 2400 spectrophotometer. Microbial P (P_{micro}) was measured as the difference in P between the fumigated and non-fumigated extracts. Concentration values in the microbial fraction were not corrected for extraction efficiency, as the main objective of the study is to analyze the effect of the different treatments on nutrient availability rather than determine the total nutrient immobilization. For simplicity, we refer to SOM, extractable soil N and P, dissolved organic fractions (DON and DOC), and microbial nutrient content (C, N and P) as the soil-nutrient fractions and microbial fractions, respectively, hereafter. The C and N content in litter were determined by combustion at 850ºC (Leco TruSpec autoanalyzer), and P by the molybdovanadate method (AOAC 1975). The proportion of C, N, and P was referred to the dry weight of the 10 × 10 cm sample and expressed as g/m^2. *Q. ilex* seedlings were also analyzed for N, and P following the same procedure.

DATA ANALYSIS

Differences in soil volumetric water content were analyzed between habitats and climatic scenarios by a repeated-measures ANOVA. The effect of habitat and climatic scenarios on SOM and C, N, and P forms in soil and microbes was analyzed using a factorial ANOVA for each nutrient form followed by Bonferroni correction. As simulations of climatic scenarios were applied only in summer, and we used a different number of factors for each season (spring and summer), we analyzed the two seasons separately instead of analyzing all the data together with rm-ANOVA. For spring, we used habitat and climatic scenario as independent factors. For the summer analysis, we also included year (2007 and 2008) as a factor, which allowed us to evaluate both inter-annual variability and the cumulative effect of one or two climatic simulations (one in 2007 and a second in 2008). As depth had a consistent effect on all soil and microbial nutrient fractions (higher concentrations in the upper soil profile), we eliminated this factor from the analysis, pooling data from two depths (differences among depths are shown in Appendix 3). For litter, we performed similar analyses for the concentration and total pool of C, N, and P. For N and P concentrations and pools in *Q. ilex* seedlings, we performed one-way ANOVAs to test the effect of climatic scenario within the habitat. We used this approach instead a two-way factorial model because we were using seedlings as a live control of the effect of climatic conditions, rather than focusing on differences among habitats. To fulfill normality and homoscedasticity assumptions, variables were log-transformed when necessary.

Fisher's *post hoc* PSLD test was used for differences within groups. Values are given throughout this paper as mean ± standard error. Analyses were made using JMP 7.0 (SAS Institute Inc. 2007).

RESULTS

SOIL-WATER AVAILABILITY

The volumetric soil-water content was significantly different among habitats ($F_{2,715} = 499.68$; $P < 0.0001$) and climatic scenarios ($F_{2,715} = 1214.71$; $P < 0.0001$; Fig. 1). Forest was the habitat with the highest soil moisture (15.1 ± 0.2%), followed by shrubland (14.6 ± 0.2%) and open (12.4 ± 0.2%). Among climatic scenarios, the highest values in soil moisture appeared under the wet-summer scenario (16.4 ± 0.2%), followed by control (13.8 ± 0.2%), and dry-summer scenario (11.9 ± 0.2%; Fig. 1). The climatic-scenario simulations translated therefore in concordant differences in soil moisture.

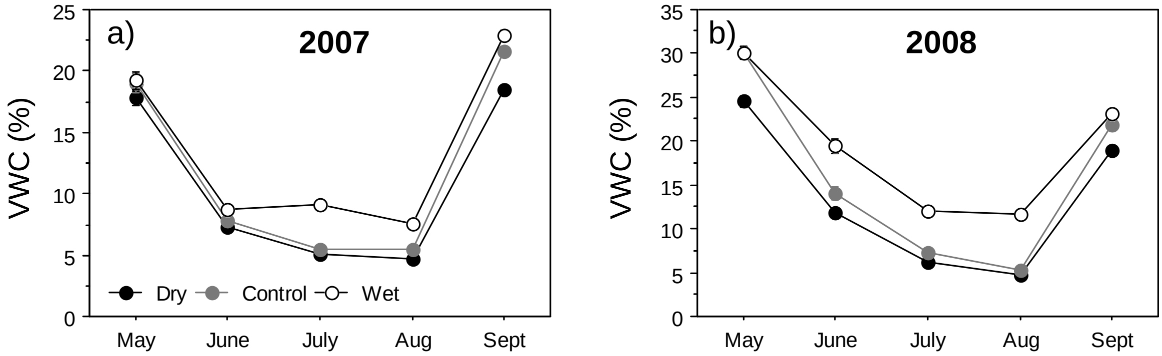


Figure 1*: Volumetric water content (VWC, in %) during experiment development (2007 and 2008) under the different climatic scenarios simulations: dry summer (black circles), control (grey circles), and wet summer (open circles). Habitats are pooled. Means are given ± SE.*

LITTER NUTRIENT POOL AND CONCENTRATION

The litter-nutrient concentration was determined mainly by habitat effect (Table 1), with higher values in open (C: 34.67 ± 0.37%; N: 1.07 ± 0.04%; P: 1.11 ± 0.04 mg/g) than in shrubland (C: 34.68 ± 0.51%; N: 1.13 ± 0.03%; P: 0.81 ± 0.02 mg/g) or forest (C: 35.94 ± 0.77 %; N: 0.73 ±

0.02%; P: 0.60 ± 0.01 mg/g) pooling scenarios and seasons. However, as different habitats differed in the total litter mass, (forest 5,762 ± 231 g/m^2; shrubland 1,684 ± 111 g/m^2; open 801 ± 59 g/m^2), the total nutrient pool contained in litter followed the opposite pattern (Table 1), with higher values in forest (C: 2,690 ± 183.94 g/m^2; N: 56.07 ± 3.98 g/m^2; P: 4.41 ± 0.28 g/m^2) than in shrubland (C: 506.52 ± 22.51 g/m^2; N: 16.97 ± 1.02 g/m^2; P: 1.21 ± 0.08 g/m^2) or open (C: 214.76 ± 16.56 g/m^2; N: 6.64 ± 0.81 g/m^2; P: 0.70 ±0.07 g/m^2). The climatic scenario had no effect, either on litter concentration or total nutrient pool during spring or summer. No differences among years appeared in total litter-nutrient pool or concentration, except a slight decrease in summer-N concentration in 2008 (0.99 ± 0.03% in 2007 and 0.97 ± 0.03% in 2008; Table 1).

Table 1: *Results of the factorial ANOVA testing differences in nutrient total carbon (C), nitrogen (N) and phosphorus (P) pool and concentrations in A) litter, and B) Q. ilex seedlings. Significative values (P < 0.05) after Bonferroni correction are bold-signaled: * 0.05 ≤ P < 0.01; ** 0.01 ≤ P < 0.001; *** P ≤ 0.001.*

	C	N	P
A) Litter			
Spring concentration			
Habitat	**4.59***	**34.43*****	**22.69*****
Scenario	1.85	0.19	0.01
H × S	1.20	0.96	0.99
Spring pool			
Habitat (H)	**69.17*****	**34.16*****	**49.74*****
Scenario (S)	0.83	0.89	2.03
H × S	1.10	1.74	2.53
Summer concentration			
Habitat	1.89	**32.55*****	**80.05*****
Scenario	0.88	0.79	0.17
Year	2.14	**14.26*****	1.85
H × S	0.63	0.87	1.24
H × Y	1.44	0.22	**6.53****
S × Y	0.96	2.35	1.52
H × S × Y	0.35	2.40	1.29
Summer pool			
Habitat	**87.09*****	**76.94*****	**84.94*****
Scenario	0.23	0.04	0.20
Year (Y)	0.04	0.97	0.11
H × S	0.17	0.30	1.01
H × Y	0.26	0.78	0.04
S × Y	0.25	0.08	0.17
H × S × Y	0.16	0.16	0.21

SOIL AND MICROBIAL NUTRIENT CONTENT

Soil and microbial nutrient fractions differed among seasons, habitats, and climatic scenarios (Table 2). In spring 2008, habitat affected SOM, C_{org}, DOC, N_{tot}, N_{inorg} and C_{micro}, but had no effect on DON, P_{inorg}, N_{micro}, or P_{micro}. For those fractions with a significant habitat effect, overall there were higher values in open for most of them, except for DOC (highest in forest) and N_{inorg} (highest in shrubland). Despite the heterogeneous effect of habitat, none of the nutrient fractions was affected by climatic scenario (Table 2), indicating that the effect exerted by this factor in the previous summer (see below) is lost during spring.

By contrast, soil and microbial nutrient fractions during summer were globally affected by habitat and climatic scenarios. Habitat affected most fractions (Table 2), generally with lower values in forest (Fig. 2). Climatic scenario effect was significant for all soil and microbial nutrient fractions except N_{inorg} (Table 2). A significant interaction between habitat and climatic scenario appeared in some cases as a consequence of changes in the pattern of climatic simulation across habitats (Table 2; Fig. 2). However, two general trends could be distinguished. First, either the dry or the control scenario showed the highest values for most of the soil fractions, whereas the wet scenario showed the lowest values. This was particularly consistent for DON, DOC and P_{inorg} (Fig. 2c-e) and also appeared in some habitats for SOM, C_{org} and N_{tot}. Second, microbial N and P showed the opposite pattern, with a clear trend to increase in the wet scenario (Fig 2i,j). Microbial carbon peaked for the dry scenario in shrubland, but did not show a clear pattern across climatic scenarios. Overall, forest was the habitat where differences among climatic scenarios were lower, especially patent for P fractions (Fig. 2e,j). All soil and microbial fractions with the exception of SOM and N_{tot} varied among seasons (Fig. 3). In general terms, soil nutrients increased their concentrations during summer, whereas microbial nutrient concentrations increased in spring (Fig. 3). The pattern across seasons and across climatic scenarios was therefore similar, with an increase in

Table 2*: Results of the factorial ANOVA for differences in habitat, climatic scenario, and their interactions on the different elements: soil organic matter (SOM), organic carbon (C_{org}), dissolved organic carbon (DOC), total nitrogen (N_{tot}), inorganic nitrogen (N_{inorg}), dissolved organic nitrogen (DON), phosphorus (P_{inorg}), microbial carbon (C_{micro}), microbial nitrogen (N_{micro}), and microbial phosphorus (P_{micro}) during spring (2008) and summer (2007 and 2008). All variables except C_{org} were log-transformed. Year factor join both the effect of inter-annual variation and the cumulative effect of one or two climatic simulations. Significative values ($P < 0.05$) after Bonferroni correction are bold-signaled: * $0.05 \leq P < 0.01$; ** $0.01 \leq P < 0.001$; *** $P \leq 0.001$.*

Factor	SOM	C_{org}	DOC	N_{tot}	N_{inorg}	DON	P_{inorg}	C_{micro}	N_{micro}	P_{micro}	D.F.
Spring											
Habitat (H)	**11.20*** **	**5.85** **	**10.83*** **	**17.58*** **	**4.19* **	1.65	1.97	**4.12* **	1.87	3.00	2
Scenario (S)	1.23	0.45	0.78	1.49	1.55	0.68	0.41	1.45	1.47	0.49	2
H × S	0.61	0.66	0.63	0.69	1.26	0.87	0.35	0.59	1.51	0.38	4
Summer											
Habitat	**45.79*** **	**33.02*** **	1.80	**71.21*** **	**9.04*** **	**15.94*** **	**5.15** **	**6.44** **	**7.51*** **	**4.27* **	2
Scenario	**13.69*** **	**7.45** **	**28.11*** **	**10.41*** **	3.03	**21.81*** **	**15.34*** **	**8.62*** **	**7.54*** **	**9.36*** **	2
Year (Y)	3.05	2.17	0.28	0.71	2.02	**18.91*** **	**66.47*** **	4.60	**8.36** **	**3.80* **	1
H × S	5.06	**3.80** **	**3.71* **	**4.11** **	**6.41*** **	3.12	**5.74** **	**4.32** **	0.91	0.60	4
H × Y	1.21	0.13	1.49	1.82	**10.25*** **	0.82	0.26	0.75	1.10	0.80	2
S × Y	0.17	0.67	0.56	0.21	1.85	0.22	0.02	0.21	0.11	2.02	2
H × S × Y	0.07	0.14	0.88	0.09	1.21	0.56	0.33	0.53	0.33	0.93	4

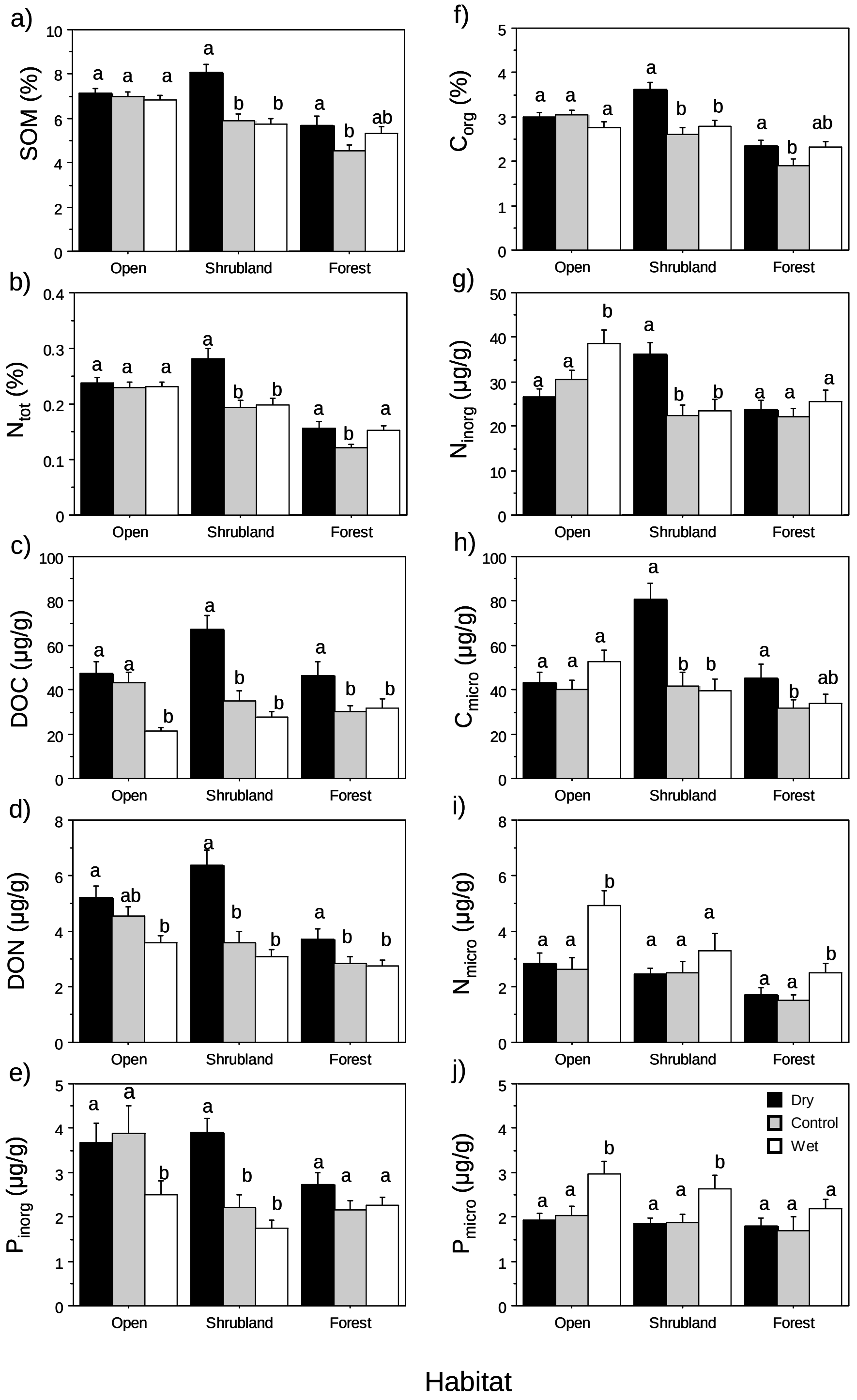


Figure 2: *Mean soil organic matter (SOM), and soil (C_{org}, DOC, N_{tot}, N_{inorg}, DON, and P_{inorg}) and microbial (C_{micro}, N_{micro}, and P_{micro}) fractions during summer among the three studied habitats: open, shrubland, and forest, and the three different climatic scenarios: dry summer scenario (black bars), control (grey bars), and wet summer scenario (open bars). Differences among climatic scenarios within habitat are indicated by different letter. Depths and years are pooled. Error bars represents SE. Concentration values in the microbial fractions were not corrected for extraction efficiency.*

inorganic nutrient availability under dry conditions and a reduction with higher moisture availability.

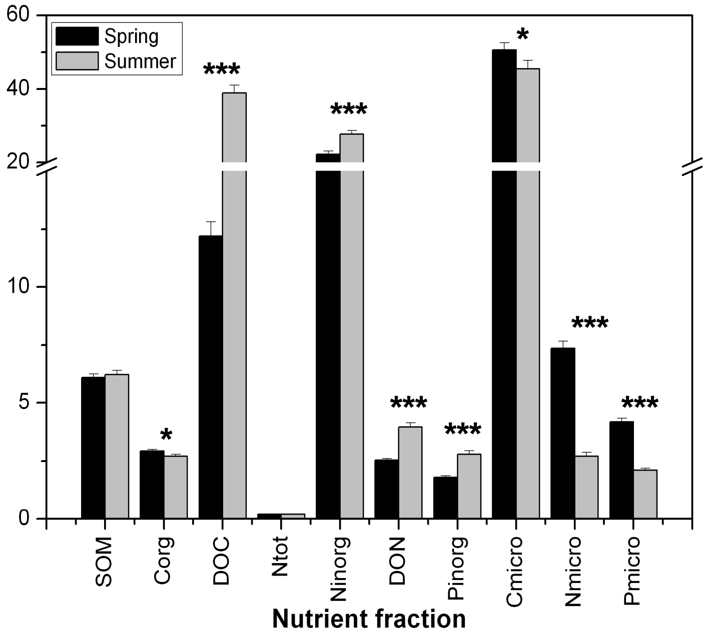


Figure 3: *Soil and microbial nutrients variations among seasons: spring (2007, black bars), and summer (2007 and 2008 data pooled, grey bars). Different habitats, climatic scenarios and depths are pooled. Significative differences among depths after Bonferroni correction are indicated: * 0.05 ≤ P < 0.01; ** 0.01 ≤ P < 0.001; *** P ≤ 0.001. Error bars represents standard error. SOM: soil organic matter, in %;* C_{org}*: organic carbon, in %; DOC: dissolved organic carbon, in µg/g;* C_{micro}*: microbial carbon, in µg/g;* N_{tot}*: total nitrogen, in %;* N_{inorg}*: inorganic nitrogen, in µg/g; DON: dissolved organic nitrogen, in µg/g;* N_{micro}*: microbial nitrogen, in µg/g;* P_{inorg}*: inorganic phosphorus, in µg/g;* P_{micro}*: microbial phosphorus, in µg/g. Concentration values in the microbial fractions were not corrected for extraction efficiency.*

Yearly variation presented a significant effect for DON, P_{inorg}, N_{micro}, and P_{micro} (Table 2), and had a consistent effect across habitats. DON and N_{micro} followed the same pattern, being consistently lower in 2008, after two climatic simulations in the three habitats (Fig. 4a,b). P_{inorg} also was lower after two climatic simulations, but the trend reversed for P_{micro} except in the dry scenario (Fig. 4d). Although yearly variations were found, the same pattern across climatic scenarios persisted in both study years.

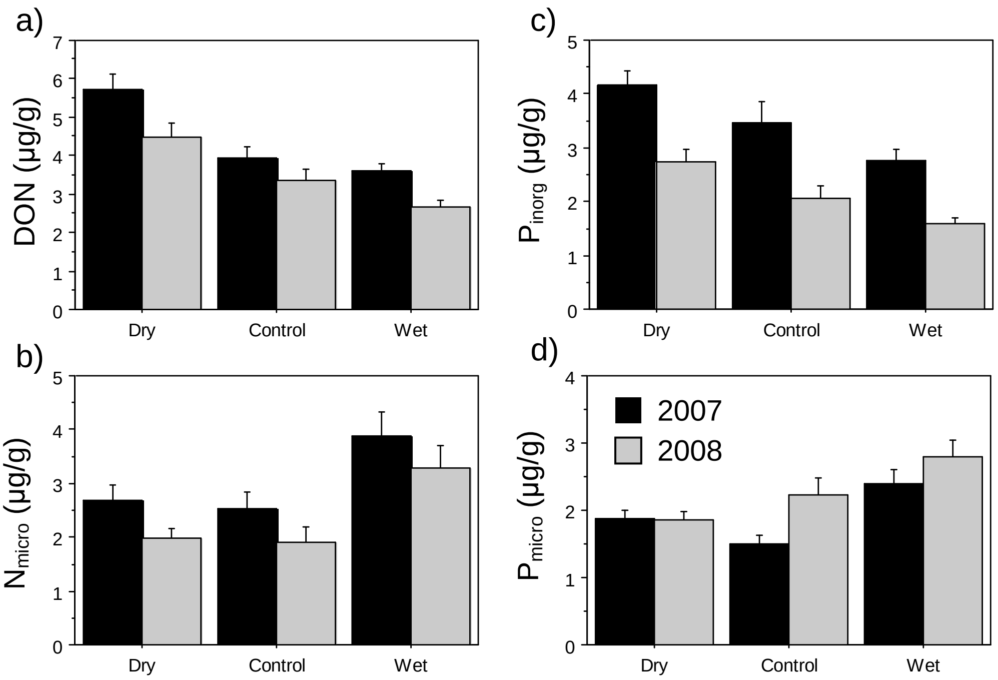


Figure 4: *Mean concentrations of dissolved organic nitrogen (a), microbial nitrogen (b), inorganic phosphorus (c), and microbial phosphorus (d) during 2007 (black bars) and 2008 (grey bars). Error bars represents SE. Concentration values in the microbial fractions were not corrected for extraction efficiency.*

MICROBIAL C:N RATIO

Microbial C to N relations significantly varied among seasons (F = 53.21, $P < 0.0001$), with higher values in summer (27.7 ± 1.9) than in spring (8.4 ± 0.5; pooling habitats and scenarios), indicating higher N immobilization by microbes during the spring period. No effect of habitat was detected in C:N relation during spring or summer but, in contrast, although climatic scenario showed no effect in spring, it had a strong effect during summer (F = 12.27, $P < 0.0001$): overall, the C:N ratio was higher under the dry-summer scenario (mean 39.6 ± 4.4; pooling habitats and depths), whereas this relation was lower under the wet-summer scenario (18.6 ± 2.0; Fig. 5). Differences among seasons and among climatic scenarios indicated higher N immobilization by microbes per mass unit with higher soil moisture.

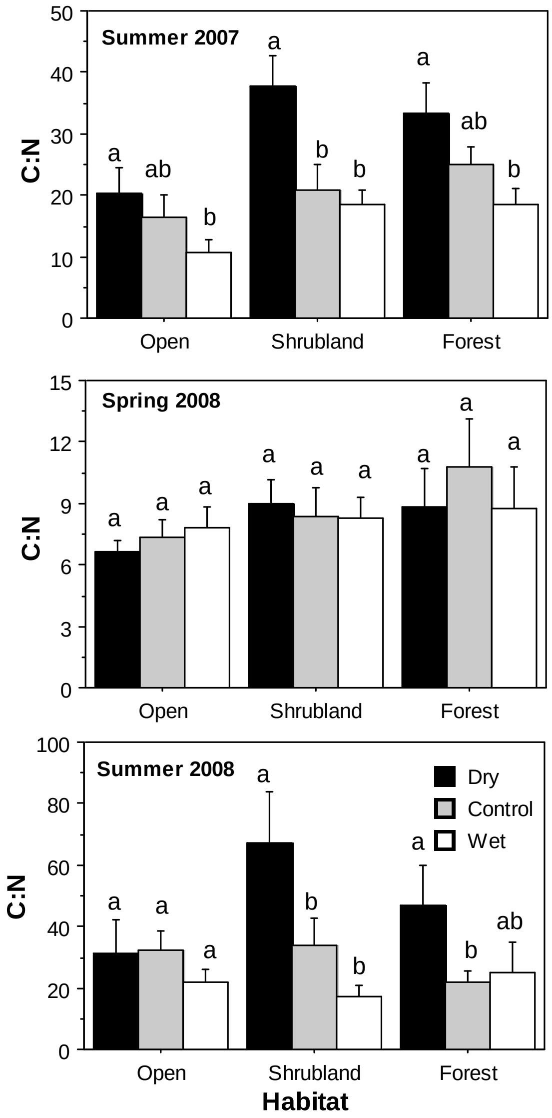


Figure 5: *Mean microbial C:N ratios among the three studied habitats: open, shrubland, and forest, and the three different climatic scenarios: dry summer scenario (black bars), control (grey bars), and wet summer scenario (open bars). Differences among climatic scenarios within habitat are indicated by different letter. Depths are pooled. Error bars represents SE.*

SEEDLINGS N AND P

There were no differences in N and P concentrations in *Q. ilex* seedlings among climatic scenarios for any of the habitats studied (*P* > 0.05 in all cases). The overall mean N and P concentrations were 0.62 ± 0.02% and 0.68 ± 0.04 mg/g respectively. However, as seedling mass was slightly greater for those seedlings growing under the wet-summer scenario, N and P total pool contained tended to increase (Table 3), although these differences were not significant.

Table 3: *Mean ± SE values of N and P total pool contained in Q. ilex seedlings growing under the different climatic scenarios.*

	Dry	**Control**	**Wet**
N			
Open	13.64 ± 2.32	17.61 ± 7.24	20.92 ± 3.28
Shrubland	9.36 ± 1.54	7.38 ± 0.49	9.13 ± 1.52
Forest	11.56 ± 0.94	10.01 ± 1.18	12.82 ± 1.20
P			
Open	1.20 ± 0.29	1.21 ± 0.41	2.07 ± 0.46
Shrubland	0.97 ± 0.19	0.76 ± 0.14	1.20 ± 0.23
Forest	1.32 ± 0.18	1.33 ± 0.14	1.55 ± 0.17

DISCUSSION

Changes in soil-moisture availability as a consequence of climate change have the potential to alter soil-nutrient availability and soil-plant-microbial interactions (Emmett *et al*. 2004). The possible impact has been analyzed considering either drier (Sardans and Peñuelas 2007, Andersen *et al*. 2010) or milder conditions (Illeris *et al*. 2003, Johnson *et al*. 2008), trying to simulate the most plausible scenario for the coming decades in those ecosystems. However, this is the first study considering simultaneously these two possible scenarios across different habitats that make up the ecosystem, despite that climate and land-use changes are the two main drivers of global change at the planetary scale. Only by integrating the different climatic scenarios under a gradient of habitat quality could we precisely assess the impact of climate change and predict its consequences on the different compartments involved in nutrient dynamics (i.e. nutrient input to soil and plant assimilation).

Our experimental results reveal strong differences in soil and microbial nutrient concentrations among seasons, years, habitats, and climatic scenarios. Microbial nutrient content increased in spring, whereas soil nutrients did during summer. Seasonal variations in soil and microbial nutrients have been reported from different ecosystems worldwide (Díaz-Raviña *et al*. 1993, Miller *et al*. 2009), and are a response of temperature and soil-moisture differences (Mlambo *et al*. 2007). During spring, when the temperature is not too high and water is not a limiting factor, nutrients available in soil can be lower due to the greater nutrient demand by plants and microbes (Wardle 1992), as well as to higher leaching or run-off (Singh *et al*. 1989, Srivastava *et al*. 1992). By contrast, during the summer drought, plant and microbes demand and leaching decline, at the same time that evaporation could augment upward nutrient movement (Austin *et al*. 2004 and references therein), thereby increasing the soil extractable nutrient concentration. Finally, habitats and climatic scenarios showed a strong effect for almost all the nutrient forms analyzed in summer. Moreover, there were differences related to the number of simulations of the climatic events (one or two years). We could not ascertain whether this was due solely to the number of events, to inter-annual variability, or both, but in any case provided consistent effects for key components of the nutrient cycle such as DON, P_{inorg}, N_{micro}, and P_{micro}. All this support the idea that climate change may profoundly alter nutrient availability and soil-plant-microbial interactions in a short period, and that these changes may happen across the main habitats at the ecosystem level.

CLIMATIC SCENARIOS EFFECT

Climatic scenarios consistently affected all soil and microbial fractions except N_{inorg} during summer. In general terms, soil-nutrient concentrations decreased with soil-moisture increase, from dry to wet climatic scenarios, whereas the pattern was the opposite for microbial fractions. Usually, soil-nutrient availability is inversely related to microbial activity (Ross and Sparling 1993, Killham 1994, Criquet *et al*. 2004, Monokrousos *et al*. 2004), as our results confirm. The decrease in soil nutrients under the wet scenario is explained by the positive relationship between microbial biomass and soil moisture (Santruckova 1992, Kandeler and

Bohm 1996), as well as the higher plant uptake (Kozlowski and Pallardy 2002), resulting in the opposite case with drier conditions. Mild years have therefore strong implications for ecosystem functioning, since they activate N and P cycling, two of the most limiting resources in Mediterranean areas (Sardans and Peñuelas 2007). Furthermore, this pattern was reinforced by seasonal variations increasing microbial uptake during spring as well as the soil fractions during summer. However, differences in soil moisture also altered microbial C:N relations. In general terms, lower soil moisture increased C:N relation, both among seasons and scenarios, this pattern being consistent through habitats. These differences have been commonly addressed to an alteration of microbial community composition (Austin *et al*. 2004, Schimel *et al*. 2007). Lower C:N relations should be interpreted as a bacterial-dominated community, whereas higher C:N values indicates a fungi dominance (Ross and Sparling 1993, Schimel *et al*. 2007). In our case, bacteria may be responsible for most microbial activity during spring, whereas fungi predominate in summer, especially under the dry summer scenario. Thus, fungi dominance would increase under the drier conditions expected for the coming decades.

Although differences were found between the two sampled summers, the same pattern among climatic scenarios was repeated in 2007 and 2008 for some soil and microbial nutrients. The overall between-year pattern reinforced the results found by climatic scenarios simulations, increasing soil nutrients (DON and P_{inorg}) during the drier 2007, whereas the milder 2008 increased microbial P immobilization. However, this was not the case of N_{micro}, which was higher in 2007. Because 2007 was the first year with scenarios simulations and in 2008 accumulated two consecutive years of simulations, it is difficult to determine the source of variation.

Differences in soil and microbial nutrient availabilities were not reflected in the *Q. ilex* N and P concentrations. The lack of difference in nutrient seedling concentration among those scenarios in which soil and microbial concentrations varied may indicate two important facts: on one hand, seedlings did not take advantage of the greater soil-nutrient availability under the dry scenario, probably for the lack of enough water to take them up in solution (Kozlowski and Pallardy 2002). On the other hand, seedlings did not reduce nutrients under the wet scenario, where microbial retention was higher and soil availability was lower. In fact, the higher seedling growth under this scenario increased the total N and P pool. Thus, higher soil moisture increased nutrient uptake by microbes and plants, boosting nutrient circulation among the different compartments of the cycle. This result indicates that seedlings and microorganisms are not competing for resources, and that a higher microbial nutrient immobilization has positive effects for seedling performance at a seasonal scale (Jonasson *et al*. 2006).

PATTERNS ACROSS HABITAT

Habitat was an important factor determining nutrient availability, microbial immobilization, and overall plant-microbial interactions as reported in many other studies (Gallardo and Schlesinger 1995, Criquet *et al*. 2004, Monokrousos *et al*. 2004, Cookson *et al*. 2007). Differences among habitats are expected, since differences in plant cover determine the soil-nutrient input by different litter quantity and quality (Santa Regina *et al*. 1997, Holmgren *et al*. 2000). Besides these differences among habitats, litter nutrient content was not affected by climatic scenarios, probably due to the small scale of the study plots, which did not affect to leaf-nutrient content of trees or shrubs or decomposition in the soil.

Although the main effects of the different climatic scenarios were consistent, increasing soil nutrients under the drier conditions and increasing microbial immobilization under the wetter scenario, habitat was able to modulate them. The lack of differences between dry scenario and control in open for all soil and microbial fractions suggest the already limiting conditions of current summers in this habitat, where drought reduces microbial biomass to the minimum levels. Due to this drought limitation, it is in this habitat where a wet summer is especially important to activate microbial activity and nutrient cycling. However, shrubland, and especially forest, had the capacity to partially compensate the effect of the different climatic scenarios. Although differences in reduction appeared in covered habitats for various fractions (N_{inorg}, DON, N_{micro}), this was particularly clear in the case of P, which did not vary its concentration in soil or microbes among scenarios in forest. According to these results, soil sensitivity to changes in precipitation (either higher or lower) varies with the plant cover. That is, denser canopies increase habitat resilience (*sensu* Holling 1973), making them less prone to alter nutrient dynamics. This interaction of climatic scenarios effects by the different habitats should be taken into account for predictions and forecasting models for the effect of climate change on nutrient cycling.

CONCLUSIONS

The interaction between soil, microbes, and plants in relation to nutrient cycle is a complex network deeply affected by habitat structure and climatic conditions. Under a global-change scenario, where climatic and land-use change are expected (Houghton *et al*. 2001), this interaction will be altered. Wetter scenarios induce higher microbial activity, increasing therefore the mineralization rate (Killham 1994) and mid-term nutrient availability for plants (Jonasson *et al*. 2006). On the contrary, a dryer climate reduces microbial nutrient uptake, increasing soil availability. However, these effects would be modulated by the different habitats: dense-covered habitats as forests are able to ameliorate the effects of the different climatic scenarios. By contrast, habitats with sparse plant cover are more dependent on milder conditions to enhance microbial activity and nutrient cycling. The higher nutrient availability in soil under drier conditions could not be exploited by plants, presumably due to the lack of enough water to take them up in solution. This higher nutrient pool in soil, together with the higher torrential rainfall predicted for the coming decades (Houghton *et al*. 2001) may increase the risk of nutrient loss by leaching or erosion (De Luis *et al*. 2003, Ramos and Martínez-Casanovas 2004), leading to a short to middle-term nutrient loss and soil impoverishment.

ACKNOWLEDGEMENTS

We thank the Consejería de Medio Ambiente (Andalusian Government) and the Direction of the Sierra Nevada National Park for facilities and support to carry out the experiment. We also wish to thank to Nacho Villegas, Ramón Ruiz, and Eulogio Corral for field and lab assistance. This work would be not possible without the invaluable help and advice of Susana Hitos with chemical analyses. David Nesbitt checked the English of this paper. This study was supported by the coordinated Spanish MEC Project DINAMED (CGL2005-05830-C03), GESBOME (P06-RNM-1890), Subprograma de Técnicos de Apoyo MICINN (PTA2009-1782-I), and by a grant FPI-MEC (BES-2006-13562) to LM. This research is part of the GLOBIMED (www.globimed.net) network in forest ecology.

LITERATURE CITED

Allison, S. D. and K. K. Treseder. 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. Global Change Biology **14**:2898-2909.

Andersen, L.C., A. Michelsen, S. Jonasson, I.K. Schmidt, T.N. Mikkelsen, P. Ambus, C. Beier. 2010. Plant nutrient mobilization in temperate heathland responds to elevated CO_2, temperature and drought. Plant and Soil **328:**381-396.

AOAC, Association of Official Analytical Chemists. 1975. Methods of analysis. (12th edn.), Washington, DC, USA.

Austin, A. T., L. Yahdjian, J. M. Stark, J. Belnap, A. Porporato, U. Norton, D. A. Ravetta, and S. M. Schaeffer. 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia **141**:221-235.

Beniston, M., D. B. Stephenson, O. B. Christensen, C. A. T. Ferro, C. Frei, S. Goyette, K. Halsnaes, T. Holt, K. Jylha, B. Koffi, J. Palutikof, R. Scholl, T. Semmler, and K. Woth. 2007. Future extreme events in European climate: an exploration of regional climate model projections. Climatic Change **81**:71-95.

Bremner, J. M. and D. R. Keeney. 1965. Steam distillation methods for determination of ammonium nitrate and nitrate. Analytica Chimica Acta **32**:485-495.

Casals, P., C. Gimeno, A. Carrara, L. Lopez-Sangil, and M. J. Sanz. 2009. Soil CO_2 efflux and extractable organic carbon fractions under simulated precipitation events in a Mediterranean Dehesa. Soil Biology and Biochemistry **41**:1915-1922.

Castro, J., R. Zamora, J. A. Hodar, and J. M. Gomez. 2005. Alleviation of summer drought boosts establishment success of *Pinus sylvestris* in a Mediterranean mountain: an experimental approach. Plant Ecology **181**:191-202.

Cookson, W. R., M. Osman, P. Marschner, D. A. Abaye, I. Clark, D. V. Murphy, E. A. Stockdale, and C. A. Watson. 2007. Controls on soil nitrogen cycling and microbial community composition across land use and incubation temperature. Soil Biology and Biochemistry **39**:744-756.

Criquet, S., E. Ferre, A. M. Farnet, and J. Le petit. 2004. Annual dynamics of phosphatase activities in an evergreen oak litter: influence of biotic and abiotic factors. Soil Biology and Biochemistry **36**:1111-1118.

Delgado, R., G. Delgado, J. Párraga, E. Gámiz, M. Sánchez, M.A. Tenorio. 1989. Mapa de suelos, hoja 1027 (Güejar Sierra). Instituto para la Conservación de la Naturaleza, Madrid, Spain.

De Luis, M., J. C. Gonzalez-Hidalgo, and J. Raventos. 2003. Effects of fire and torrential rainfall on erosion in a Mediterranean gorse community. Land Degradation and Development **14**:203-213.

Díaz-Raviña, M., M. J. Acea, and T. Carballas. 1993. Seasonal fluctuations in microbial populations and available nutrients in forest soils. Biology and Fertility of Soils **16**:205-210.

Emmett, B. A., C. Beier, M. Estiarte, A. Tietema, H. L. Kristensen, D. Williams, J. Penuelas, I. Schmidt, and A. Sowerby. 2004. The response of soil processes to climate change: Results from manipulation studies of shrublands across an environmental gradient. Ecosystems **7**:625-637.

Ettema, C. H. and D. A. Wardle. 2002. Spatial soil ecology. Trends in Ecology and Evolution **17**:177-183.

Gallardo, A. and W. H. Schlesinger. 1994. Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. Soil Biology and Biochemistry **26**:1409-1415.

Gallardo, A. and W. H. Schlesinger. 1995. Factors determining soil microbial biomass and nutrient immobilization in desert soils. Biogeochemistry **28**:55-68.

Gomez-Aparicio, L., I. M. Perez-Ramos, I. Mendoza, L. Matías, J. L. Quero, J. Castro, R. Zamora, and T. Maranon. 2008. Oak seedling survival and growth along resource gradients in Mediterranean forests: implications for regeneration in current and future environmental scenarios. Oikos **117**:1683-1699.

Graciano, C., J. J. Guiamet, and J. F. Goya. 2005. Impact of nitrogen and phosphorus fertilization on drought responses in *Eucalyptus grandis* seedlings. Forest Ecology and Management **212**:40-49.

Holling, C. S. 1973. Resilience and stability of ecological systems. Annual Review in Ecology and Systematics **4**:1-23.

Holmgren, M., R. Aviles, L. Sierralta, A. M. Segura, and E. R. Fuentes. 2000. Why have European herbs so successfully invaded the Chilean matorral? Effects of herbivory, soil nutrients, and fire. Journal of Arid Environments **44**:197-211.

Holmgren, M., P. Stapp, C. R. Dickman, C. Gracia, S. Graham, J. R. Gutierrez, C. Hice, F. Jaksic, D. A. Kelt, M. Letnic, M. Lima, B. C. Lopez, P. L. Meserve, W. B. Milstead, G. A. Polis, M. A. Previtali, R. Michael, S. Sabate, and F. A. Squeo. 2006. Extreme climatic events shape arid and semiarid ecosystems. Frontiers in Ecology and the Environment **4**:87-95.

Houghton, J.T., Y. Ding, D.J. Griggs, M. Noguer, P.J. Van der Linden, and D. Xiaosu. 2001. Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change (IPCC), Cambridge, England.

Illeris, L., A. Michelsen, and S. Jonasson. 2003. Soil plus root respiration and microbial biomass following water, nitrogen, and phosphorus application at a high arctic semi desert. Biogeochemistry **65**:15-29.

IPCC, Intergovernmental Pannel on Climate Change. 2001. Climate change 2001: impacts, adaptation and vulnerability. Summary for policymakers – A report of working group II of the Intergovernmental Pannel on Climate Change. IPCC, Geneva, Swiss.

Jenkinson, D. S. and D. S. Powlson. 1976. Effects of biocidal treatments on metabolism in soil. 5. Method for measuring soil biomass. Soil Biology and Biochemistry **8**:209-213.

Jensen, K. D., C. Beier, A. Michelsen, and B. A. Emmett. 2003. Effects of experimental drought on microbial processes in two temperate heathlands at contrasting water conditions. Applied Soil Ecology **24**:165-176.

Johnson, D. W., D. E. Todd, and P. J. Hanson. 2008. Effects of throughfall manipulation on soil nutrient status: results of 12 years of sustained wet and dry treatments. Global Change Biology **14**:1661-1675.

Jonasson, S., J. Castro, and A. Michelsen. 2006. Interactions between plants, litter and microbes in cycling of nitrogen and phosphorus in the arctic. Soil Biology and Biochemistry **38**:526-532.

Kandeler, E. and K. E. Bohm. 1996. Temporal dynamics of microbial biomass, xylanase activity, N-mineralisation and potential nitrification in different tillage systems. Applied Soil Ecology **4**:181-191.

Killham, K. 1994. Soil ecology. Cambridge University Press, Cambridge, UK.

Kosmas, C., N.G. Danalatos, F. López-Bermúdez, and M.A. Romero-Díaz. 2002. The effect of land use on soil erosion and land degradation under Mediterranean conditions. Pages 57-70 *in* N. A. Geeson, C. J. Brandt, and J. B. Thornes, editors. Mediterranean desertification: a mosaic of processes and responses. Wiley, Chichester, UK.

Kozlowski, T. T. and S. G. Pallardy. 2002. Acclimation and adaptive responses of woody plants to environmental stresses. Botanical Review **68**:270-334.

Lambers, H., F.S. Chapin III, and T.L. Pons. 1998. Plant physiological ecology. Springer-Verlag, New York, New York, USA.

Lindenmayer, D.B., and J. Fischer. 2006. Habitat fragmentation and landscape change. An ecological and conservation synthesis. Island Press, Washington DC, USA.

Matesanz, S., A. Escudero, and F. Valladares. 2009. Impact of three global change drivers on a Mediterranean shrub. Ecology **90**:2609-2621.

Mendoza, I., R. Zamora, and J. Castro. 2009. A seeding experiment for testing tree-community recruitment under variable environments: Implications for forest regeneration and conservation in Mediterranean habitats. Biological Conservation **142**:1491-1499.

Michelsen, A., E. Graglia, I.K. Schimdt, S. Jonasson, D. Sleep, and C. Quarmby. 1999. Differential responses of grass and dwarf shrub to long-term changes in soil microbial biomass C, N and P following factorial addition of NPK fertilizer, fungicide and labile carbon to a heath. New Phytologist **143:**523-538.

Miller, A. E., J. P. Schimel, J. O. Sickman, K. Skeen, T. Meixner, and J. M. Melack. 2009. Seasonal variation in nitrogen uptake and turnover in two high-elevation soils: mineralization responses are site-dependent. Biogeochemistry **93**:253-270.

Mlambo, D., E. Mwenje, and P. Nyathi. 2007. Effects of tree cover and season on soil nitrogen dynamics and microbial biomass in an African savanna woodland dominated by *Colophospermum mopane*. Journal of Tropical Ecology **23**:437-448.

Monokrousos, N., E. M. Papatheodorou, J. D. Diamantopoulos, and G. P. Stamou. 2004. Temporal and spatial variability of soil chemical and biological variables in a Mediterranean shrubland. Forest Ecology and Management **202**:83-91.

Nadelhoffer, K. J., A. E. Giblin, G. R. Shaver, and J. A. Laundre. 1991. Effects of temperature and substrate quality on element mineralization in six arctic soils. Ecology **72**:242-253.

Ramos, M. C. and J. A. Martinez-Casasnovas. 2004. Nutrient losses from a vineyard soil in Northeastern Spain caused by an extraordinary rainfall event. Catena **55**:79-90.

Rinnan, R., A. Michelsen, E. Baath, and S. Jonasson. 2007. Fifteen years of climate change manipulations alter soil microbial communities in a subarctic heath ecosystem. Global Change Biology **13**:28-39.

Rivas-Godoy, S. and S. Rivas-Martínez. 1971. Vegetación potencial de la provincia de Granada. Trabajos del Departamento de Botánica y F. Vegetal **4**:3-85.

Rodrigo, F. S. 2002. Changes in climate variability and seasonal rainfall extremes: a case study from San Fernando (Spain), 1821-2000. Theoretical and Applied Climatology **72**:193-207.

Ross, D. J. and G. P. Sparling. 1993. Comparison of methods to estimate microbial C and N in litter and soil under *Pinus radiata* on coastal sand. Soil Biology and Biochemistry **25**:1591-1599.

Sala, O. E., F. S. Chapin, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, H. A. Mooney, M. Oesterheld, N. L. Poff, M. T. Sykes, B. H. Walker, M. Walker, and D. H. Wall. 2000. Biodiversity - Global biodiversity scenarios for the year 2100. Science **287**:1770-1774.

Santa-Regina, I., M. Rapp, A. Martin, and J. F. Gallardo. 1997. Nutrient release dynamics in decomposing leaf litter in two Mediterranean deciduous oak species. Annales Des Sciences Forestieres **54**:747-760.

Santruckova, H. 1992. Microbial biomass, activity and soil respiration in relation to secondary succession. Pedobiologia **36**:341-350.

Sardans, J. and J. Penuelas. 2007. Drought changes phosphorus and potassium accumulation patterns in an evergreen Mediterranean forest. Functional Ecology **21**:191-201.

Schimel, J., T. C. Balser, and M. Wallenstein. 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology **88**:1386-1394.

Schinner, F., R. Öhlinger, E. Kandeler, R. Margesin. 1995. Methods in soil biology. Springer, Berlin, Germany.

Singh, J. S., A. S. Raghubanshi, R. S. Singh, and S. C. Srivastava. 1989. Microbial biomass acts as source of plant nutrients in dry tropical forests and savanna. Nature **338**:499-500.

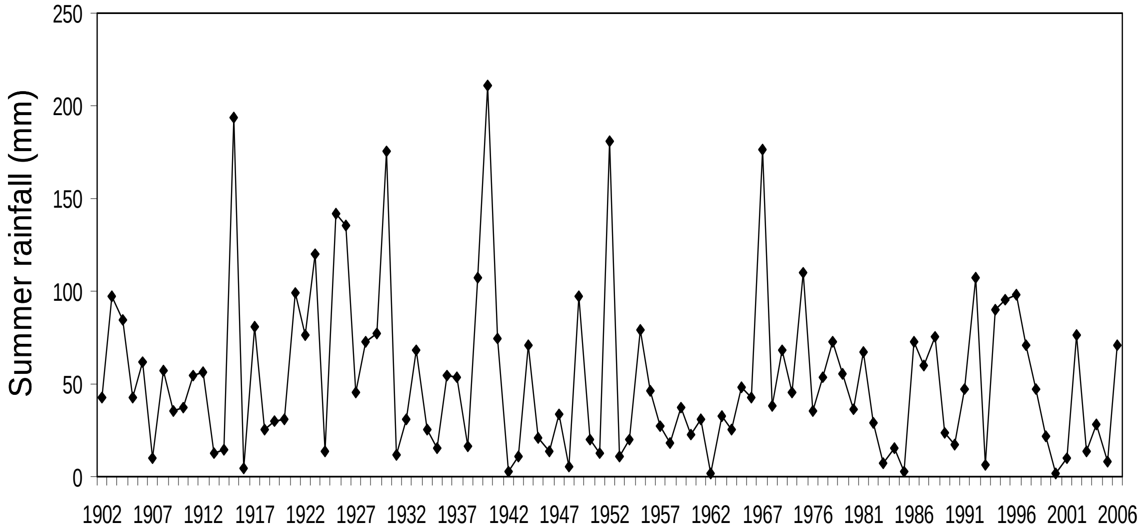
Sparks, D.L. 1996. Methods of soil analysis. Part 3. Chemical Methods Soil Science Society of America and American Society of Agronomy, Madison, Wisconsin, USA.

Srivastava, S. C. 1992. Influence of soil properties on microbial C, N and P in dry tropical ecosystems. Biology and Fertility of Soils **13**:176-180.

Wardle, D. A. 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biological Reviews of the Cambridge Philosophical Society **67**:321-358.

Watanabe S. and R.S. Olsen. 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Science Society of America Journal **29**:677-678.

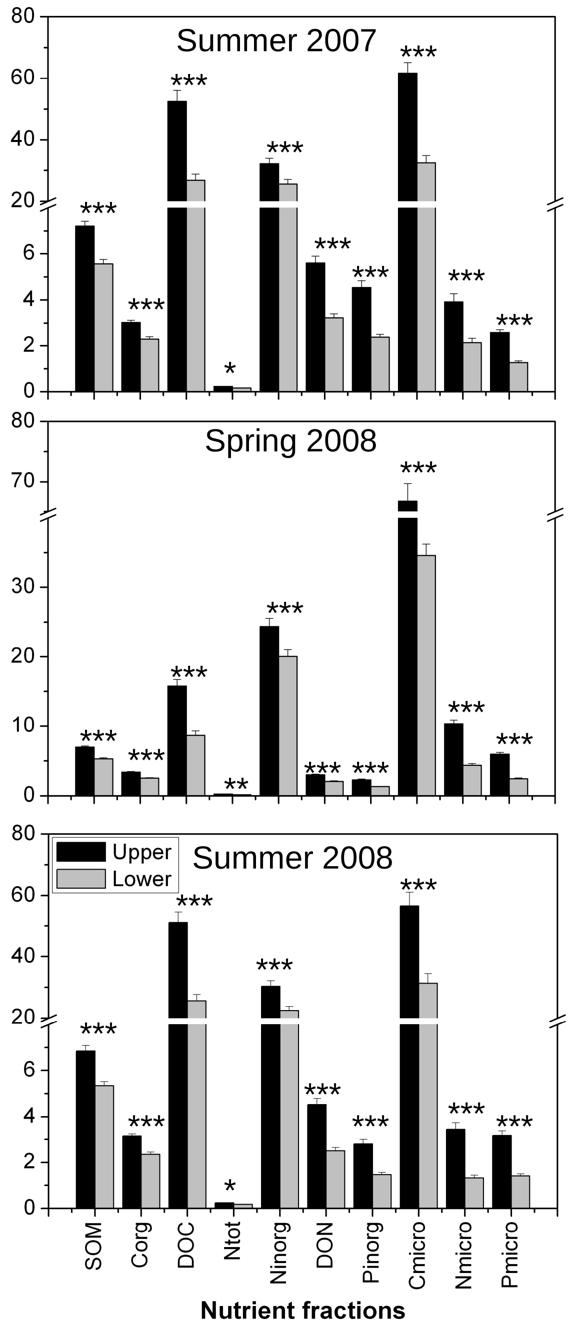
Yahdjian, L. and O. E. Sala. 2002. A rainout shelter design for intercepting different amounts of rainfall. Oecologia **133**:95-101.



Appendix 1: *Summer rainfall (June-August) for the study area during the 1902-2006 series. Data from 1902-1990 are inferred from La Cartuja meteorological station (Granada; $R^2 = 0.75$; $P < 0.0001$). Water amount for the simulated rainy summer was added according to the mean summer precipitation of the five mildest years (1915, 1930, 1940, 1952 and 1967), giving a value of 180 mm. We took the five highest values by two principal reasons: in one hand we wanted to simulate extreme (although natural) events; in the other hand, during these mild years evapotranspiration diminishes, making differences even stronger.*

Appendix 2: *Mean values and results of one way-ANOVA (d.f = 2; N = 144) exploring differences among plots where climatic scenarios were later simulated during the previous summer (2006) and spring (2007) to experiment development of the different elements: soil organic matter (SOM), organic carbon* (C_{org})*, dissolved organic carbon (DOC), microbial carbon* (C_{micro})*, total nitrogen* (N_{tot})*, inorganic nitrogen* (N_{inorg})*, dissolved organic nitrogen (DON), microbial nitrogen* (N_{micro})*, inorganic phosphorus* (P_{inorg})*, and microbial phosphorus* (P_{micro})*. The two depths (0-8 and 8-16 cm) are pooled. Concentration values in the microbial fractions were not corrected for extraction efficiency. As expected, there were no differences among plots prior to climatic scenarios simulation for any of the nutrient forms analyzed. We may thus consider that differences detected in the following years were due to the treatment.*

		Summer			**Spring**		
	Units	**Mean ± SE**	**F**	***P***	**Mean ± SE**	**F**	***P***
Soil							
SOM	%	7.63 ± 0.21	2.33	0.10	7.96 ± 0.30	0.20	0.82
C_{org}	%	3.09 ± 0.10	1.50	0.23	3.46 ± 0.10	0.47	0.62
DOC	µg/g	39.31 ± 1.92	0.56	0.57	35.76 ± 2.25	0.36	0.70
N_{tot}	%	0.25 ± 0.01	2.19	0.12	0.26 ± 0.01	0.06	0.94
N_{inorg}	µg/g	27.89 ± 0.87	0.27	0.77	31.52 ± 1.24	0.80	0.45
DON	µg/g	5.01 ± 0.18	0.79	0.45	3.56 ± 0.15	0.69	0.50
P_{inorg}	µg/g	3.24 ± 0.24	0.33	0.72	2.57 ± 0.11	0.51	0.60
Microbial							
C_{micro}	µg/g	56.53 ± 2.47	1.50	0.23	88.71 ± 5.04	0.55	0.58
N_{micro}	µg/g	6.23 ± 0.37	1.34	0.27	11.58 ± 0.47	0.01	0.99
P_{micro}	µg/g	4.45 ± 0.19	2.22	0.11	6.15 ± 0.24	0.18	0.84
Litter							
C	g/m^2	1266.20 ± 213.01	0.31	0.74	1240.49 ± 205.18	0.02	0.98
N	g/m^2	37.20 ± 5.27	0.09	0.91	36.61 ± 5.34	0.13	0.88
P	g/m^2	2.62 ± 0.31	0.09	0.92	2.45 ± 0.31	0.11	0.90



Appendix 3: *Soil and microbial nutrients variations (SOM: soil organic matter, in %; C_{org}: organic carbon, in %; DOC: dissolved organic carbon, in µg/g; C_{micro}: microbial carbon, in µg/g; N_{tot}: total nitrogen, in %; N_{inorg}: inorganic nitrogen, in µg/g; DON: dissolved organic nitrogen, in µg/g; N_{micro}: microbial nitrogen, in µg/g; P_{inorg}: inorganic phosphors, in µg/g; P_{micro}: microbial phosphors, in µg/g) among soil depths: upper (0-8 cm, black bars), and lower (8-16 cm, grey bars). Different habitats and climatic scenarios are pooled. Concentration values in the microbial fractions were not corrected for extraction efficiency. Significant differences among depths after Bonferroni correction are indicated: * $0.05 \leq P < 0.01$; ** $0.01 \leq P < 0.001$; *** $P \leq 0.001$. Error bars represents standard error. Overall, SOM was 1.3 times higher in the upper profile than in the lower, C_{org} 1.3 times, DOC 2.0 times, C_{micro} 1.9 times, N_{tot} 1.4 times, N_{inorg} 1.3 times, DON 1.7 times, N_{micro} 2.3 times, P_{inorg} 1.9 times, and P_{micro} 2.3 times.*

CHAPTER 2:

REDUCTION OF RAINY EVENTS IS MORE CRITICAL THAN DROUGHT INCREASE FOR WOODY SEEDLING REGENERATION: A FIELD EXPERIMENT SIMULATING CLIMATE CHANGE

Luis Matías*, Regino Zamora, and Jorge Castro

ABSTRACT

In this study we analyze the way in which vegetation will cope with global change alterations predicted for the coming decades. Besides drier summers are predicted to be the norm, exceptional rainy summers are possible to appear in Mediterranean mountains. Different species cohabiting within the same community has the potential to respond in a different way to an alteration of their optimum conditions in the regeneration niche generated by the global change alterations. The objective of this work is to assess the effect of direr, wetter, and current summer conditions on the recruitment probabilities and growth of seedlings from eight woody species representatives of the most important functional groups in the woody community: pioneer and mid-successional shrubs, and trees. For this, we analyzed the response in emergence, growth and survival of these species to an experimentally manipulated rainfall input under field conditions across the main habitats in the study area: open, shrubland, and forest. We found an effect of the habitat on emergence, survival and final biomass, whereas different climatic scenarios affected seedling survival and biomass. Wet summer scenario boosted growth and survival, whereas a drought increase only reduced it in some cases. These results were modulated by the habitat type. Overall, shrub species presented higher survival and growth, being less affected by drought increase, whereas some tree species were extremely dependent on wet summer conditions. Thus, the reduction in frequency of mild summers predicted for the coming decades in Mediterranean areas will have stronger consequences for species recruitment than drought increase. The differential response of the species from the different functional groups has the potential to alter future community composition and structure.

Keywords: Climate change, woody community, regeneration, rainfall variability, summer drought, irrigation.

INTRODUCTION

To what extent are global change drivers shaping the diversity, distribution and dynamics of forest ecosystems? There are a great amount of papers trying to gain some insights into this important question from different approaches, from ecophysiology (Martínez-Vilalta & Piñol 2002; Linares *et al*. 2009; McDowell *et al*. 2010) to community-landscape level (Allen & Beshears 1998; Walker *et al*. 2006; Allen *et al*. 2010), or modelling vegetation dynamics at broad scale (Thuiller *et al.* 2005; Malcolm *et al.* 2006; Randin *et al.* 2009). Understanding and predicting the consequences of these changes on ecosystems is emerging as one of the major challenges for scientists, and forecasting the impacts on forests is particularly important (Boisvenue & Running 2006; Bonan 2008).

Most of the studies investigating the impacts of global change on world's forests have used adult trees as the study target, from those more focused on the vigor of trees as indicator of forest responses to impacts —*i.e.*, the case of forest dieback studies (Allen *et al*. 2010)—, to those more focused in modelling and forecasting changes in latitudinal/altitudinal distribution of species (Thuiller *et al.* 2005; Nogués-Bravo *et al.* 2008). However, from a demographic point of view, a forest is more than a single vital stage (the adult one), and from a community point of view, a forest is more than a single species belonging to a particular functional group (*e.*g. the dominant tree). Real-world forests are usually composed by a diverse sample of species differing in requirements, biogeographical origin and life-history traits. Thus, for fully understand forest responses, we must consider as study case a sample of species representing the true diversity of life-forms existing in a real forest. Furthermore, from a demographic point of view, real-world tree populations in any forest are age structured, being the bank of recruits a key forest compartment to understand forest dynamic.

The use of the recruits bank as study system represents some additional advantages respect to the use of adults: first, the high sensitivity to abiotic conditions of recruits, because their small root system in comparison with adults (Houle 1994; Hampe & Arroyo 2002; Castro *et al*. 2005); second, the faster response to environmental changes respect to adult phase (Lloret *et al*. 2009). For this reasons, recruits bank can be used as a reliable indicator of near-future forest community, since the forest of tomorrow is the bank of recruits of today. Thus, to properly assess the dynamic response of forest ecosystems to very fast changes derived from global-change impacts we should pay special attention to the bank of recruits, considering a diverse representation of functional groups.

Forest Regeneration dynamics can be affected simultaneously by diverse drivers that frequently generate non-additive responses which cannot be predicted based on single-factor studies (Sala *et al*. 2000). Land-use change is an important driver not only for ecosystem functioning itself (Sala *et al*. 2000, Lindenmayer & Fischer 2006) but also interacts with climate change, modulating it effects (Matesanz *et al*. 2009). Specifically, in Mediterranean areas, spring and summer precipitation is expected to decline up to 30% during the last part of present century (Giorgi & Lionello 2008). However, in the Mediterranean mountains, rainy summers may eventually occur with a low frequency and irregular time intervals (7 to 40 years, Esteban-Parra *et al.* 1998; Rodrigo 2002), and might represent a window of opportunity (*sensu* Eriksson & Fröborg 1996) for the recruitment of long-lived woody species (García & Zamora 2003; Castro *et al*. 2005; Mendoza *et al*. 2009) or for those forming a persistent seed bank (Díaz-Villa *et al*. 2003).

The complex scenario created by different climatic conditions and habitat types may alter the range of optimum requirements for the different species' regeneration niche. Coexisting species usually differ in their tolerance to resource availability (Grubb 1998;

Schreeg *et al*. 2005), allowing regeneration only inside a range of specific conditions that would locally vary within the same ecosystem. Grubb's (1977) concept of the regeneration niche emphasizes that the niche differences among coexisting plant species need only be manifested during the early stages of life histories, determining the abundance and distribution of plant species. As species regeneration niche is highly dependent on small scale variations (Quero *et al*. 2008a) and usually differ from realized niche in adults (García *et al*. 1999; Chase & Leibold 2003; Miriti 2006), experimental studies analyzing the effects of different climatic scenarios on recruits bank among the possible habitats in which it may establish are a strong way to predict community dynamics for the coming decades.

Despite the amount of studies addressing the effect of higher water availability (Morris *et al*. 2000; Cabin *et al*. 2002; Castro *et al.* 2005; Mendoza *et al.* 2009) or drier conditions (Holmgren 2000; Weltzin & McPherson 2000; Lloret *et al.* 2004; Sánchez-Gómez *et al*. 2006) on woody species regeneration, we are not aware of studies explicitly analysing the effect of these contrasted climatic and land-use scenarios on the bank of recruits performance at community level. This is a key point to understand the effect of global change on woody species regeneration and community composition, as both drier and milder years still will occur in many ecosystems types even under the current scenario of increase in aridity.

According to this, it is possible to expect a different functional groups response of the species of the woody-community, *i.e.* pioneer shrubs, mid-successional shrubs, and trees. This may result in a differential recruitment probability at community level in different ecological templates (habitats and precipitation patterns), which would be able to deeply alter community regeneration dynamics, as hypothesized in Fig. 1. This alteration may ultimately affect to the composition, relative abundance and community dynamics (Peñuelas & Boada 2003; Peñuelas *et al.* 2004; Thuiller *et al.* 2005; Loret *et al.* 2009).

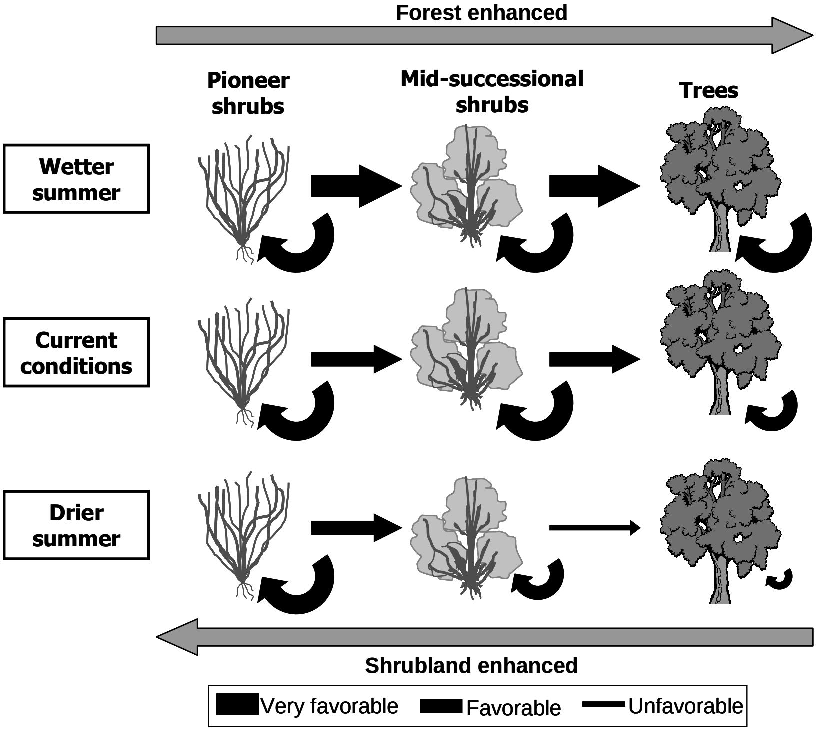


Figure 1: *Schematic diagram representing the community dynamics under the different climatic scenarios for the coming decades in Mediterranean areas. Arrows represent the hypothesized dynamics: within functional groups, represents the autoregeneration capacity; across groups, represents the probability of transition between stages. Arrow width is proportional to success probability, from unfavorable to very favorable. Our hipotesis proposes that: 1) wet summer conditions would represent a good oportunity for tree species regeneration, enhancing both forest maintenance, and forest colonization and expansion to nearby degraded habitats. 2) On the contrary, a drier summer scenario would limit forest regeneration, and probably collapse the colonization of nearby open and shrubland habitats. 3) In turn, this drier scenario would be very favourable for a shrubland expansion, enhancing both shrubland maintenance and colonization and expansion, even to nearby forest.*

The objective of this work is to assess the effect of direr and wetter summer conditions on the recruitment probabilities and growth of seedlings from woody species representatives of the entire woody community in a Mediterranean mountain. To do this, we carried out a field experimental approach during two consecutive years simulating a) sporadic wetter conditions during the growing season (within the limits of historical records); b) drier climatic conditions during the growing season (according to A-2 scenario from IPCC); and c) current climatic conditions. As targets, we considered species from the three principal functional groups in Mediterranean areas, that is, tress, mid-successional shrubs, and pioneer shrubs. In addition, we tested the effect of the different climatic scenarios across diverse habitats. For this, we selected the three principal habitat types in Mediterranean mountains, *i.e.* open areas, under shrub cover, and under tree canopies. Main novelties here encompassed with respect to previous literature are: *i*) the multi-specific approach using species from the three principal functional groups representatives of the whole woody community; *ii*) the field experimental approach to compare three different climatic scenarios; and *iii*) the use of the three principal habitats where seedlings could establish, representing the natural heterogeneity of abiotic conditions in Mediterranean forest Ecosystems. The main questions that we tried to answer were: 1) What is the response of the species from the three functional groups to the different combination of climatic scenarios and habitats in terms of survival and growth? 2) Which of the successive demographic stages implied in recruitment (emergence, growth, and survival) is the most sensible to the different climatic scenarios? 3) What is more important for plant recruitment, the increase in frequency of drier years, or the reduction of milder ones? Answering these questions led us to envisage the suitable paths of forest dynamics in Mediterranean mountains under a diverse combination of habitats and climatic scenarios.

MATERIALS AND METHODS

STUDY AREA

The study was conducted from December 2006 to September 2008 in La Cortijuela area, within the limits of Sierra Nevada National Park (37º05' N, 3º28' W, Granada, SE Spain). The bedrock is calcareous, with regosols and cambisols as predominant soil types (Delgado *et al.* 1989) and the elevation range around 1650 m a.s.l. This mountain area has a continental Mediterranean climate, with cold winters and hot dry summers. Rainfall is 811 mm yr^{-1} (mean 1990-2008), accumulated mostly during spring and autumn. Vegetation in the area is composed by patches with different management predominating pine plantations, shrublands, open areas and patches of native forest (see Matías *et al.* 2009 for more details). The experiment was conducted inside a natural 12.4 ha fenced area with ungulates exclosure since 1986. Predominant tree species inside the fenced area are *Pinus sylvestris*, *Pinus nigra*, and *Quercus ilex*. Predominant mid-successional shrubs are *Crataegus monogyna*, *Berberis vulgaris*, and *Prunus ramburii*, whereas predominant pioneers are *Salvia lavandulifolia* and *Cytisus scoparius*.

STUDY SPECIES

We selected eight target species representative from the three principal successional stages on Mediterranean mountains, all of them native in the study area: four trees, from which two were deciduous (*Sorbus aria* and *Acer opalus*) and two were evergreen *(Pinus sylvestris* and *Quercus ilex*); two mid-successional shrubs (*Crataegus monogyna* and *Berberis vulgaris*); and two pioneer shrubs: (*Salvia lavandulifolia* and *Cytisus scoparius*). Hereafter, all of them will be named by their *Genus* names. Seeds and acorns from these species were collected on the surrounding area during 2006 fructification period

(September to December). Then, they were visually inspected to reject clearly-aborted or predated ones and stored at 4 °C until the sowing. Seed mass and species characteristics are shown in Table 1.

Table 1: *Principal characteristics of the eight species selected for the experiment. Functional groups of the species is indicated as Tr = trees, MSS = mid-successional shrubs, and PS = pioneer shrubs. Distribution is based on the ecological requirements and actual distribution of the species and is obtained from (Blanco et al. 2005). Seed mass is given as mean ± SD, n = 100 for all species except for Q. ilex (n = 360), where all seeds were weighted. Last two columns indicate number of seeds sown in each plot and in the whole experiment respectively.*

Species	Functional group	Distribution	Seed Mass	Sown per plot	N sown
Acer	Tr	Sub-Mediterranean	0.046 ± 0.011	15	1080
Sorbus	Tr	Sub-Mediterranean	0.036 ± 0.038	10	720
Pinus	Tr	Boreal-Alpine	0.012 ± 0.01	15	1080
Quercus	Tr	Mediterranean	4.480 ± 1.25	5	360
Berberis	MSS	Mediterranean	0.018 ± 0.008	15	1080
Crataegus	MSS	Mediterranean	0.079 ± 0.018	15	1080
Cytisus	PS	Mediterranean	0.011 ± 0.002	15	1080
Salvia	PS	Mediterranean	0.005 ± 0.002	15	1080

EXPERIMENTAL DESIGN

We used a fully-factorial field experiment crossing two main factors: habitat and climatic scenario, each one with three levels. For habitat, we selected three common habitats in Mediterranean mountains differing in woody coverage: 1) open: areas of bare soil or covered with sparse herbaceous vegetation, representing the lowest canopy cover; 2) shrubland: composed of the dominant shrubs in the study area, principally *Crataegus monogyna*, *Prunus ramburii* and *Genista speciosa*., representing the mid canopy cover; 3) forest: composed of a mixed forest of *Pinus sylvestris*, *Pinus nigra* and scattered *Quercus ilex*, and represent the denser canopy cover. For the climatic scenario treatment, we selected three levels differing in their water availability: 1) dry summer: based on the SRES A-2 model by Intergovernmental Panel on Climate Change (IPCC 2007), where a reduction on summer rainfall of 30% is predicted for Mediterranean areas, as well as a longer dry period, occupying end of the spring. For this treatment, we built rain exclusion shelters following the main design by Yahdjian & Sala (2002). Rainout shelters had a metal frame supporting V-shaped clear methacrylate bands without UV filter (Barlocast®; Faberplast S.L., Madrid), intercepting only a small proportion of light (ultraviolet type b, < 250 nm). Each shelter had an area of 4 m^2 (2×2 m) and a mean height of 1 m. The roof had 11.3° inclination, and a gutter to collect the excluded water in the lowest side. Each shelter had 7 bands, with a distance between bands of 21 cm, covering the 35 % of the total surface. Moreover, a 20 cm deep ditch was excavated along the entire shelter to intercept runoff water supply. Rainout shelters were placed from April to September, simulating drier growing seasons. Hereafter, this scenario will be named *dry summer*. 2) Sporadic rainy summer: to simulate rainy events, we placed 2×2 m squares on the soil with a water addition system composed of 4 sprinklers with 90° angle and 2 m range located on the corners. Each week from mid June to end September we added a water quantity of 12 $l\ m^{-2}$, simulating a summer storm. If one week a natural storm occurred, irrigation pulse was not added. The total water added during the summer was 180 l, equivalent to the mean summer rainfall of the five milder summers of the 1902-2006 series on the study area (chapter 1 of this thesis). Hereafter, this scenario will be named *wet summer*. 3) Current conditions: natural rainy conditions during experiment development. We placed 2×2 m squares without water addition or exclusion exposed to current climatic conditions during the experiment development, acting as control for the experiment. Hereafter, this scenario will be named *control*. In each one of the three habitats we placed 24 plots of 2×2 m, and haphazardly assigned 8 of them to each one of the climatic scenarios, giving a total amount of 72 plots (3 habitats × 3 climatic scenarios × 8 replicates).

In each plot, the eight species were sown inside individualized 15×25 cm side and 1-cm wire mesh cages to avoid seed predation by small mammals as well as herbivory losses. The number of seeds sown (7,560 in total) was different on each species depending on seed mass and germination rates, checked on a previous experiment (Mendoza *et al.* 2009), ranging from 5 to 15 (Table 1). The sowing was carried out on December 2006, and seeds stayed on the soil during all winter period, suffering a natural cold stratification period on the field. From beginning May 2007, each seedling that emerged on the cages was individually tagged, and its survival was monitored during two complete growing seasons (June to September 2007 and 2008). At the end of the experiment, on September 2008, all surviving seedlings were harvested, extracting completely the root with the help of a pneumatic hammer. Each seedling was separated in aboveground and belowground components, oven-dried at 70 ºC for at least 72h, and weighted. In all study plots, soil water content was monthly measured by the time domain reflectometry method (TDR-100, Spectrum Technologies Inc., USA). Study plots were sampled by two perpendiculars transects recording the volumetric water content every 0.5 m.

DATA ANALYSIS

Differences in soil water content were tested between habitats and climatic scenarios using a repeated-measures ANOVA. The effects of the two main factors (habitat and climatic scenario) were tested by a Generalized Linear Model with emergence rate (proportion of the number of emerged seedlings to the total number of sowed seeds) and survival rate (proportion of the number of surviving seedlings to the total number of emerged seedlings) as the response variables, binomial probability distribution, and logit as link function for each species separately (Quinn & Keough 2002). Aboveground and belowground biomass were transformed by their natural logarithms and analysed by factorial ANOVA for each species. Due to the low number of surviving seedlings in some treatments, *Pinus* was eliminated from the biomass analysis, as well as data from *Acer* and *Sorbus* in open, and *Salvia* in forest. Values are given throughout this paper as mean ± standard error. All analyses were made using JMP v. 7.0 (SAS Institute Inc., 2007).

RESULTS

SOIL MOISTURE

Soil volumetric water content was significantly different among habitats ($F_{2,715} = 499.68$; $P < 0.0001$), being higher in forest (15.1 ± 0.18%) than shrubland (14.6 ± 0.17%) or open (12.4 ± 0.15%). Differences between climatic scenarios were also significant ($F_{2,715} = 1214.71$; $P < 0.0001$), with higher values in the wet summer scenario (16.4 ± 0.16%) than in control plots (13.8 ± 0.18%) or under the dry summer scenario (11.9 ± 0.15%).

SEEDLING EMERGENCE

From the 7,560 sown seeds, 2,139 seedlings emerged (28%). The habitat had a significant effect for all species (Table 2), with higher values in forest for *Acer*, *Sorbus*, *Berberis* and *Cytisus*, in shrubland for *Pinus* and *Crataegus*, and in open for *Quercus* and *Salvia* (Table 3). However, climatic scenario only showed a significant effect for *Cytisus* (Table 2), with lower emergence under the wet scenario (Table 3).

Table 2: *Summary of the logistic model for emergence and survival after the second summer under different habitats and climatic scenarios. Analyses were separated for each species. Emergence rate was calculated as the proportion of emerged seedlings to the total number of sown seeds per plot. Survival rate was calculated as the proportion of surviving seedlings to the total number of emerged seedlings per plot. Due to high mortality, Pinus was extracted from the survival analysis. Significant P values are shown in bold.*

		Habitat (H)		Scenario (S)		H × S	
	Species	χ^2	*P*	χ^2	*P*	χ^2	*P*
Emergence							
	Acer	11.992	**0.0025**	3.628	0.1630	2.851	0.5831
	Pinus	120.701	**<0.0001**	2.635	0.2678	11.799	**0.0189**
	Sorbus	98.315	**<0.0001**	0.274	0.8719	5.946	0.2032
	Quercus	7.368	**0.0251**	0.879	0.6444	1.720	0.7870
	Crataegus	100.576	**<0.0001**	1.837	0.3992	3.523	0.4744
	Berberis	165.305	**<0.0001**	5.976	0.0504	1.639	0.8018
	Cytisus	21.786	**<0.0001**	8.405	**0.0150**	9.569	**0.0483**
	Salvia	37.428	**<0.0001**	1.667	0.4344	2.039	0.7286
Survival							
	Acer	19.833	**<0.0001**	12.037	**0.0024**	9.554	**0.0486**
	Pinus	-	-	-	-	-	-
	Sorbus	9.762	**0.0076**	34.339	**<0.0001**	11.298	**0.0234**
	Quercus	11.945	**0.0043**	26.733	**<0.0001**	3.686	0.4501
	Crataegus	32.212	**<0.0001**	30.314	**<0.0001**	9.578	**0.0482**
	Berberis	23.640	**<0.0001**	0.802	0.6696	8.555	0.0732
	Cytisus	9.436	**0.0089**	13.951	**0.0009**	11.647	**0.0202**
	Salvia	0.661	0.7186	10.756	**0.0046**	7.857	0.0970
	DF	2		2		4	

Table 2: *Mean emergence percentage (proportion of emerged seedlings to total number of sown seeds) and survivorship percentage (proportion of surviving saplings to the total number of emerged seedlings) for the eight studied species under the three habitats, and the three climatic scenarios: dry summer, control summer, and wet summer. Means are given ± SE.*

		Emergence		
Scenario	**Species**	**Forest**	**Shrubland**	**Open**
Dry	*Acer*	13.3 ± 2.8	10.0 ± 2.2	8.3 ± 3.3
	Pinus	31.7 ± 6.7	25.8 ± 8.1	5.8 ± 3.4
	Sorbus	48.7 ± 11.4	47.5 ± 10.1	16.2 ± 3.7
	Quercus	85.0 ± 8.2	85.0 ± 5.0	95.0 ± 3.3
	Crataegus	45.0 ± 6.6	45.0 ± 6.9	15.0 ± 3.5
	Berberis	40.0 ± 6.0	37.5 ± 8.9	5.0 ± 1.7
	Cytisus	66.7 ± 3.6	43.3 ± 6.5	38.3 ± 5.9
	Salvia	1.6 ± 1.6	7.5 ± 2.6	10.8 ± 4.0
Control	*Acer*	15.0 ± 3.7	10.8 ± 2.8	4.2 ± 1.7
	Pinus	33.3 ± 6.5	35.8 ± 5.8	8.3 ± 3.5
	Sorbus	47.5 ± 5.9	46.2 ± 9.9	13.7 ± 7.0
	Quercus	87.5 ± 5.3	75.0 ± 12.9	92.5 ± 3.6
	Crataegus	31.7 ± 3.7	43.3 ± 8.1	14.2 ± 3.7
	Berberis	41.6 ± 6.7	34.2 ± 6.3	3.3 ± 1.7
	Cytisus	55.0 ± 5.3	45.8 ± 8.8	36.7 ± 7.4
	Salvia	0.83 ± 0.8	11.7 ± 3.0	11.7 ± 3.5
Wet	*Acer*	8.3 ± 3.3	9.1 ± 3.3	3.3 ± 1.8
	Pinus	30.8 ± 4.3	51.7 ± 5.7	5.0 ± 2.4
	Sorbus	63.7 ± 6.5	42.5 ± 9.2	11.2 ± 3.5
	Quercus	82.5 ± 8.8	82.5 ± 5.9	90.0 ± 3.8
	Crataegus	37.5 ± 5.0	49.2 ± 9.1	11.7 ± 3.7
	Berberis	28.3 ± 7.3	29.2 ± 5.3	1.7 ± 1.1
	Cytisus	40.0 ± 3.9	41.7 ± 5.7	35.0 ± 5.8
	Salvia	2.5 ± 1.7	14.2 ± 4.6	11.7 ± 4.1

SEEDLING SURVIVAL

From the 2,139 emerged seedlings, 1,306 survived to the second summer at the end of the experiment (61%). We found differences on the habitat effect on the final survival for all species except salvia (Table 2; data of *Pinus* were not analyzed due to the elevated presence of ceros, but survival was clearly different among habitats, Fig. 2). Overall, open was the and *Salvia*), whereas for the rest of species the highest survival was in the shrubland or the forest habitats (Fig. 2).

Climatic scenarios showed a strong effect on the final survivorship for all the species except *Berberis* (Table 2; data of *Pinus* not analyzed due to presence of ceros, but survival clearly differed among climatic scenarios; Fig. 2). Indeed, lack of overall differences for *Berberis* are likely related to the low (cero) survival in Open for dry and wet scenarios, but climatic scenarios differences still appeared within habitats (Fig. 2). As a general trend, the wet scenario increased survival for most species at least in some of the habitats (Fig. 2). Also as a general trend, the climatic scenario provoked the highest differences in open habitat, whereas in shrubland or forest habitats its effect was more reduced. Pioneer shrubs (except *Salvia* in forest) and *Q. ilex* were the species less susceptible to climatic scenarios. Finally, survival was not reduced in the dry scenario in relation to the control scenario (with the exception of *Acer* and *Salvia* in forest; Fig. 2). Overall, survival was 45.6 ± 3.2 % under dry summer scenario, 47.0 ± 3.1 % in control, and 75.0 ± 2.8 under wet summer scenario (all species pooled). The effect of the dry scenario was particularly patent for *Acer* and *Pinus*, even with no one survivor under this scenario (Fig. 2). We also found a significant interaction between habitat and climatic scenario for four of the species, induced by the different intensity of the dry summer effect on different habitats (Table 2, Fig.2). For example, for *Sorbus*, survivorship was lower under the dry summer scenario in open and forest, whereas increased in shrubland.

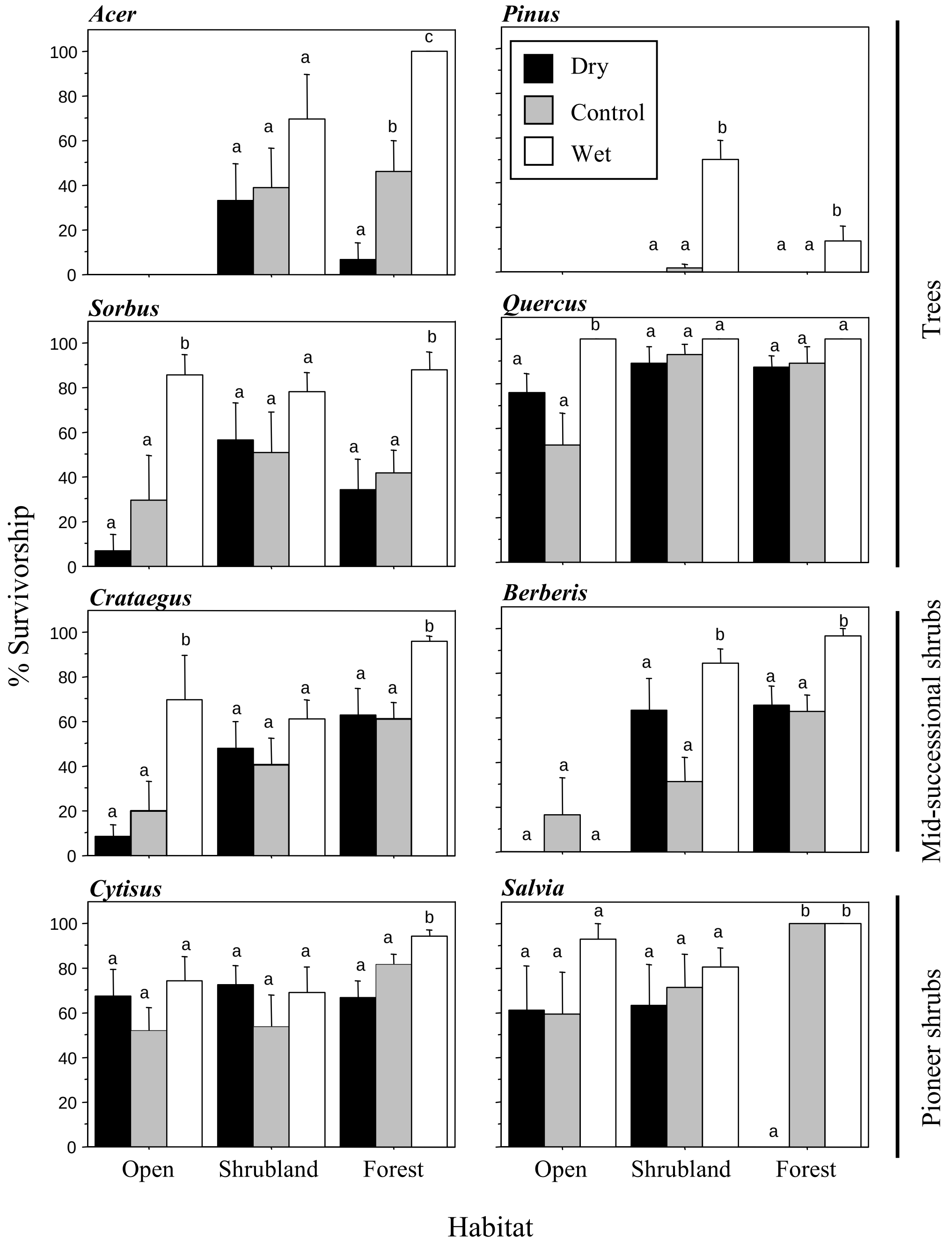


Figure 2: *Mean (+SE) survivorship percentage at the end of the experiment for the eight species in different habitats and climatic scenarios. Survivorship was calculated as proportion of the number of surviving seedlings from the number of emerged seedlings per plot. Different letters are indicating differences between climatic scenarios within habitat by a Fisher's PLSD test.*

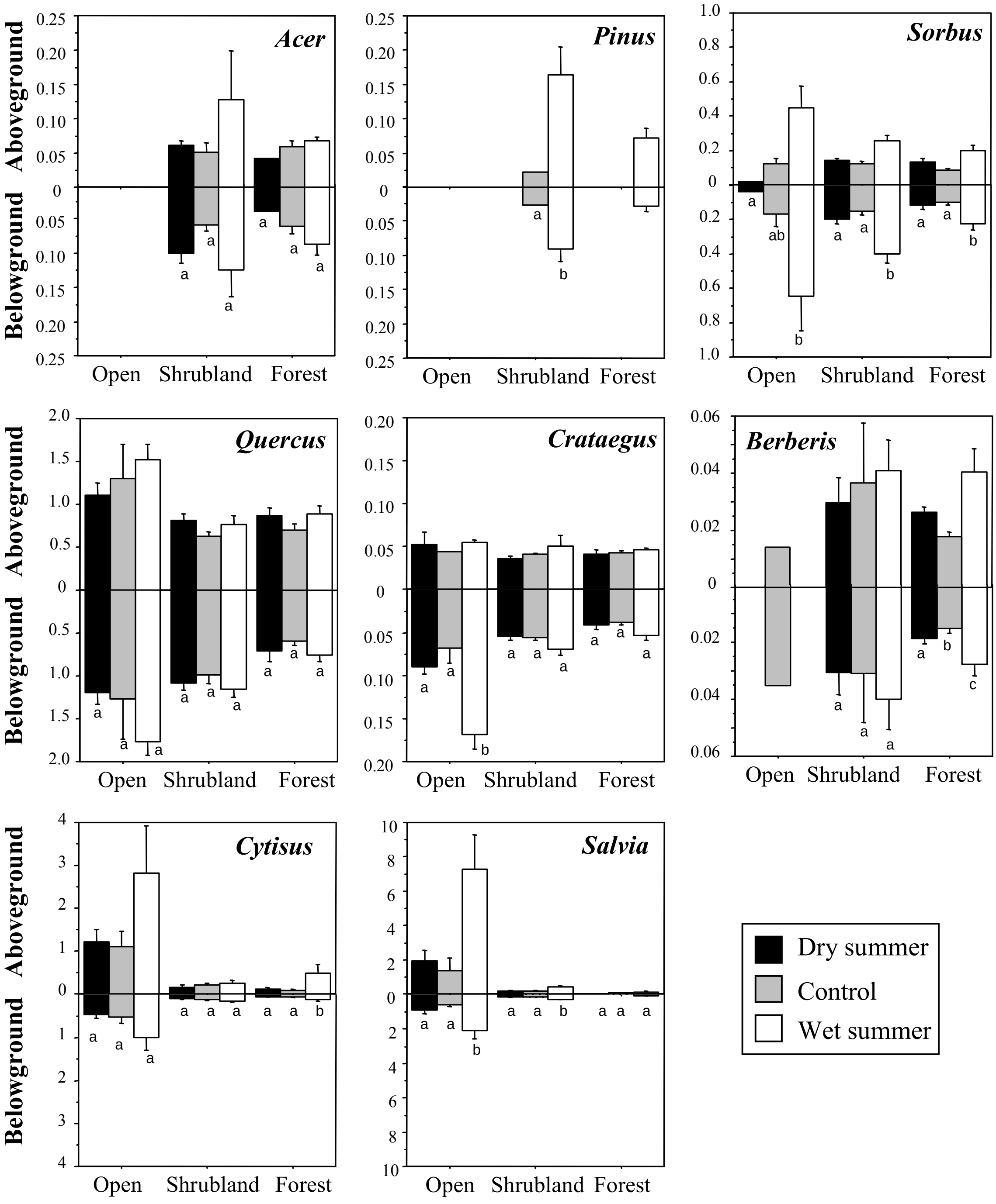


Figure 3: *Final biomass (aboveground and belowground), expressed in grams, at the end of the experiment for the eight species on the three habitats. Black bars represent dry summers, streaked bars are control, and open bars are wet summers. Mean are given + SE. Different letters indicate significant differences between climatic scenarios within habitat for total biomass (aboveground plus belowground) by a Fisher's PLSD test.*

FINAL BIOMASS

Table 4: *Results of the factorial ANOVA using aboveground and belowground biomass, transformed by natural logarithms, as dependent variable. Habitat factor have three levels (forest, shrubland and open) as well as the scenario factor (dry summer, control, and wet summer). Due to low number of surviving seedlings, some data were extracted from the analysis (Pinus: all data, Acer and Berberis: data from open habitat, Salvia: data from forest habitat). Significant P values are shown in bold.*

	Habitat (H)			Scenario (S)			H × S		
Species	F	*P*	DF	F	*P*	DF	F	*P*	DF
Aboveground									
Acer	0.16	0.695	1	0.93	0.415	2	0.60	0.562	2
Sorbus	3.79	**0.032**	2	24.00	**<0.0001**	2	5.48	**0.001**	4
Quercus	13.72	**<0.0001**	2	2.57	0.085	2	0.79	0.537	4
Crataegus	1.76	0.184	2	1.26	0.295	2	0.24	0.914	4
Berberis	0.17	0.683	1	4.37	**0.020**	2	0.39	0.675	2
Cytisus	26.55	**<0.0001**	2	2.93	**0.046**	2	0.71	0.589	4
Salvia	55.06	**<0.0001**	1	7.91	**0.002**	2	0.37	0.696	2
Belowground									
Acer	2.16	0.161	1	1.74	0.207	2	0.85	0.446	2
Sorbus	5.17	**0.010**	2	22.99	**<0.0001**	2	2.70	**0.045**	4
Quercus	23.00	**<0.0001**	2	4.75	**0.012**	2	0.82	0.520	4
Crataegus	28.30	**<0.0001**	2	12.29	**<0.0001**	2	1.77	0.154	4
Berberis	4.19	**0.048**	1	4.15	**0.024**	2	0.08	0.926	2
Cytisus	56.38	**<0.0001**	2	3.53	**0.036**	2	0.46	0.762	4
Salvia	67.15	**<0.0001**	1	8.33	**0.002**	2	0.17	0.847	2

Habitat had an effect on seedlings biomass, being significant for a higher number of species on belowground than aboveground (Table 4). As a general trend across species, forest was the habitat where lower biomass was reached, whereas it augmented in shrubland and specially in open (Fig. 3). Climatic scenario also had an effect on seedling biomass, being the differences significant in most of species for aboveground biomass, and principally for belowground biomass (Table 4). Overall, the wet summer scenario increased aboveground biomass 2.1 times respect control and belowground biomass 1.6 times. The effect of the dry summer scenario was less pronounced, showing no differences in most cases with the control treatment (Fig. 3). Interactions between these two main factors only appeared in *Sorbus*, where the biomass reduction under the drier scenario was more evident in open habitat.

DISCUSSION

Changes in precipitation predicted under a global-change scenario may alter the recruitment pattern of woody species (Lloret *et al*. 2004). In a similar way, regeneration success of plant species is strongly affected by microclimatic conditions among the different habitats (Grubb 1998; Schreeg *et al*. 2005). Our results show that these two factors interact, and that the recruitment response may be different depending on the habitat and on the particular precipitation pattern. This creates a complex situation for seedling recruitment in which the strong effect of the climatic scenario on species-specific regeneration niches is modulated by the habitat type at different levels. The differential response of the species from the different functional groups has the potential to alter future community composition and structure.

RESPONSES TO HABITAT AND CLIMATIC SCENARIOS

Seedling emergence was mainly affected by the habitat type, but by contrast was scarcely affected by the climatic scenario, probably because climatic scenario simulations started in April 2007, once that seedling germination had started, explaining the lack of effect (see Castro *et al*. 2004a, 2005; Mendoza *et al*. 2009 for similar results). The climatic scenario could impact seedling emergence only in case of species with strong physiological seed dormancy that requires more than one year for germination (*e.g*. *Taxus baccatta*, Mendoza *et al*. 2009). On the other hand, the effect of habitat was not the same for all species. Most of them emerged preferably under woody canopy, whether forest or shrubland, and only three of the species had a comparatively high emergence in open: the two pioneer shrubs and the Mediterranean tree *Quercus*. These experimental results indicate that different climatic scenarios changing summer dryness will not likely modify in a significative way the seedling emergence pattern, being principally affected by spring conditions, as well as by the habitat effect.

By contrast, climatic scenario strongly altered seedling survival of almost all studied species. Wet summer scenario was the most important factor, boosting seedling survival of most species, except for the two pioneer species *Cytisus* and *Salvia*, from which the effect was almost null. These two species, due to their pioneer character (Fogarty & Facelli 1999; Palacio & Monserrat-Martí 2006; Gaudio *et al*. 2008), are the most drought-tolerant ones, being able to survive under a wide range of water availability. Mid-successional shrubs, and especially tree species, have higher water requirements (Kollmann & Reiner 1996; Castro *et al*. 2005, Gómez-Aparicio *et al*. 2008; Mendoza *et al*. 2009) and thus would presumably be more beneficiated by a sporadic mild summer in terms of recruitment. However, one of the tree species, *Quercus*, only showed small differences across scenarios. From studied tree species, this is the only one with a Mediterranean origin (Blanco *et al*. 2005), having some drought adaptations (Quero *et al*. 2006), which together with the higher seed reserves, confer to this species some independence of abiotic conditions during the early-life stages (Quero *et al*. 2007), resulting in a high survival rate under any of the studied climatic scenarios. On the opposite way, *Pinus* was the species with higher dependence of sporadic wet summers, being almost unable to recruit new individuals under any other climatic scenario. These two species (*Quercus* and *Pinus*) are the dominant trees in the community, and an alteration in their recruitment pattern may imply important consequences for ecosystem structure.

Besides the important effect of climatic scenarios on seedling survival, different habitats modulated its intensity. As a general trend, plant cover (whether shrubland or forest) increased seedling survival, especially for trees and mid-successional shrubs. The importance of plant coverage for woody-species seedling survival is a well known pattern in many areas over the world (Callaway *et al*. 1996; Kitzberger *et al*. 2000; Kozlowski & Pallardy 2002) and in Mediterranean in particular (Maestre *et al*. 2003; Castro *et al*. 2004a; Gómez-Aparicio *et al*. 2004). But habitat not only altered seedling survival itself, it also was interacting with climatic scenarios modulating its effect for some species. Whereas the differences among scenarios were ameliorated in shrubland, major contrast appeared in open or forest habitats depending on species identity. This complex interaction among climatic scenarios, habitat types and species highlight the importance of taking into account the habitat structure heterogeneity to accurately adjust forecasting projections of climatic variation effects at broader scales.

EFFECTS ON BIOMASS

The biomass increase, both at aboveground and belowground level, induced by wet summer scenario was consistent for all species and habitats. Wet summers not only represent an important factor for the seedling survival increase on that season, but also allow a higher aerial growth as well as deeper roots. Longer and deeper roots allow recruits to take water from deeper and moister soil profiles (Padilla & Pugnaire 2007), conferring a delay advantage for next summers, and increasing therefore the survival probabilities onwards (Rey-Benayas & Camacho-Cruz 2004; Castro *et al.* 2005; Holmgren *et al*. 2006). The higher growth under wet scenario, together with the fact that dry summer did not represent a significant biomass reduction respect control scenario, are probably indicating that trees (except *Quercus*), as well as mid-successional shrubs growing under current climatic conditions are growth-limited by summer drought (Gómez-Aparicio *et al*. 2005; Mendoza *et al*. 2009). As occurred with survival, the effect of climatic scenarios on seedling growth was modulated by the different habitats. Overall, open was the habitat where seedlings from most species were able to take maximum advantage in terms of growth from the higher water input under the wet summer scenario. Is under this specific combination of conditions (open habitat and wet scenario) where the two most important factors determining seedling growth (*i.e.* light and water; Valladares & Pearcy 2002; Sánchez-Gómez *et al*. 2006; Quero *et al*. 2008b) are highest, and recruits are able to reach higher biomass, being growth partially limited by light in the other habitats.

CONSEQUENCES FOR COMMUNITY DYNAMICS UNDER A CHANGING CLIMATE

Results here presented strongly support our previous hypothesis. The increase in aridity expected for large areas of the planet under a climate change scenario, and particularly for Mediterranean-type ecosystems (Giorgi & Lionello 2008), may have a negative effect on the recruitment ability of some species, inducing changes in the community structure (Peñuelas & Filella 2001; Lloret *et al.* 2004, 2009). From our results, we could expect a change in the community composition due to the differential response of seedlings to an increase in summer drought intensity and frequency. Thus, some tree species, like *Pinus*, will be almost unable to recruit under drier conditions, and its populations would be near to collapse. Other tree species less sensitive to drought (*Acer* and *Sorbus*), could maintain their populations, mainly nursed by shrubs (Callaway *et al*. 1996; Kozloswki & Pallardy 2002; Castro *et al*. 2004b; Gómez-Aparicio *et al*. 2004), whereas *Quercus*, the most drought-tolerant species, could be expected to be the dominant tree species. These results are not completely in concordance with the previous hypothesis, since not all species are affected in the same way by a drought increase, being the success probability determined by species identity and habitat characteristics. Shrubs (especially pioneers) will be less affected by an increase in summer drought, being able to expand their habitat and colonize open areas and gaps in forest. In consequence, community structure is expected to change, with two main consequences: the loss of drought-intolerant tree species like *Pinus*, inducing a biodiversity loss, and the dominance of drought-tolerant and typically Mediterranean species: *Quercus* and shrubs. Both, biodiversity loss and species-dominance change are in concordance with other modelled predictions (Gitay *et al.* 2002; Thuiller *et al.* 2005; Malcolm *et al.* 2006; Randin *et al.* 2009), giving them an empirical support, although future models should take into account habitat variations at smaller scales. As proposed in our previous hypothesis, wetter summer conditions improve tree recruitment and colonization to nearby degraded areas (especially shrubland, but even open areas for some species), enhancing therefore forest maintenance and expansion.

According to the results here presented, the sporadic wet summers have a strong role boosting seedling regeneration abilities of the species from the different successional phases, whereas a drought increase only tended to reduce seedling growth or survival. As the risk of death normally decreases in plant populations from emergence onwards (Watkinson 1997), mild summers represents a window of opportunity for plant regeneration (Holmgren & Scheffer 2001; Scwinning & Sala 2004; Mendoza *et al*. 2009), since establishment is otherwise hampered by drought (Traveset *et al*. 2003; Urbieta *et al*. 2008; Mendoza *et al*. 2009). Is for this reason because we can affirm that the reduction in frequency of mild summers predicted for the coming decades in Mediterranean areas (Rodrigo 2002; Christensen *et al*. 2007; Giorgi & Lionello 2008) will have stronger consequences for species recruitment than drought increase. The expected decrease in the frequency of mild summers, as well as the increase of drier ones may induce a species loss in Mediterranean mountains, reducing therefore the biodiversity, as well as a change in the community dominance. For the coming decades, in the driest scenarios, these climatic conditions may induce a "regressive encroachment", in the case of some tree species are replaced by shrubs.

ACKNOWLEDGEMENTS

We thank the Consejería de Medio Ambiente (Andalusian Government) and the Direction of the Sierra Nevada National Park for facilities and support to carry out the experiment. We also thank Nacho Villegas, Ramón Ruiz, and Asier Herrero for invaluable field assistance. This study was supported by the coordinated Spanish MEC Project DINAMED (CGL2005-05830-C03) and GESBOME (P06-RNM-1890), and by a grant FPI-MEC (BES-2006-13562) to L.M. This research is part of the GLOBIMED (www.globimed.net) network in forest ecology.

REFERENCES

Allen, C.D., Breshears, D.P. 1998. Drought-induced shift of a forest-woodland ecotone: Rapid landscape response to climate variation. *Proceedings of the National Academy of Sciences* 95: 14839-14842.

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H., Gonzalez, P., Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J.H., Allard, G., Running, S.W., Semerci, A., Cobb, N. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* 259: 660-684.

Blanco, E., Costa, M., Escribano, R. 2005. Los bosques ibéricos: una interpretación geobotánica. Planeta, Barcelona.

Boisvenue, C., Running, S.W. 2006. Impacts of climate change on natural forests productivity: Evidences since the middle 20th century. *Global Change Biology* 12: 862-882.

Bonan, G.B. 2008. Forests and climate change: Forcing, feedbacks, and the climate benefits of forests. *Science* 320: 1444-1449.

Cabin, R.J., Weller, S.G., Lorence, D.H., Cordell, S., Hadway, L.J. 2002. Effects of microsite, water, weeding, and direct seeding on the regeneration of native and alien species within a Hawaiian dry forest preserve. *Biological Conservation* 104: 181-190.

Callaway, R. M., Delicia, E.H., Moore, D., Nowak, R., Schlesinger, W.H. 1996. Competition and facilitation: Contrasting effects of Artemisia Tridentata on desert vs. montane pines. *Ecology* 77: 2130-2141.

Castro, J., Zamora, R., Hódar, J.A., Gómez, J.M. 2004a.Seedling establishment of a boreal tree (*Pinus sylvestris*) at its southernmost distribution limit: Consequences of being in a marginal Mediterranean habitat. *Journal of Ecology* 92: 266-277.

Castro, J., Zamora, R., Hódar, J.A., Gómez, J.M., Gómez-Aparicio, L. 2004b. Benefits of using shrubs as nurse plants for reforestation in Mediterranean mountains: A 4-year study. *Restoration Ecology* 12: 352-358.

Castro, J., Zamora, R., Hódar, J.A., Gómez, J.M. 2005. Alleviation of summer drought boosts establishment success of *Pinus sylvestris* in a Mediterranean mountain: an experimental approach. *Plant Ecology* 181: 191-202.

Chase, J.M., Liebold, M.A. 2003. Ecological niches: linking classical and contemporary approaches. Chicago & London: The University of Chicago Press.

Christensen, J. H., Hewitson, B., Busuioc, A., Chen, A., Gao, X., Held, R., Jones, R., Kolli, R. K., Kwon, W. K.,Laprise, R., Magana Rueda, V., Mearns, L., Menendez, C. G., Räisänen, J., Rinke, A., Sarr, A., Whetton, P.,Arritt, R., Benestad, R., Beniston, M., Bromwich, D., Caya, D., Comiso, J., de Elia, R., Dethloff, K. 2007.Regional climate projections, climate change: the physical science basis. Contribution of working group I to the fourth assessment report of the IPCC, University Press, Cambridge, pp. 847-943.

Díaz-Villa, M.D., Marañón, T., Arroyo, J., Garrido, B. 2003. Soil seed bank and floristic diversity in a forest-grassland mosaic in southern Spain. *Journal of Vegetation Science* 14: 701-709.

Delgado, R., Delgado, G., Párraga, J., Gámiz, E., Sánchez, M., & Tenorio, M.A. (1989) Mapa de suelos, hoja 1027 (Güejar Sierra). Instituto para la Conservación de la Naturaleza, Madrid.

Eriksson, O., Fröborg, H. 1996. "Windows of opportunity" for recruitment in long lived clonal plants: Experimental studies of seedling establishment in *Vaccinum* shrubs. *Canadian Journal of Botany* 74: 1369-1374.

Esteban-Parra, M.J., Rodrigo, F.S., Castro-Diez, Y. 1998. Spatial and temporal patterns of precipitation in Spain for the period 1880-1992. *International Journal of Climatology* 18: 1557-1574.

Fogarty, G., Facelli, J.M. 1999. Growth and competition of *Cytisus scoparius*, an invasive shrub, and Australian native shrubs. *Plant Ecology* 144: 27-35.

García, D., Zamora, R., Hódar, J.A., Gómez, J.M. 1999**.** Age structure of *Juniperus communis* L. in the Iberian peninsula: conservation of remnant populations in Mediterranean mountains. *Biological Conservation* 87: 215-220.

García, D., Zamora, R. 2003.Persistence and multiple demographic strategies in long-lived Mediterranean plants. *Journal of Vegetation Science* 14: 921-926.

Gaudio, N., Balandier, P., Marquier, A. 2008. Light-dependent development of two competitive species (Rubus idaeus, Cytisus scoparius) colonizing gaps in temperate forest. *Annals of Forest Science* 65: 104p1-104-p5.

Giorgi, F., Lionello, P. 2008. Climate change projections for the Mediterranean region. *Global and Planetary Change*, **63**, 90-104.

Gitay, H., Suárez, A., Watson, R.T., Dokken, D.J. 2002. Climate change and biodiversity. *IPCC Technical Paper V*. Intergovernmental Panel on Climate Change.

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

Gómez-Aparicio, L., R. Zamora and J.M. Gómez. 2005. Analysis of the regeneration status of the endangered *Acer opalus* subsp. *granatense* throughout its geographical distribution in the Iberian Peninsula. *Biological Conservation* 121: 195-206.

Gómez-Aparicio, L., Pérez-Ramos, I.M., Mendoza, I., Matías, L., Quero, J.L., Castro, J., Zamora, R., Marañón, T. 2008. Oak seedling survival and growth along resource gradients in Mediterranean forests: implications for regeneration under current and future environmental scenarios. *Oikos* 117: 1683-1699.

Grubb, P.J. 1977. The maintenance of species richness in plant communities. The importance of regeneration niche. *Biological Review* 52: 107-145.

Grubb, P.J. 1998. A reassessment of the strategies of plants which cope with shortages of resources. *Perspectives in Plant Ecology, Evolution and Systematics* 1: 3-31.

Hampe, A., Arroyo, J. 2002. Recruitment and regeneration in populations of an endangered South Iberian Tertiary relict tree. *Biological Conservation* 107: 236-271.

Holmgren M. 2000. Combined effects of shade and drought on tulip poplar seedlings: trade-off in tolerance or facilitation? *Oikos* 90: 67–78.

Holmgren, M., Scheffer, M. 2001. El Niño as a window of opportunity for the restoration of degraded arid ecosystems. *Ecosystems* 4: 151-159.

Holmgren, M., López, B.C., Gutiérrez, J.R. Squeo, F.A. 2006. Herbivory and plant growth determine the success of El Niño Southern Oscillation-driven tree establishment in semiarid South America. *Global Change Biology* 12: 2263-2271.

Houle, G. 1994. Spatiotemporal patterns in the components of regeneration of four sympatric tree spacies, *Acer rubrum*, *A. saccharum*, *Betula alleghaniensis* and *Fagus grandifolia*. *Jounal of Ecology* 82: 39-53.

IPCC. 2007. Climate Change, 2007. The Physical Science Basis: Working Group I Contribution to the Fourth Assessment Report of the IPCC. Cambridge University Press, Cambridge.

Kitzberger, T., Steinaker, D.F., Veblen, T.T. 2000. Effects of climatic variability on facilitation of tree establishment in Northern Patagonia. *Ecology* 81: 1914-1924.

Kollmann, J., Reiner, S.A. 1996. Light demands of shrub seedlings and their establishment within scrublands. *Flora* 191: 191-200.

Kozlowski T.T., Pallardi, S.G. 2002. Acclimation and adaptive responses of woody plants to environmental stresses. *Botanical Review* 68: 270-334.

Linares, J.C., Delgado-Huertas, A., Camarero, J.J., Merino, J., Carreira, J.A. 2009. Competition and drought limit the response of water-use efficiency to rising atmospheric carbon dioxide in the Mediterranean fir *Abies pinsapo*. *Oecologia* 161: 611-624.

Lindenmayer, D.B., and J. Fischer. 2006. Habitat fragmentation and landscape change. An ecological and conservation synthesis. Island Press, Washington DC, USA.

Matesanz, S., A. Escudero, and F. Valladares. 2009. Impact of three global change drivers on a Mediterranean shrub. *Ecology* 90:2609-2621.

Lloret, F., Peñuelas, J., Estiarte, M. 2004. Experimental evidence of reduced diversity of seedlings due to climate modification in a Mediterranean-type community. *Global Change Biology* 10: 248-258.

Lloret, F., Peñuelas, J., Prieto, P., Llorens, L., Estiarte, M. 2009. Plant community changes induced by experimental climate change: Seedling and adult species composition. *Perspectives in Plant Ecology, Evolution and Systematics* 11: 53-63.

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

Malcolm, J.R., Liu, C., Neilson, R.P., Hansen, L., Hannah, L. 2006. Global warming and extinctions of endemic species from biodiversity hotspots. *Conservation Biology* 20: 538-548.

Martínez-Vilalta, J., Piñol, J. 2002. Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian Peninsula. *Forest Ecology and Management* 161: 247-256.

Matías, L., Mendoza, I., Zamora, R. 2009. Consistent pattern of habitat and species selection by post-dispersal seed predators in a Mediterranean mosaic landscape. *Plant Ecology* 203: 137-147.

McDowell, N.G., Allen, C.D., Marshall, L. 2010. Growth, carbon-isotope discrimination, and drought-associated mortality across a *Pinus ponderosa* elevational transect. *Global Change Biology* 16: 399-415.

Mendoza, I., Zamora, R., Castro, J. 2009. A seeding experiment for testing tree-community recruitment under variable environments: Implication for forest regeneration and conservation in Mediterranean habitats. *Biological Conservation* 142: 1491-1499.

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

Morris, M.H., Negrero-Castillo, P., Mize, C. 2000. Sowing date, shade, and irrigation affect big-leaf mahogany (Swietenia macrophylla King). *Forest Ecology and Management* 132: 173-181.

Nogués-Bravo, D., Araújo, M.B., Romdal, T., Rahbek, C. 2008. Scale effects and human impact on the elevational species richness gradients. *Nature* 453: 216-220.

Padilla, F.M., Pugnaire, F.I. 2007. Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. *Functional Ecology* 21: 489-195.

Palacio, S., Monserrat-Martí, G. 2006. Comparison of the bud morphology and shoot growth dynamics of four species of Mediterranean subshrub growing along an altitudinal gradient. *Botanical Journal of the Linnean Society* 151: 527–539.

Peñuelas, J., Filella, I. 2001. Phenology: Responses to a warming world. *Science* 294: 793-795.

Peñuelas, J., Boada, M. 2003. A global change-induced biome shift in the Montseny mountains (NE Spain). *Global Change Biology* 9: 131-140.

Peñuelas, J., Filella, I., Zhang; X., Llorens, L., Ogaya, R., Lloret, F., Comas, P., Estiarte, M., Terradas, J. 2004. Complex spatiotemporal phenological shifts as a response to rainfall changes. *New Phytologist* 161: 837–846

Quero, J.L.,Villar, R., Marañón, T., Zamora, R. 2006. Interactions of drought and shade effects on seedlings of four *Quercus* species: physiological and structural leaf responses. *New Phytologist* 170: 819-834

Quero, J.L., Villar, R., Marañón, T., Zamora, R., Poorter, L. 2007. Seed mass effect in four Mediterranean *Quercus* species (*Fagaceae*) growing in contrasting light environments. *American Journal of Botany* 94: 1795-1803.

Quero, J.L., Gómez-Aparicio, L., Zamora, R., Maestre, F.T. 2008a. Shift in the regeneration niche of an endangered tree (*Acer opalus* ssp. *granatense*) during ontogeny: Using an ecological concept for application. *Basic and Applied Ecology* 9: 635-644.

Quero, J.L., Villar, R., Marañón, T., Zamora, R., Veja, D., Sack, L. 2008b. Relating leaf photosyntetic rate to whole-plant growth: drought and shade effects on seedlings of four *Quercus* species. *Functional Plant Biology* 35: 725-737.

Quinn, G.P., Keough, M.J. 2002. Experimental design and data analysis for biologist. Cambridge, Cambridge University Press.

Randin, C.F., Engler, R., Normand, S., Zappa, M., Zimmermann, N.E., Pearman, P.B., Vittoz, P., Thuiller, W., Guisan, A. 2009. Climate change and plant distribution: local models predict high-elevation persistence. *Global Change Biology* 15: 1557-1569.

Rey-Benayas, J.M., Camacho-Cruz, A. 2004. Performance of *Quercus ilex* saplings planted in abandoned Mediterranean cropland after long-term interruption of their management. *Forest Ecology and Management* 194: 223-233.

Rodrigo, F.S. 2002. Changes in climate vatiability and seasonal rainfall extremes: a case study from San Fernando (Spain), 1821-2000. *Theoretical and Applied Climatology* 72: 193-207.

Sala, O. E., F. S. Chapin, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, H. A. Mooney, M. Oesterheld, N. L. Poff, M. T. Sykes, B. H. Walker, M. Walker, and D. H. Wall. 2000. Biodiversity - Global biodiversity scenarios for the year 2100. *Science* 287:1770-1774.

Sánchez-Gómez, D., Valladares, F., Zavala, M.A. 2006. Performance of seedlings of Mediterranean woody species under experimental gradients of irradiance and water availability: trade-offs and evidence for niche differentiation. *New Phytologist* 170: 795-806.

Schwinning, S., Sala, O.E. 2004. Hierarchy of responses to resource pulses in arid and semi-arid ecosystems. *Oecologia* 141: 211-220.

Schreeg, L., Kobe, R.K., Walters, M.B. 2005. Tree seedling growth, survival and morphology in response to landscape-level variation in soil resource availability in northern Michigan. *Canadian Journal of Forest Research* 35: 263-273.

Thuiller, W., Lavorel, S., Araújo, M., Sykes, M.T., Prentice, C. 2005. Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Science* 102: 8245-8250.

Traveset, A., Gulias, J., Riera, N., Mus, M. 2003. Transition probabilities from pollination to establishment in a rare dioecious shrub species (*Rhamnus ludovici-salvatoris*) in two habitats. *Journal of Ecology* 91: 427-437.

Urbieta, I.R., Pérez-Ramos, I.M., Zavala, M.A., Marañón, T., Kobe, R.K. 2008. Soil water content and emergence time control seedling establishment in three co-occurring Mediterranean oak species. *Canadian Journal of Forest Research* 38: 2382-2393.

Valladares, F., Pearcy, R.W. 2002. Drought can be more critical in the shade than in the sun: a field study of carbon gain and photo-inhibition in a Californian shrub during a dry El Niño year. *Plant, Cell and Environment* 25: 749-759.

Walker, M.D., Wahren, C.H., Hollister, R.D., Henry, G.H.R., Ahlquist, L.E., Alatalo, J.M., Bret-Harte, M.S., Calef, M.P., Callahan, T.V., Carroll, A.B., Epstein, H.E., Jónsdóttir, I.S., Klein, J.A., Magnússon, B., Molau, U., Oberbauer, S.F., Rewan, S.P., Robinson, C.H., Shaver, G.R., Suding, K.N., Thompson, C.C., Tolvanen, A., Totland, O., Turner, P.L., Tweedie, C.E., Webber, P.J., Wookey, P.A. 2006. Plant community responses to experimental warming across the tundra biome. *Proceedings of the National Academy of Sciences* 103: 1342-1346.

Watkinson, A.R. 1997. Plant population dynamics. In: Crawley, M.J. (ed.) Plant ecology. 2nd ed., pp. 359-400. Blackwell Science, Oxford, UK.

Weltzin, J.F., McPherson, G.R. 2000. Implications of precipitation redistribution for shifts in temperate savanna ecotones. *Ecology* 81: 1902-1913.

Yahdjian, L., Sala, O. (2002). A rainout shelter design for intercepting different amounts of rainfall. *Oecologia* 133: 95-101.

CHAPTER 3:

TO GROW OR TO SURVIVE. EFFECTS OF CLIMATE CHANGE ON WOODY SEEDLINGS FROM DIFFERENT FUNCTIONAL GROUPS IN MEDITERRANEAN MOUNTAINS

Luis Matías, José Luis Quero, Regino Zamora, Jorge Castro

ABSTRACT

Light and water are the two main factors determining seedling growth and recruitment probability. Climatic and land use changes are expected to alter the availability of these two resources as well as their relationships, and this alteration may ultimately affect seedling performance and morphological traits, although not all the species in the community will respond in a similar way to these changes. We have designed an experiment under natural field conditions to investigate the physiological and structural responses of woody seedlings to nine combinations of light (three habitats) and water (three climatic scenarios) differing in light and water availability, respectively. Eight woody species with different life form and successional stages (trees, mid-successional shrubs, and pioneer shrubs) were selected for the comparison, including therefore species representatives from the whole community, and explored the drought resistance in terms of survival (D_S), the relations of the different morphological traits to D_S, and the possible trade-off between growth and survival under the different conditions of light and water availability. D_S ranged from 0% to 99% for the different species, and overall *Quercus* and *Cytisus* were the most drought-resistant species, whereas *Acer* and *Pinus* were the most drought-sensitive ones. Morphological traits scarcely explained D_S, although was positively related with total biomass and leaf mass ratio (LMR) and negatively with leaf area-root mass ratio (LARMR). We did not found evidences of trade-off between growth and survival on Mediterranean seedlings. Accordingly with these results, some tree species with low D_S would be deselected under the future climatic conditions. But on the contrary, those species having morphologic characteristics to increase their resistance under expected dry years on coming decades (*i.e.*, high LMR or low LARMR) would be positively selected, altering therefore the community structure, functioning and processes.

Keywords: climate change, drought resistance, morphological traits, trade-off,

INTRODUCTION

Plant survival and growth have been suggested as major bottlenecks controlling community structure in many ecosystems over the world (Clark & Clark 1992; Kobe 1999; Mendoza *et al*. 2009). Light and water are two main resources affecting these variables (Hastwell & Facelli 2003; Maestre *et al*. 2003), and are ultimately responsible of plant distributions both at global and local scale (Archibold 1995; Montgomery & Chazdon 2002; Engelbretch *et al*. 2007). Climatic and land use changes are expected to alter water and light availability as well as their relationships at a planetary scale (Vitousek 1994; Houghton *et al*. 2001). Global circulation models forecast drier conditions for the coming decades, increasing summer drought up to 30% in Mediterranean areas (Giorgi & Lionello 2003; IPCC 2007). In Mediterranean mountains, rainy summers are rare events, appearing with a low frequency and irregular time intervals (7 to 40 years, Esteban-Parra *et al.* 1998; Rodó & Comín 2001; Rodrigo 2002). These exceptional climatic conditions may alter plant performance, causing ultimately changes in demography (García *et al*. 2005) and community structure (Mendoza *et al*. 2009).

Within ecosystems, consequences of summer rainfall variability should be modulated in different ways on different habitats (Callaway & Pugnaire 1999; Shumway 2000; Bey 2003; Gómez-Aparicio *et al.* 2004; Pyke & Andelman 2007), since light availability is altered by different habitat canopies. These changes in water and light availability directly affect plant physiology such as relative and absolute growth (Quero *et al*. 2008), photosynthetic rate (Quero *et al*. 2006), leaf traits (Wright *et al*. 2005), nutrients concentrations (Cunningham *et al*. 1999) and survival (Castro *et al*. 2005). Despite these multiple studies, we have scarce knowledge concerning to plant performance and its implications at community-level under a global change scenario (Valladares & Pearcy 1997; Lei 2001; Prieto *et al*. 2009; Peñuelas *et al*. 2009). Therefore, it is still needed to explore plant responses to different water availability levels among main habitats conforming the ecosystem, since these resources interact in a complex way under field conditions (Valladares & Pearcy 2002; Michalet *et al*. 2003), and their relationships need more knowledge to be clarified (Holmgren 2000; Valladares & Pearcy 2002; Sack 2004; Aranda *et al* 2005; Quero *et al*. 2006).

From a demographical point of view, seedling stage is likely to be more sensitive to light and water alterations due to their limited root systems, representing a good study subject to explore specific responses to combinations of these two main factors. At community level, species from different successional stages (*i.e.* pioneer shrubs, successional shrubs, or trees) present different tolerances to these two stressors (Engelbrecht & Kursar 2003; Sack 2004), and therefore are expected to respond in a different way to climatic alterations (either wetter or drier) as well as to differences in light availability offered by different habitats. The specific capacity to cope with a combination gradient of these two factors is considered a key factor determining community structure and dynamics (Pigott & Pigott 1993; Castro *et al.* 2004; Gómez-Aparicio *et al*. 2004; Zavala & Zea 2004) and ecosystem functioning (Díaz & Cabido 1997). Only investigating these responses to drought we will be able to understand the direction of the community succession in the coming decades.

Although specific drought responses in terms of survival and growth vary in a continuous gradient, strategies of coping with drought would be classified as 1) little effect on both growth and survival; 2) little effect on survival, but strong effect on growth, 3) strong effect on survival, with the surviving individuals showing a low to moderate growth reduction (Englebercht & Kursar 2003). These responses have resulted in some cases in a growth-survival trade-off among a continuous resource gradient, but the outcome and sign of this trade-off is highly dependent on the species, seed size, and microhabitat (Walter & Reich 1996; Holmgren 2000; Dalling & Hubbell 2002; Seiwa 2007). The different responses to drought across the light gradient and the result of the trade-off would be determined by differences in biomass allocation patterns either on leaves, shoot or roots (Smith & Huston

1989), but the relative effect of different morphological traits is not clear enough (Sack 2004).

The common way of analysing the relationships between light and water availability with plant performance is under controlled conditions in greenhouse experiments (Quero *et al*. 2006; Sánchez-Gómez *et al*. 2006). But natural regeneration, however, takes place in a much more complex scenario, where many abiotic and biotic factors act simultaneously and interactively (Ibáñez & Schupp 2001; Gómez 2004). Studies that explore seedling performance through environmental gradients under field conditions are necessary to discern the ranking and interactions among factors determining plant recruitment (Gómez-Aparicio *et al*. 2008). We have designed an experiment under natural field conditions to investigate the physiological and structural responses of woody seedlings to nine combinations of light (three habitats) and water (three climatic scenarios) differing in light and water availability, respectively. Eight woody species with different life form and successional stages (trees, successional shrubs, and pioneer shrubs) were selected for the comparison, including therefore species representatives from the whole community. The specific questions to solve are: *i*) what is the drought resistance of seedlings from species representatives of the principal successional strategies conforming Mediterranean mountains in terms of survival? *ii*) what is the effect of the habitat on seedling drought resistance? *iii*) which morphological traits are contributing to increase the drought resistance along the different strategies? *iv*) is there a trade-off between growth and survival in Mediterranean mountains?

METHODS

STUDY AREA AND SPECIES

Present study was conducted in La Cortijuela site, within the limits of Sierra Nevada National Park (37º05’ N, 3º28’ W, Granada, SE Spain), with an elevation around 1650 m a.s.l. This mountain area has a continental Mediterranean climate, with cold and snowy winters and hot dry summers. The mean minimum temperature in the coldest month (January) is -1.1 ºC, and the mean maximum of the hottest month (July) is 29.2 ºC. Rainfall is 811 mm yr^{-1}, accumulated mostly during spring and autumn (means 1990-2008). The experiment was conducted inside a natural 12.4 ha fenced area with ungulates exclosure since 1986, covered by trees (mainly *Pinus sylvestris*, *Pinus nigra*, and *Quercus ilex*), shrubs (such *Crataegus monogyna*, *Berberis vulgaris*, *Salvia lavandulifolia* and *Cytisus scoparius*) and open areas without woody cover.

We selected eight woody species from a diverse range on successional status from pioneer shrubs to trees: *Sorbus aria* L., *Acer opalus* Mill., *Pinus sylvestris* L., *Quercus ilex* L., *Crataegus monogyna* Jacq., *Berberis vulgaris* L., *Salvia lavandulifolia* Vahl., and *Cytisus scoparius* L. Hereafter, studied species will be named by their *Genus* names. More information about species characteristics can be found in Table 1. All of them are naturally presents inside the fenced area and were representatives of the three principal successional stages: pioneers, mid-successional, and late-successional (Thompson 2005). Seeds and acorns from these species were collected on the surrounding area during 2006 fructification period (September to December). Then, they were visually inspected to reject clearly-aborted or predated ones and stored at 4 °C until the sowing.

Table 1. *Summary of the species studied: life form (T, tree; Sh, shrub; B, broom; Sc, scrub), foliar longevity (D, deciduous; E, evergreen), strategy (LS, late-successional; MS, mid-successional; P, pioneer), origin (SM, sub-Mediterranean; BA, Boreal-Alpine; M, Mediterranean). Seed mass indicate the mean seed mass value (n = 360 for Quercus, n = 100 for the rest). Sowed seeds represent the number of seeds sowed from each species per plot.*

Species	**Family**	**Life form**	**Foliar Longevity**	**Strategy**	**Origin**	**Seed mass**	**Sowed seeds**
Acer opalus	Aceraceae	T	D	LS	SM	0.046	15
Pinus sylvestris	Pinaceae	T	E	LS	BA	0.012	15
Sorbus aria	Rosaceae	T	D	LS	SM	0.036	10
Quercus ilex	Fagaceae	T	E	LS	M	4.480	5
Crataegus monogyna	Rosaceae	Sh	D	MS	M	0.079	15
Berberis vulgaris	Berberidaceae	Sh	D	MS	M	0.018	15
Cytisus scoparius	Fabaceae	B	E	P	M	0.011	15
Salvia lavandulifolia	Lamiaceae	Sc	E	P	M	0.005	15

EXPERIMENTAL DESIGN

To test the effect of light and water availability on growth and morphological characteristics of woody seedlings, we performed a fully-factorial field experiment crossing these main factors, each one with three levels. For light treatment, we selected the three principal habitats in Mediterranean mountains where seedling emergence naturally occurs (Castro *et al*. 2005, Mendoza *et al*. 2009), ranging from high to low light availability: open areas, under shrub canopy, and under tree canopy. Hereafter, these habitats will be named *open*, *shrubland* and *forest* respectively. For water treatment, we selected three levels based on different climatic scenarios: 1) dry summer: based on the SRES A-2 model by Intergovernmental Panel on Climate Change (IPCC 2007), where a reduction on summer rainfall of 30% is predicted for Mediterranean areas. We built rain exclusion shelters (Yahdjian & Sala 2002) formed by a 2 ×2 m metal frame supporting V-shaped clear methacrylate bands without UV filter (Barlocast®; Faberplast S.L., Madrid), covering 35 % of the surface, and intercepting the same percentage of natural water supply by rain. A 20 cm deep ditch was excavated along the entire shelter to intercept runoff water supply. Rainout shelters were placed from April to September, simulating drier and longer summers. 2)

Sporadic rainy summer: to simulate rainy events, we placed 2 × 2 m squares on the soil with a water addition system composed of 4 sprinklers at the corners. Each week, from mid June to end September, we added a water quantity of 12 mm m^{-2}, simulating a summer storm. If one week a natural storm occurred, irrigation pulse was not added. The total water added during the summer was 180 mm, equivalent to the mean summer rainfall of the five milder summers of the 1902-2006 series on the study area (La Cortijuela meteorological station); 3) normal conditions: natural rainy conditions during experiment development. We placed 2 × 2 m squares without water addition or exclusion, acting as control for the experiment. Hereafter, these climatic scenarios will be named as *dry summer*, *wet summer*, and *control* respectively. Eight replicated plots of each climatic scenario were placed in each one of the three habitats, giving a total amount of 72 study plots (8 replicates × 3 climatic scenarios × 3 habitats).

In each plot, the eight studied species were sown inside individualized cages (1cm × 1cm wire mesh width) to avoid seed predation by small mammals as well as herbivory losses by insects. The number of seeds sown was different on each species depending on seed mass and germination rates, checked on a previous experiment (Mendoza *et al*. 2009), ranging from 5 to 15 (Table 1). The sowing was carried out on December 2006, and seeds stayed on the soil during all winter period, suffering a natural cold stratification period on the field. Light availability was measured by hemispherical photography (Valladares & Guzmán 2006), estimating the global site factor (GSF), representing the total amount of light, ranging from 0 (light absence) to 1 (100% light availability). Soil water content was monthly measured during growing seasons (May to September) by the time domain reflectometry method (TDR-100, Spectrum Technologies Inc., USA). Study plots were sampled by two perpendiculars transects recording the volumetric water content every 0.5m.

On September 2008, after two complete growing seasons, all surviving seedlings were harvested. The whole plants were extracted by excavating the whole root system with a pneumatic hummer. Roots were carefully washed to remove soil rests, plants were introduced into individualized plastic bags with moist atmosphere to maintain leaves hydration, and carried under colder conditions to the lab, where morphological and structural measures were taken. Stem and root length and leaf area were determined in fresh. Leaf area was calculated for the whole plant, by scanning all leaves and processing the images with an image analysis software (Image Pro-Plus v 4.5, Media Cybernetic Inc., Silver Spring, MD, USA). Then, plants were divided into leaves, stem and roots, oven dried at 70 ºC for at least 72 h, and weighted. From the primary data, the following variables were derived: root mass ratio (RMR; root mass/total plant mass, in g g^{-1}), stem mass ratio (SMR; stem mass/total plant mass, in g g^{-1}), leaf mass ratio (LMR; leaf mass/total plant mass, in g g^{-1}), specific leaf area (SLA; total leaf area/total leaf mass, in m^2 kg^{-1}), leaf area ratio (LAR; total leaf area/ total plant mass, in m^2 kg^{-1}), leaf area root mass ratio (LARMR; total leaf area/root mass, in m^2 kg^{-1}), and specific stem length (SSL; stem length/stem mass, in cm g^{-1}). These variables refer, respectively, to biomass allocation (RMR, SMR, LMR), leaf display (SLA, LAR), the balance between investment in light intercepting organs *vs* water and nutrient uptaking organs (LARMR), and the efficiency of biomass investment for height gain (SSL) (Poorter 1999).

DATA ANALYSIS

Soil volumetric water content and light availability (expressed as GSF) were analysed among habitats and climatic scenarios by repeated-measures ANOVA and factorial ANOVA respectively. To avoid pseudoreplication, we calculated the mean value for the different variables (Total biomass, RMR, SMR, LMR, SLA, LAR, LARMR, and SSL) from all seedlings surviving in each plot per species. These values were used to test the effects of habitats and climatic scenarios on each morphological variable and species by linear models

(R 2.9.1, R Development Core Team, 2009). All variables were transformed to their natural logarithms before analysis following Poorter & Garnier (1996) recommendations.

We also calculated the drought resistance of each species growing in the different habitats in terms of survival (D_S) as the percent survival in the dry summer treatment relative to the wet summer treatment:

$$D_S = \frac{S_D}{S_W} \times 100$$

where S_D and S_W are the number of seedlings surviving at the end of the second growing season relative to the number of emerged seedlings in the dry and wet summer scenarios respectively (Engelbrecht & Kursar 2003). This index was correlated for each species and habitat with morphological variables to asses their importance in drought resistance. In a same way, survival and total biomass were correlated to find any relation between growth and survival under different scenarios. Results throughout this paper are given as mean ± SE.

RESULTS

EFFICACY OF WATER AND LIGHT TREATMENTS IMPOSED

Light availability (expressed as GSF) ranged from 0.10 to 0.91, representing almost totally the range of natural light availability in Mediterranean areas (Valladares *et al.* 2004). Open was the habitat with the higher radiation values (0.87 ± 0.01; 100% of available radiation), followed by shrubland (0.42 ± 0.02; 48.5%), and forest (0.22 ± 0.01; 25.2%). These values significantly differed between habitats ($F_{2,63}$ = 451.5; $P < 0.0001$), but there were significant differences neither among climatic scenarios ($F_{2,63}$ = 1.30; $P = 0.28$), nor the interaction of these two factors (F_4 = 0.36; $P = 0.84$). Soil volumetric water content was also significantly different among habitats ($F_{2,715}$ = 499.7, $P < 0.0001$; forest 15.1% ± 0.18, shrubland 14.6% ± 0.17, open 12.4% ± 0.15), and among climatic scenarios ($F_{2,715}$ = 1214.7, $P < 0.0001$; wet summer 16.4% ± 0.16, control 13.8% ± 0.18, dry 11.9% ± 0.15).

DROUGHT RESISTANCE

Seedling survival after the second summer was higher in the wet scenario than in the dry scenario in all species, although differences varied among functional groups, and habitats. Accordingly, drought resistance (D_S) ranged from 0% for *Pinus* in all habitats to 99% for *Cytisus* in shrubland (Fig. 1). Overall, *Quercus* and *Cytisus* were the most drought-resistant species, whereas *Acer* and *Pinus* were the most drought-sensitive ones. Considering successional strategies, pioneer shrubs showed a high D_S in all habitats, except *Salvia* in forest. Mid-successional shrubs had also a high D_S in forest and shrubland habitats and low in open. For tree species, *Pinus* and *Acer* showed a low D_S in all habitats; *Sorbus* had the highest D_S in shrubland, followed by forest and open; finally *Quercus* had a similar high D_S across habitats.

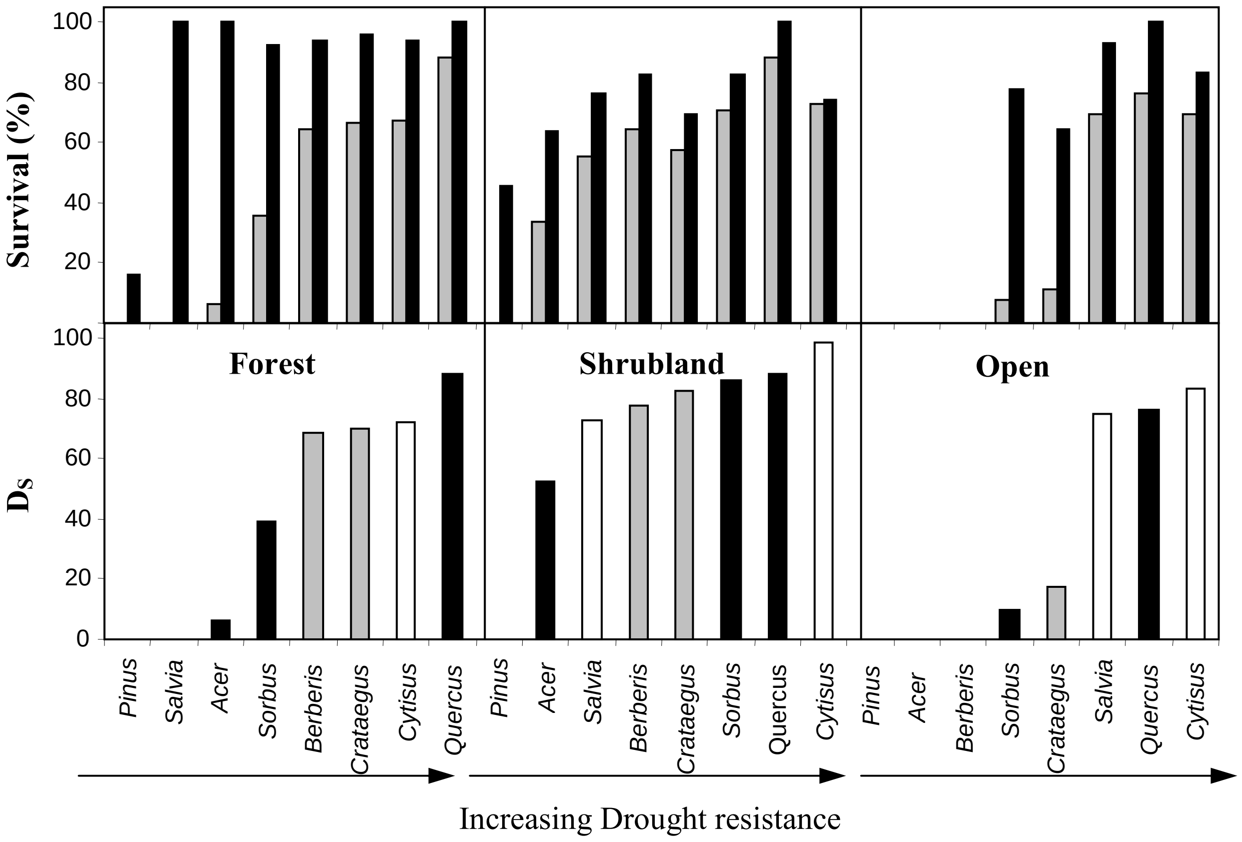


Figure 1: *Survival in the dry summer scenario relative to the wet summer scenario* (D_S=S_D/S_W×100) *in each habitat. Species are sorted by increasing drought resistance in terms of survival.* D_s *index is represented by black bars for trees, by grey bars for mid-successional shrubs, and by white bars for pioneer shrubs.*

EFFECT OF HABITAT AND CLIMATIC SCENARIOS ON SEEDLINGS' MORPHOLOGICAL TRAITS AND ITS RELATIONSHIP WITH DROUGHT RESISTANCE

Habitat and climatic scenario affected differentially morphological traits across species and functional groups (Table 2). Pioneer and mid-successional shrubs were mainly affected by habitat, although climatic scenario also affected those groups except *Cytisus*. Within trees, *Sorbus* and *Quercus* were more affected by habitat than climatic scenario for most of the studied variables (on average, 36.7% of variance for habitat vs. 22.6% variance for scenario). *Pinus* was mainly affected by climatic scenario (46.1% of variance), whereas for *Acer* we did not found strong effects either habitat or climatic scenario. Some variables such as RMR, SLA, LAR or LARMR were affected mainly by habitat effect for most species, whereas the other variables were affected also by climatic scenario. Interactions between the main factors were scarce, appearing only in *Acer* (LAR), *Sorbus* (total biomass, SMR and SSL), *Crataegus* (SMR) and *Berberis* (LMR and SMR) and explained a low percentage of the model (on average 16.1% of variance).

Overall, morphological traits scarcely explained D_S among species and within habitat. However, D_S was partially explained by total biomass and LMR in open habitat: plants with higher total biomass and LMR had also D_S (Fig. 2). Furthermore, LARMR negatively contributed to D_S in shrubland: plants with higher leaf area per unit of root mass had lower drought resistance.

Table 2: *Results of the linear model for seedling Ln-transformed morphological traits (Total biomass; RMR, root mass ratio; LMR, leaf mass ratio; SMR, stem mass ratio; SLA, specific leaf area; LAR, leaf area ratio; LARMR, leaf area root mass ratio; SSL, specific stem length), according to the factors habitat (open, shrubland, and forest) and climatic scenario (wet summer, control, and dry summer). The proportion of the explained variance (SSx/SStotal) and the level of significance (a, $P \leq 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$) for each factor and their interaction are indicated.*

Species	**Factor**	**Total Biomass**	**RMR**	**LMR**	**SMR**	**SLA**	**LAR**	**LARMR**	**SSL**	df
Acer	Habitat	4.24	11.72	6.17	12.49	50.52***	31.85**	32.67**	0.02	1
	Scenario	13.73	5.47	34.81*	4.67	3.04	16.92*	15.51	20.90	2
	H×S	6.17	2.80	14.12	10.05	10.77	17.43*	9.44	4.56	2
	R^2	0.241	0.200	0.551	0.272	0.643	0.662	0.576	0.255	16
Pinus	Habitat	35.55	37.78*	4.26	49.48***	44.85**	14.05	26.86*	0.17	1
	Scenario	54.94*	17.82	45.82	33.69**	16.75	32.08*	29.34*	80.29***	1
	R^2	0.905	0.556	0.501	0.832	0.616	0.461	0.562	0.805	10
Sorbus	Habitat	13.49**	30.38***	7.60	37.08***	70.49***	20.87**	26.41**	20.19***	2
	Scenario	36.12***	3.45	5.43	1.88	13.64	6.34	6.19	23.65***	2
	H×S	15.30**	8.46	6.95	25.27***	0.49	5.37	3.83	26.59***	4
	R^2	0.649	0.423	0.200	0.642	0.846	0.356	0.364	0.704	37
Quercus	Habitat	32.05***	47.71***	18.35***	44.61***	67.80***	45.39***	48.66***	27.75***	2
	Scenario	7.93*	1.82	1.92	1.27	1.40	1.50	1.30	1.52	2
	H×S	2.92	2.66	1.97	3.60	1.30	0.75	1.01	11.17*	4
	R^2	0.429	0.522	0.222	0.495	0.705	0.476	0.510	0.404	61
Crataegus	Habitat	41.84***	49.75***	18.66**	54.12***	65.96***	61.44***	65.73***	48.16***	2
	Scenario	12.96**	11.88**	26.73***	0.56	3.40	6.23*	10.12**	11.41**	2
	H×S	4.73	4.34	6.31	9.54*	2.01	2.58	1.15	1.27	4
	R^2	0.595	0.660	0.517	0.642	0.714	0.702	0.770	0.608	42
Berberis	Habitat	4.73	39.87***	2.16	50.36***	55.51***	27.94***	37.00***	3.08	2
	Scenario	19.29*	0.08	22.16**	6.16*	3.36	9.72	4.02	13.21	2
	H×S	0.65	7.19	12.09*	11.79**	1.77	2.04	1.86	4.20	2
	R^2	0.247	0.471	0.364	0.683	0.606	0.397	0.429	0.205	37
Cytisus	Habitat	50.57***	23.73***	-	24.90***	51.80***	70.30***	52.17***	53.06***	2
	Scenario	4.93*	2.30	-	1.37	0.76	0.52	0.73	2.49	2
	H×S	1.91	4.62	-	3.24	3.38	1.82	2.65	1.04	4
	R^2	0.574	0.306	-	0.295	0.559	0.726	0.555	0.566	59
Salvia	Habitat	65.14***	23.84*	7.41	5.01	56.23***	49.77***	34.01***	76.67***	2
	Scenario	12.30**	7.24	23.14*	20.42*	1.01	13.14*	15.79*	4.97*	2
	H×S	1.22	6.35	4.52	0.19	5.02	4.22	8.60	0.87	3
	R^2	0.787	0.374	0.351	0.256	0.623	0.671	0.584	0.825	25

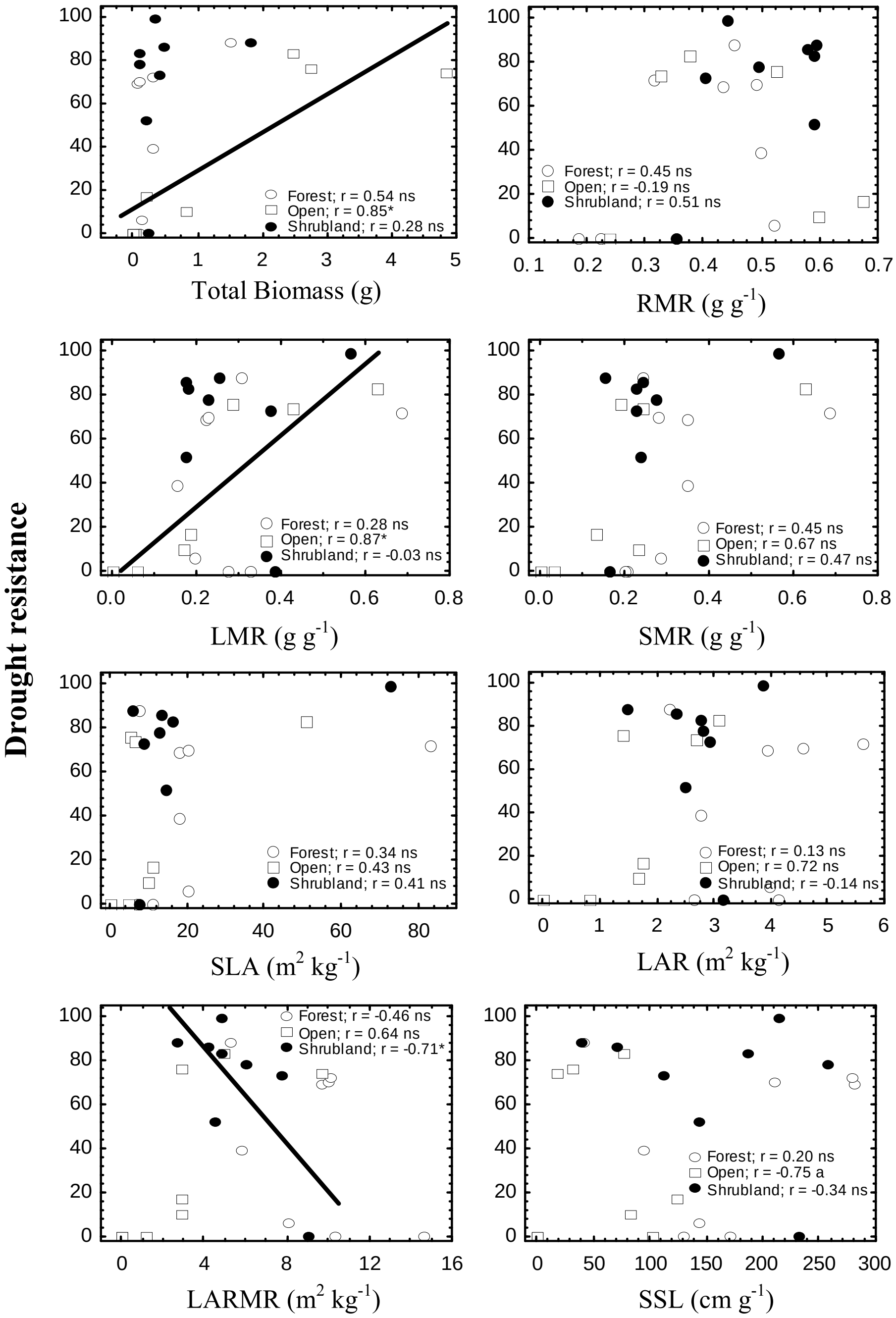


Figure 2: *Relations between drought resistance and seedling morphological traits: Total biomass (g); RMR, root mass ratio (g g^{-1}); LMR, leaf mass ratio (g g^{-1}); SMR, stem mass ratio (g g^{-1}); SLA, specific leaf area (m^2 kg^{-1}); LAR, leaf area ratio (m^2 kg^{-1}); LARMR, leaf area root mass ratio (m^2 kg^{-1}); and SSL, specific stem length (cm g^{-1}). White circles represent species growing in forest, white squares in open, and black circles in shrubland. Significant correlations are represented within habitats.*

GROWTH-SURVIVAL RELATIONS

Total growth, expressed as total biomass at the end of the experiment, and survival were related in some of the studied habitats and climatic scenarios. Among habitats, a positive relation appeared in open and shrubland, whereas any relation was found in forest (Fig. 3A). Among climatic scenarios, growth and survival only were related in the control treatment, being this relation also positive, as well as a positive trend was found in dry scenario (Fig. 3B). According to this, species with higher growth presents also higher survival, finding therefore no evidences of a trade-off between growth and survival. Furthermore, we did not found any trade-off between survival in the shade and growth in high light conditions (r^2 = 0.34; P = 0.021, positive relationship), or between survival in high light and growth in shade (r^2 = 0.23; P = 0.073).

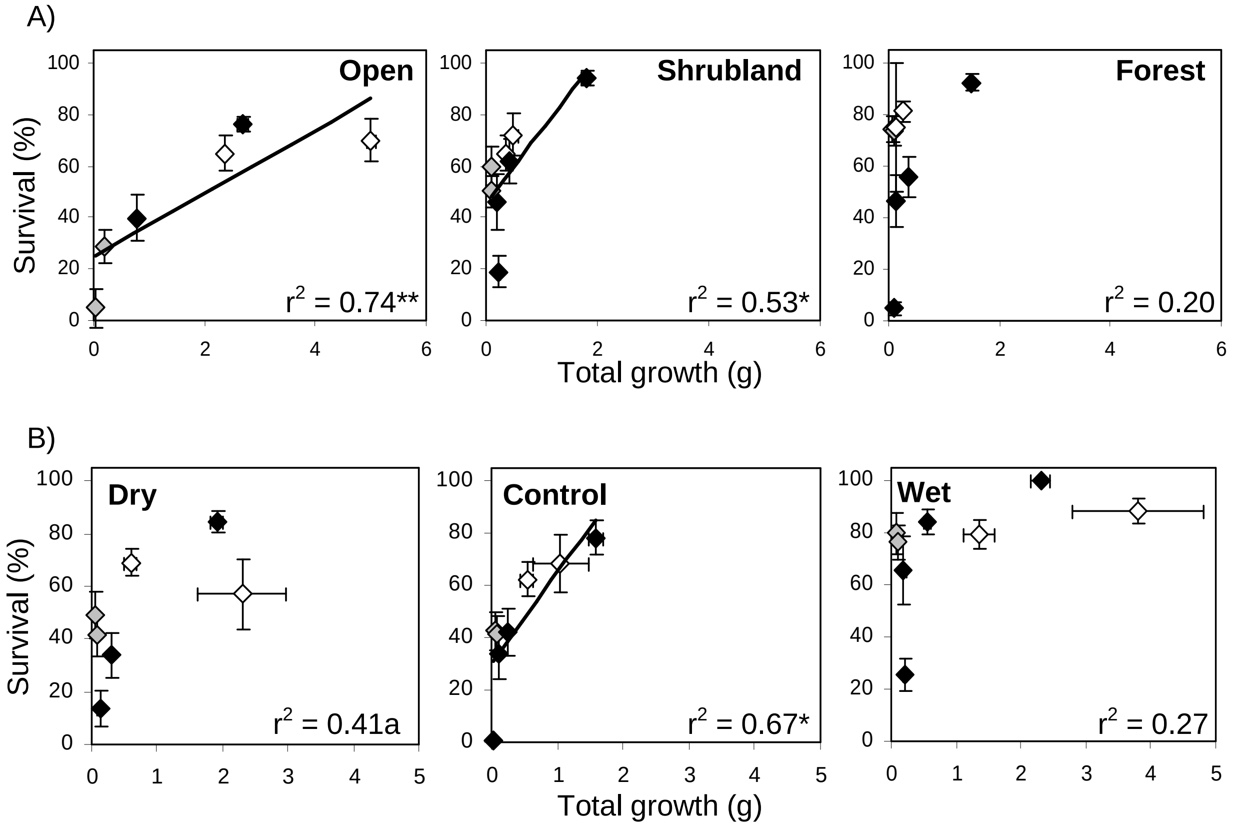


Figure 3: *Growth-survival relations under the different habitats (A), and climatic scenarios (B), where each point represents one of the studied species. Tree species are represented in black, mid-successional shrubs are in grey, and pioneer shrubs are in white. Only significant regressions are represented (a, $P \leq 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$).*

DISCUSSION

In this study we analyzed woody-seedling responses to a combination of different conditions of light and water availability from the main successional strategies within a community. Under field conditions, most studies exploring morphological trait variations in response to light or water alterations have been carried out in tropical environments, and principally with tree species (Kitajima 1994; Kobe 1999; Dalling & Hubbell 2002; Engebrecht & Kursar 2003; Wright *et al*. 2005). To our knowledge, there are no studies explicitly analysing the response of species from different successional groups to different climatic and successional scenarios. Many studies, both under field and greenhouse conditions, have evidenced that the only way to really assess consequences of drought effects is to analyze it in terms of survival more than in terms of growth (Engelbrecht & Kursar 2003; Tyree et al. 2003). In this study, we asked what is the drought resistance of different successional strategies under different habitats, what morphological traits are contributing to increase the drought resistance, and whether there is a trade-off between growth and survival in Mediterranean ecosystems during first stages of community recruitment.

DROUGHT RESISTANCE UNDER DIFFERENT HABITATS

Species from different successional strategies presented different D_S. By groups, pioneer shrubs showed high D_S in general terms, although habitat conditions modulated the resistance of the pioneer *Salvia*, which was very low under forest canopy whereas was very high in open habitat. The other pioneer species (*Cytisus*) presented high D_S in all habitats, being the highest in shrubland and open. For both pioneer species, we found that higher canopies reduced D_S, confirming therefore their pioneer status. Mid-successional shrubs presented very similar D_S (around 75%) when woody canopy was present, but being low resistant in open, especially *Berberis*. This may be indicating the necessity of the previous establishment of drought tolerant species (pioneers shrubs) which may alter microclimatic conditions to successfully establish (Broncano *et al*. 1998; Rodá *et al*. 1999). Regarding tree species, we found high D_S variation among them, *Pinus* was the species with lowest drought resistance, being zero in all habitats. This species was only able to recruit new individuals in the milder years, as was indicated in previous studies in the same habitat (Castro *et al*. 2004; Mendoza *et al*. 2009). *Acer* only presented high D_S in shrubland, whereas was very low in the other habitats. For establishment, *Acer* seedlings need to be nursed by other previously established shrub species (Gómez-Aparicio *et al*. 2005; Quero *et al*. 2008). *Sorbus* was able to establish in any habitat, but showed the highest D_S in shrubland. As occurred with *Acer*, *Sorbus* seedlings establish better under shrub canopies (Herrero *et al*. 2008; Quero *et al*. *under review*), but was also able to resist in open. The tree species with highest D_S in all habitats was *Quercus*.

Surprisingly, the two species with higher resistance to drought, independently to the canopy, were *Cytisus* and *Quercus*, a pioneer and a tree species. These two species present very different strategies to cope with drought: *Cytisus* is a drought avoider (*sensu* Valladares *et al*. 2008) because of their leaf shedding in summer to reduce transpiration, maintaining stem photosynthetic function, whereas *Quercus* is a drought resistant species *sensu stricto*, presenting different mechanisms to resist drought effects such as stomatal control, embolism resistance, or isohidric strategy (Martinez-Vilalta *et al*. 2002; Corcuera *et al*. 2004).

MORPHOLOGICAL TRAITS AND DROUGHT RESISTANCE

Overall we found that D_S was partially explained by some morphological traits, although this relation did not appear in all habitats. Total biomass as well as LMR, were positively related to D_S, but only in open habitat. Plants with higher biomass, or higher leaf mass per plant unit were more resistant to drought in terms of survival. We could explain this as a consequence of the higher development of the root and photosynthetic systems, which allow the intake of water from deeper soil profiles, higher photosynthetic efficiency, or higher reservoir storage

(Lloret *et al*. 1999; Paula & Ojeda 2009). The absence of any relation in the two habitats covered by plants (forest or shrubland) may be induced by a growth limitation (but no survival limitation). In these habitats, seedlings may be partially limited by light being unable to efficiently adapt their morphology to increase their drought resistance, and expressing therefore the relations only in the habitat where the light is not a limiting factor (Aranda *et al*. 2005). We also found a significant relation between LARMR and D_S in shrubland, indicating that species with higher inversions in light intercepting organs in relation to water and nutrient uptaking organs are less able to cope with drought. An increase in leaf area is generally a response to lower light availability (Ellsworth & Reich 1992, 1993; Niinemets & Kull 1994; Niinemets & Tenhunen 1997), at expenses of higher root development (Smith & Houston 1989), promoting higher efficiency in water uptaking, and may be a competitive advantage in conditions where other factors limit growth and survival more than light (Walters & Reich 1996).

TRADE-OFF BETWEEN GROWTH AND SURVIVAL

Most studies relating growth and survival have been developed in tropical and temperate forests, but there is still scarce information about this relationship in Mediterranean areas under field conditions (but see Zavala *et al*. 2000). Contrary to the general pattern found in tropical (Kitajima 1994; Kobe 1999; Dalling & Hubbell 2002) or temperate environments (Kobe *et al*. 1995; Pacala *et al.* 1996), we did not find any trade-off between growth and survival in Mediterranean mountains (Zavala *et al*. 2000; Zavala & Zea 2004). Furthermore, a positive growth-survival relationship was found in open and shrubland, whereas no relationship was found in forest. Among scenarios, the growth-survival positive relationship was only found in control scenario, as well as a positive trend in dry. Plants growing without water limitations, that is, in wet scenario, increased its survivorship being relatively growth-independent. The lack of relationship in forest may be due to a growth limitation by light deficit (light availability in forest is 25.2% respect light in open; Poorter 1999; Holmgren 2000; Sánchez-Gómez *et al.* 2006; Quero *et al.* 2008). Our data suggest that the growth-survival relationship may be general since it appears to be continuous regardless of whether the source of variation is habitat or climatic scenario. The shape of the growth-survival relationship reveals that growth close to zero results in a high death probability, with survival increasing strongly with growth (except in the case of forest). The linear dependence of survival on final growth suggests that seedlings may be contingent on maximizing growth rates and not on merely maintaining a positive growth rate (Walter & Reich 1996). This positive relationship supports the result of the previous objective where we found an increase in D_S when total biomass was higher. The lack of trade-off between growth and survival under field conditions contrast with the results found by Sánchez-Gómez *et al.* (2006) in a greenhouse experiment also with Mediterranean trees and shrubs. They found a negative relationship between relative growth rate and survival under different light intensities, but our results did not support this pattern under field conditions. However, we should have into account that our calculations of growth are based on populations of plants where mortality has occurred. We observed positive relationships between growth and survival across our experimental treatments in this study. Thus, growth calculations based on survivors may overestimate true population growth as the survivors would be the individuals with greater growth, and therefore some relations should still undetected.

Plant successional groups are the convergent response from different species to similar climatic conditions (Díaz & Cabido 1997). Some of these different groups have more success under a specific climatic scenario, thus some successional strategies are better adapted to cope with future climatic conditions. Species-specific alterations as a response of climatic variations results in a community change (biodiversity loss, assemblage alterations; Lloret *et al.* 2004; Thuiller *et al.* 2005). If lost species belongs to a specific successional group, it is therefore possible to expect an alteration in the group dominance. These changes on the functional group dominance would result in an alteration of the ecosystem functioning and processes (Schulze & Mooney 1993) such as productivity, nutrient cycling or the capacity for C-sequestration (Díaz & Cabido 1997). Accordingly with the results presented in this paper, some tree species with low D_S would be deselected under the future climatic conditions (*Acer* and especially *Pinus*). But on the contrary, those species having morphologic characteristics to increase their resistance under expected dry years on coming decades (*i.e.*, high LMR or low LARMR) would be positively selected, altering therefore the community structure, functioning and processes.

ACKNOWLEDGMENTS

We thank the Consejería de Medio Ambiente (Andalusian Government) and the Direction of the Sierra Nevada National Park for facilities and support to carry out the experiment. We also thank Ramón Ruiz and Eulogio Corral for invaluable field and lab assistance, and Luis Cayuela for statistical advice. This study was supported by the coordinated Spanish MEC Project DINAMED (CGL2005-05830-C03) and GESBOME (P06-RNM-1890), by a grant FPI-MEC (BES-2006-13562) to LM, and by a postdoctoral contract (2007-0572) from Spanish Ministry of Innovation and Science to JLQ. This research is part of the GLOBIMED (www.globimed.net) network in forest ecology.

REFERENCES

Aranda, Castro, L., Pardos, M., Gil, L., Pardos, J.A. Effects of the interaction between drought and shade on water relations, gas exchange and morphological traits in cork oak (*Quercus suber* L.) seedlings. *Forest Ecology and Management* 210: 117-129.

Castro, J., Zamora, R., Hódar, J.A., Gómez, J.M. 2004. Seedling stablishment of a boreal tree species (*Pinus sylvestris*) at its shouthernmost distribution limit: consequences of being in a marginal, Mediterranean habitat. *Journal of Ecology* 92: 266-277.

Castro, J., Zamora, R., Hódar, J.A., Gómez, J.M. 2005. Alleviation of summer drought boosts establishment success of *Pinus sylvestris* in a Mediterranean mountain: an experimental approach. *Plant Ecology* 181: 191-202.

Clark, D.A., Clark, D.B. 1992. Life history diversity of canopy and emergent trees in a neotropical rain forest. *Ecological Monographs* 62:315-344.

Cunningham, S.A., Summerhayes, B., Westboy, M. 1999. Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. *Ecological Monographs* 69: 569-588.

Díaz, S., Cabido, M. 1997. Plant functional types and ecosystem function in relation to global change. *Journal of Vegetation Science* 8: 463-474.

Ellsworth, D.S., Reich, P.B., 1992.Water relations and gas exchange *Acer saccharum* seedlings in contrasting natural light and water regimes. *Tree Physiology* 10, 1-20.

Ellsworth, D.S., Reich, P.B., 1993. Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. *Oecologia* 96: 169–178.

Engelbrecht, B.M.J., Kursar, T.A. 2003. Comparative drought-resistance of seedlings of 28 species co-occuring tropical woody plants. *Oecologia* 136: 383-393.

Esteban-Parra, F.J., Rodrigo, F.S., Castro-Díez, Y. 1998. Spatial and temporal patterns of precipitation in Spain for the period 1880-1992. *International Journal of Climatology* 18: 1557-1574.

Giorgi, F., Lionello, P. 2003. Climate change projections for the Mediterranean region. *Global and Planetary Change* 63: 90-104.

Gómez, J.M. 2004. Importance of burial and microhabitat on *Quercus ilex* early recruitment: non-additive effects on multiple demographic processes. *Plant Ecology* 172: 287-297.

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

Hasstwell, G.T., Facelli, J.M. 2003. Differing effects of shade-induced facilitation on growth and survival during the establishment of a chenopod shrub. *Journal of Ecology* 91:941-950.

Herrero, A., Quero, J.L., Zamora, R. 2008. Heterogeneidad ambiental a pequeña escala y patrones espaciales de supervivencia de las especies leñosas en áreas de montaña mediterránea (Sierra Nevada, Granada). In: Maestre, F.T., Escudero, A., Bonet, A. (eds.) *Introducción al análisis espacial de datos en ecología y ciencias ambientales: métodos y aplicaciones*. Pp 637-648. Universidad Rey Juan Carlos, Madrid (Spain).

Holmgren, M. 2000. Combined effects of shade and drought on tulip poplar seedlings: trade-off in tolerance or facilitation? *Oikos* 90: 67-78.

Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Xiaosu, D. 2001. Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change (IPCC), Cambridge, England

Ibáñez, I., Schupp, E.W. 2001. Positive and negative interactions between environmental conditions affecting *Cercocarpus ledifolius* seedling survival. *Oecologia* 129: 543-550.

IPCC. 2007. Climate Change, 2007. The Physical Science Basis: Working Group I Contribution to the Fourth Assessment Report of the IPCC. Cambridge University Press, Cambridge.

Kitajima, K. 1994. Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. *Oecologia* 98: 419-428.

Kobe, R.K. 1999. Light gradient partitioning among tropical tree species through differential seedling mortality and growth. *Ecology* 80:187-201.

Kobe, R.K., Pacala, S.W., Silander, J.A., Canham, C.D. 1995. Juvenile tree survivorship as a component of shade tolerance. *Ecological Applications* 5: 517-532.

Lei, S.A. 2001. Survival and development of *Phoradendron californicum* and *Acacia greggii* during a drought. *Western North American Naturalist* 61: 78-84.

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

Mendoza, I., Zamora, R., Castro, J. 2009. A seeding experiment for testing tree-community recruitment under variable environments: Implication for forest regeneration and conservation in Mediterranean habitats. *Biological Conservation* 142: 1491-1499.

Michalet, R., Rollnad, C., Joud, D., Gafta, D., Callaway, R.M. 2003. Associations between canopy and understory species increase along a rainshadow gradient in the Alps: habitat heterogeneity or facilitation? *Plant Ecology* 165: 145-160.

Niinemets, Ü., Kull, O., 1994. Leaf weight per area and leaf size of 85 Estonian woody species in relation to shade tolerance and light availability. *Forest Ecology and Management* 70, 1-10.

Niinemets, Ü., Tenhunen, J.D., 1997. A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer saccharum*. *Plant, Cell, and Environment* 20: 845-866.

Pacala, S.W., Canham, C.D., Saponara, J., Silander, J.A., Kobe, R.K., Ribbens, E. 1996. Forest models defined by field measurements: estimation, error analysis, and dynamics. *Ecological Monographs* 66: 1-43.

Paula, S., Ojeda, F. 2009. Belowground starch consumption after recurrent severe disturbance in three resprouter species of the genus *Erica*. *Botany-Botanique* 87: 253-259.

Peñuelas, J., Rutishauser, T., Filella, I. 2009.Phenology feedbacks on climate change. *Science* 304: 887-888.

Pigott, C.D., Pigott, S. 1993. Water as a determinant of the distribution of trees at the boundary of the Mediterranean zone. *Journal of Ecology* 81: 557-566.

Poorter, H., Garnier, E. 1996. Plant growth analysis: an evaluation of experimental design and computational methods. *Journal of Experimental Botany* 47:1342-1353.

Poorter, L. 1999. Growth responses of 15 rain-forest tree species to a light gradient: the relative importance of morphological and physiological traits. *Functional Ecology* 13: 396-410.

Prieto, P., Peñuelas, J., Lluisà, J., Asensio, D., Estiarte, M. 2009. Effects of experimental warming and drought on biomass accumulation in a Mediterranean shrubland. *Plant Ecology* DOI 10.1007/s11258-009-9608-1

Quero, J.L., Villar, R., Marañón, T., Zamora, R., Veja, D., Sack, L. 2008. Relating leaf photosyntetic rate to whole-plant growth: drought and shade effects on seedlings of four *Quercus* species. *Functional Plant Biology* 35: 725-737.

Rodó, X. & Comín, F. 2001. Fluctuaciones del clima mediterráneo: conexiones globales y consecuencias regionales. In: *Ecosistemas Mediterráneos. Análisis funcional.* Zamora, R. y Pugnaire, F., eds. pp. 1-35 CSIC-AEET.

Sack, L. 2004. Responses of temperate woody seedlings to shade and drought: do trade-offs limit potential niche differentiation? *Oikos* 107:110-127.

Sánchez-Gómez, D., Valladares, F., Zavala, M.A. 2006. Performance of seedlings of Mediterranean woody species under experimental gradients of irradiance and water availability: trade-offs and evidence for niche differentiation. *New Phytologist* 170: 795-806.

Schulze, E.D., Mooney, H.A. (eds.). 1993. Biodiversity and ecosystem function. Springer-Verlag, Berlin.

Smith, T., Huston, M. 1989. A theory of the spatial and temporal dynamics of plant communities. *Vegetatio* 83: 49-69.

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

Valladares, F., Pearcy, R.W. 2002. Drought can be more critical in the shade than in the sun: a field study of carbon gain and photo-inhibition in a Californian shrub during a dry El Niño year. *Plant, Cell and Environment* 25: 749-759.

Valladares, F., Guzmán, B. 2006. Canopy structure and spatial heterogeneity of understory light in abandoned Holm oak woodland. *Annales of Forest Science* 63: 749-761.

Walters, M.B., Reich, P.B. 1996. Are shade tolerance, survival and growth linked? Low light and nitrogen effects on hardwood seedlings. *Ecology* 77: 841-853.

Wright, I.J., Reich, P.B., Westboy, M. 2001. Strategy-shifts in leaf physiology, structure and nutrient content between species of high and low rainfall, and high and low nutrient habitats. *Functional Ecology* 15:423-434.

Wright, I.J., Reich, P.B., Cornelissen, J.H.C., Falster, D.S., Groom, P.K., Hikosaka, K., Lee, W., Lusk, C.H., Niinemets, Ü., Oleksyn, J., Osada, N., Poorter, H., Warton, D.I., Westboy, M. 2005. Modulation of leaf economic traits and trait relationships by climate. *Global Ecology and Biogeography* 14: 411-421.

Yahdjian, L., Sala, O. 2002. A rainout shelter design for intercepting different amounts of rainfall. *Oecologia* 133: 95-101.

Zavala, M.A., Espelta, J.M., Retana, J. 2000. Constraints and trade-offs in Mediterranean plant communities: the case of Holm Oak-Aleppo pine forests. *The Botanical Review* 66: 119-149.

Zavala, M.A., Zea, G.E. 2004. Mechanisms maintaining biodiversity in Mediterranean pine-oak forests: insights from a spatial simulation model. *Plant Ecology* 171: 197-207.

CHAPTER 4:

BIODIVERSITY CHANGES IN MEDITERRANEAN MOUNTAINS INDUCED BY EXPERIMENTAL CLIMATIC CHANGE

Luis Matías, Regino Zamora, and Jorge Castro

ABSTRACT

Alterations in rainfall pattern expected under a global change scenario have the potential to alter plant communities in many areas around the World. In the concrete case of Mediterranean basin, an increase in summer drought is expected for the end of the present century. However, is likely that this greater aridity will not cancel the typical rainy summers on Mediterranean mountains. Furthermore, the effect of the different climatic scenarios may vary across the different habitats of the ecosystem. In this study, we experimentally investigated the effect of three contrasting climatic scenarios on the recruitment pattern of the natural seedling bank of a woody community among three characteristic habitats in a Mediterranean type ecosystem: forest, shrubland, and open areas. The climatic scenarios were dry summers, according to the 30% summer rainfall reduction projected for the coming decades in Mediterranean; wet summer, simulating summer storms to reach the maximum historical records in the study area; and current climatic conditions (control). Woody seedling emergence and survival after the first summer was recorded during four consecutive years. We found strong evidence that the water availability under different climatic scenarios is able to control the expression of the recruitment potential of the different habitats by the number of seedling and species successfully recruited after the first summer. Besides natural differences across habitats, wet summer scenario strongly boosted number of seedlings and species emerging and surviving to the first and successive summers. By contrast, dry summer scenario did not have any effect in the seedling emergence, although reduced its survival. This pattern was consistent among cohorts and the different habitats. This individual and species loss in the sapling bank under drier conditions has as ultimate consequence a community structure and dominance alteration for the coming decades in Mediterranean mountains.

Keywords: Diversity, drought, seedling, rainfall, recruitment

INTRODUCTION

Climatic circulation models predict drier conditions for many ecosystems over the World, as well as increases in the frequency and magnitude of weather extremes such as severe drought, heat waves or heavy rainfall and flooding (IPCC 2007). Climatic data of the past century indicate that the anomalous climate is already affecting the physiology, distributions and phenology of some species in ways that are consistent with theoretical predictions (Hughes 2000). Under a global change scenario, drier years are predicted for the coming decades, as well as a reduction in the frequency of mild years (Houghton *et al*. 2001). However, the ecological effects of extreme events, either drier or milder, have been identified as one of the main gaps of knowledge in community ecology (Agrawal *et al*. 2007, Jentsch & Beierkuhnlein 2008).

Consequences of these extreme events have been detected at different scales from community to species level. Its effects should be expressed as forest die-back (Allen & Breshears 1998; Breshears *et al*. 2005), species loss (Thomas *et al*. 2004; Thuiller *et al*. 2005), or growth reduction (Ogaya *et al*. 2003) in the case of extreme drought, or as a higher growth and survival for individuals (Valladares & Sánchez-Gómez 2006; Gómez-Aparicio *et al*. 2008) and higher community recruitment (Castro *et al*. 2005; Mendoza *et al*. 2009a) in the case of extremely rainy conditions. Climate-induced changes in species distribution and abundance may alter complex networks of species interactions and cause significant shifts in diversity patterns within communities (Tilman 1998; Chapin *et al*. 2001). The responsiveness of species to recent (Parmesan & Yohe 2003; Root *et al*. 2003) and past (Benton & Twitchett 2003) climate change raises the possibility that anthropogenic climate change could act as a major cause of extinctions in near future. In Mediterranean areas, where summer drought is the most important constraint factor affecting ecosystem functioning (Herrera *et al*. 1994), aridity have been increasing for the last decades (Esteban-Parra *et al.* 1998; Peñuelas *et al.* 2002), affecting communities capacity to maintain their functioning. In the specific case of Mediterranean mountains such as Sierra Nevada (SE Spain) consequences may be stronger, due to the intensification of the already limiting conditions for plant regeneration (Schröter *et al.* 2005; Christensen *et al.* 2007). However, different habitats within landscape would be able to ameliorate, or in the contrary be more sensible to these different climatic conditions (Kitzberger *et al.* 2000).

Seedling dynamics reveal the potential for future replacement of adults and are expected to be particularly sensitive to between-year climatic variability and consequently to directional climate change (Weltzin & McPherson 2000; Jump *et al*. 2007). Thus, seedling establishment has often been used to monitor the effects of climate change on plant communities (Lewis-Smith 1994; Taylor 1995; Sternberg *et al*. 1999; Kullman 2002). Shifts in the composition of the seedling assembly may occur as a result of species' different responses to directional climate change. Furthermore, seedlings are the only vital stage able to move and to colonize new areas, thus knowledge about constraints and performance under a global change scenario at this stage is highly important for the construction of forecasting species-distribution models and extinction risk predictions.

More experimental data are needed to asses the effects of climate change on the species composition of communities, and particularly on species diversity patterns. Climatic manipulation under natural field conditions is an important tool to really understand the responses of the whole set of species that conform the natural community, where demographical and ecophysiological processes are often operating at stand levels (Lloret *et al*. 2004). Only under field conditions, we would be able to adequately represent habitat heterogeneity and variations that naturally are affecting ecological processes (Quero *et al. in press*).

In this study we experimentally simulated three contrasted climatic scenarios (drier, milder, and current rainfall pattern) under field conditions, in the three principal habitats that conforms Mediterranean mountains: open areas, under shrub canopy, and under adult trees. We analyzed the consequences in the recruitment ability of the woody community in the number of natural emerged and established seedlings as well as its diversity. The specific questions that we tried to solve were: 1) what is the effect of the different climatic scenarios on the number of seedlings that emerge and survive to the first summer in the woody community? 2) What is the effect of the different climatic scenarios on the diversity of established seedlings in the woody community? 3) Is the effect of the different scenarios similar across habitats? 4) Is there an accumulated effect on the successive years of the climatic scenario, or is the first year of occurrence the most important one? 5) What is the effect of the different climatic scenarios on the community ability to maintain its population? To our knowledge, there are no studies explicitly analysing effects of different climatic scenarios to the whole woody community taking into account the different habitats that conform the landscape.

METHODS

STUDY AREA

Present study was conducted in La Cortijuela area, within the limits of Sierra Nevada National Park (1650 m.a.s.l; 37º05’ N, 3º28’ W, Granada, SE Spain). This mountain area has a continental Mediterranean climate, with cold winters and hot dry summers. Rainfall is 811 mm yr^{-1} (mean 1990-2008), accumulated mostly during spring and autumn. Mean temperatures of coldest (January) and hottest (July) months are 3.5 and

21.6 ºC respectively. Vegetation in the area is composed by patches with different management predominating pine plantations, shrublands, open areas and patches of native forest (see Matías *et al*. 2009 for more details). The experiment was conducted inside a natural 12.4 ha fenced area with ungulates exclosure since 1986. Predominant tree species inside fenced area are *Pinus sylvestris*, *Pinus nigra*, *Quercus ilex*, *Acer opalus* and *Sorbus aria*. Predominant shrubs are fleshy fruited, such as *Crataegus monogyna*, *Berberis vulgaris*, *Amelanchier ovalis* and *Prunus ramburii*, or dry fruited such as *Salvia lavandulifolia* and *Cytisus scoparius*.

EXPERIMENTAL DESIGN

To test the effect of climatic scenarios on woody-seedling establishment diversity, we performed a fully-factorial field experiment crossing two main factors: climatic scenario and habitat, each one with three levels. For the climatic scenario treatment, we selected three different levels: 1) dry summer: based on the SRES A-2 model by Intergovernmental Panel on Climate Change (IPCC 2007), where a reduction on summer rainfall of 30% is predicted for Mediterranean areas. For this treatment, we built rain exclusion shelters (Yahdjian & Sala 2002) formed by a 2 × 2 m metal frame supporting V-shaped clear methacrylate bands without UV filter (Barlocast®; Faberplast S.L., Madrid), covering 35% of the surface, and intercepting the same percentage of natural water supply by rain. A 20 cm deep ditch was excavated along the entire shelter to intercept runoff water. Rainout shelters were placed from April to September, simulating drier and longer summers. 2) Sporadic rainy summer: to simulate rainy events, we placed 2 × 2 m squares on the soil with a water addition system composed of 4 sprinklers at the corners. Each week from mid June to end September we added a water quantity of 12 mm m^{-2}, simulating a summer storm. If one week a natural storm occurred, irrigation pulse was not added. The total water added during the summer was 180 mm, equivalent to the mean summer rainfall of the five milder summers of the 1902-2006 series on the study area; 3) current climatic conditions: composed by 2 × 2 m squares exposed to natural rainy conditions during experiment development. Hereafter, these climatic scenarios will be named as *dry summer*, *wet summer*, and *control* scenarios respectively. As the habitat factor we selected the three principal habitats in Mediterranean mountains differing in plant coverage: 1) open, areas of bare soil or covered with sparse herbaceous vegetation, representing the lowest canopy cover; 2) shrubland: composed of the dominant shrubs in the study area, principally *Crataegus monogyna*, *Prunus ramburii* and *Genista speciosa*, representing the mid canopy cover; 3) forest: formed by a mixed forest of *Pinus sylvestris*, *Pinus nigra* and *Quercus ilex*, and represent the denser canopy cover. In each one of the three habitats we placed 24 plots of 2 × 2 m, and randomly assigned 8 to each one of three climatic scenarios simulated, giving a total amount of 72 plots (3 climatic scenarios × 3 habitats × 8 replicates).

SEEDLING EMERGENCE AND ESTABLISHMENT

Inside each plot we placed eight permanent sampling quadrats of 400 cm^2 (20 × 20 cm) in the same location within plot. To avoid any of border effect, a 0.4 m wide buffer area was kept. Seedling emergence was recorded once per year at the spring (beginning May). Every woody seedling that emerged in the present spring was identified to species level, and marked with a small coloured tag for future revisions. At the end of the first and successive springs and summers until September 2009 seedling survival was recorded. As the first summers is the most critical period for seedlings, accumulating the highest mortality rates (Castro *et al*. 2005; Mendoza *et al*. 2009a), we assumed a seedling as established when survived to the first growing season. In 2006 a pre-treatment seedling emergence and survival monitoring was recorded to identify any possible variability between experimental plots. Climatic scenarios simulation started in late spring 2007 and we monitored three seedling cohorts (2007, 2008, and 2009 cohorts) until end of 2009 summer, therefore with three, two, and one growing seasons monitored respectively.

DATA ANALYSIS

Soil volumetric water content was analyzed among habitats and among climatic scenarios by a repeated-measures ANOVA. Climatic scenario and habitat effects on seedling emergence and survival after first summer were analysed by factorial ANOVA's, using climatic scenario, habitat and cohort as response variables. Differences on climatic scenarios among years or habitats were tested by a Fisher's PSLD post-hoc test. We used the total number of seedlings and species per plot (sum of the eight 20 × 20 cm^2 quadrats) for the different analyses. Shannon *H'* evenness index was calculated also for both emerged and surviving after the first summer seedlings from the different cohorts using the program EstimateS Win 7.5.0 (Colwell 2005). We estimated changes in rank abundance patterns in different years by repeated-measures ANOVA, with cohort as within-subject factor, climatic scenario as between subject factor, and species-rank abundances as the response variables pooling the data of the three habitats altogether. To analyze the effect of the different climatic scenarios on time we used a survival analysis using the 2007 and 2008 cohorts survival until the end of the experiment (29 and 17 months respectively); Log-Rank Chi-square statistic was used to test homogeneity of the survival curves across climatic scenarios (Kalbfleisch &

Prentice 1980). All analyses were performed following the JMP v 7.0 package procedures (SAS Institute Inc. 2007).

RESULTS

Soil volumetric water content significantly differed among habitats ($F_{2,715} = 499.7$, $P < 0.0001$; forest 15.1% ± 0.18, shrubland 14.6% ± 0.17, open 12.4% ± 0.15), and among climatic scenarios ($F_{2,715} = 1214.7$, $P < 0.0001$; wet summer 16.4% ± 0.16, control 13.8% ± 0.18, open 11.9% ± 0.15). During the experiment development we monitored a total of 599 seedlings from 15 woody species (Table 1).There was a high heterogeneity among habitats, both in the number of seedlings and species emerged. Overall, shrubland was the habitat where a higher number of seedlings emerged (77%), followed by forest (22%) and open (almost 1%). The habitat with the highest number of species detected was forest, with 14 out 15 species, followed by shrubland with 9 species, and open with 2.

Table 1: *Seedling densities (seedlings/m^2) censored during the four study years under the different climatic scenarios: dry summer (D), control (C), and wet summer (W). Habitats were pooled within climatic scenarios and years. Note that 2006 was a pre-treatment year in which climatic scenarios simulations were not applied.*

	2006			**2007**			**2008**			**2009**		
Species	**D**	**C**	**W**	**D**	**C**	**W**	**D**	**C**	**W**	**D**	**C**	**W**
Acer opalus	0	0.1	0	0	0	0.1	0	0	0.1	0	0	0.3
Amelanchier ovalis	0	0	0	0	0.1	0.1	0	0	0.1	0	0.1	0.1
Berberis hispanica	0.1	0.1	0.3	0	0.1	0.4	0.3	0.4	0.5	0	0.1	1.6
Cotoneaster granatense	0	0	0	0	0	0	0	0	0	0	0	0.1
Crataegus monogyna	2.0	0.5	2.5	1.4	2.0	4.2	4.0	4.0	11.3	5.3	4.7	9.5
Cytisus scoparius	0	0	0	0	0	0	0	0	0	0.1	0	0
Hedera helix	0	0.7	0.4	0	0	0.5	0	0	0.8	0	0	0.3
Lonicera xylosteum	0.1	0	0.1	0	0	0	0	0	0.3	0	0.3	0.1
Ononis aragonensis	0.4	0.1	0.1	0.4	0.1	0.1	0.1	0.3	0.1	0.4	0	0.1
Pyrus bourgaeana	0	0	0	0	0	0.1	0	0	0	0	0	0
Pinus sylvestris	0.1	0	0	0	0	0.3	0	0	0.3	0	0	0.3
Quercus ilex	0.5	0.4	0.4	0.3	0.1	0.3	0	0	0.1	0.1	0.1	0.3
Rosa canina	0.1	0	0.1	0.4	0.3	1.2	0.7	0.3	3.0	0.7	1.7	1.7
Rubus ulmifolius	0	0	0	0	0	0.1	0	0.3	0.3	0	0.1	0.4
Sorbus aria	0	0	0	0.1	0	0.1	0	0.1	0	0	0	0

Using the data from 2006, the year previous to the climatic scenarios simulations, we detected differences among habitats (Table 2) in the density of emerged seedlings, but no evidences of differences among the plots where the scenarios were later applied appeared, nor the interaction between these two factors. Thus, we can assume that differences among scenarios in the following years were due to the treatments. Using the data from 2007 to 2009 (once the climatic scenarios simulation started), the differences among habitats and climatic scenarios were significant for the density of emerged and surviving seedlings after the first summer (Table 2). Cohort and the interaction between habitat and scenario were significantly different only for emergence. This inter-annual variability in seedling emergence was not detected in survival, establishing a similar proportion of seedlings during the three studied years. Among habitats, although shrubland was the habitat with higher emergence (Fig. 1b), forest was the habitat with higher survival (72% in forest *vs*. 57% in shrubland and 0% in open; Fig. 2b). Open was not only the habitat with lower seedling emergence but also had zero survival. Among climatic scenarios, wet summer scenario improved seedling emergence, but more importantly, increased seedling's survival probabilities, showing in all cases higher emergence and survival. By contrast, dry summer scenario did not have any effect in the seedling emergence, although reduced its survival.

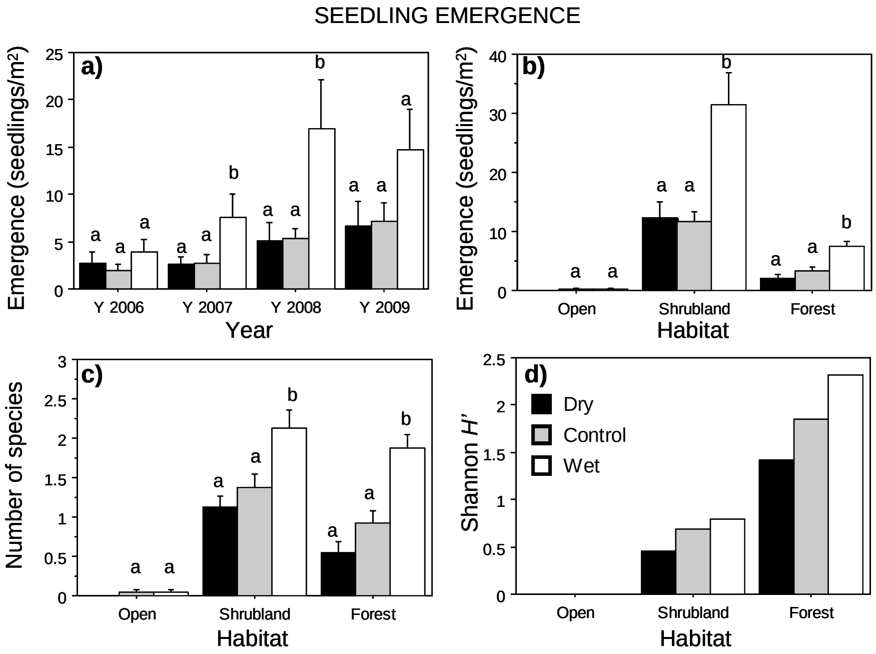


Figure 1: *Mean density of seedlings emerged under the different climatic scenarios: dry summer (black bars), control (streaky bars), and wet summer (black bars) during the four studied years (a) and in the three habitats (b). Different letters denote significant differences (P < 0.05) among scenarios within the same year (Fisher's PSLD post-hoc test). Figures c and d represents the mean number of species censored by habitat and the Shannon H' evenness index under the different climatic scenarios. Note that climatic scenarios simulations were not applied during 2006.*

Table 2: *Results of the ANOVA test for density of emerged and surviving seedlings after the first summer as dependent variables, and habitat, climatic scenario and cohort of emergence as predictors during the pre-treatment year (2006) and during the experiment (treatment years: 2007 to 2009). Note that climatic scenarios simulations were not applied during 2006. Significant factors and interactions are bold-signalled.*

		Pre-treatment year				**Treatment years**			
		Emergence		Survival		Emergence		Survival	
Factor	DF	F	*P*	F	*P*	F	*P*	F	*P*
Habitat (H)	2	5.84	**0.004**	11.65	**<0.0001**	68.61	**<0.0001**	16.76	**<0.0001**
Scenario (S)	2	0.89	0.42	0.92	0.41	16.34	**<0.0001**	8.16	**0.0005**
Cohort (C)	2	-	-	-	-	6.25	**0.0024**	0.06	0.94
H × S	4	1.12	0.36	0.48	0.75	8.51	**<0.0001**	1.44	0.23
H × C	4	-	-	-	-	5.58	**0.0003**	1.27	0.29
S × C	4	-	-	-	-	0.97	0.42	0.10	0.98
H × S × C	8	-	-	-	-	0.84	0.57	0.61	0.77

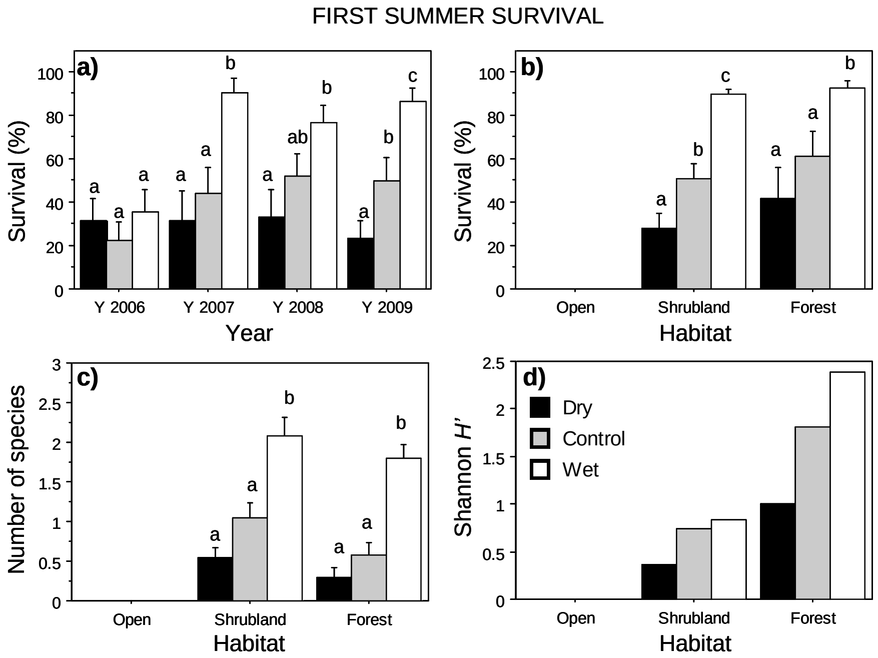


Figure 2: *Mean seedling survival after the first summer under the different climatic scenarios: dry summer (black bars), control (streaky bars), and wet summer (black bars) during the four studied years (a) and in the three habitats (b). Different letters denote significant differences (P < 0.05) among scenarios within the same year (Fisher's PSLD post-hoc test). Figures c and d represents the mean number of species censored by habitat and the Shannon H' evenness index under the different climatic scenarios. Note that climatic scenarios simulations were not applied during 2006.*

Mean number of species significantly differed among habitats and among climatic scenarios both for density of emerged seedlings (habitat: F = 91.4, $P < 0.0001$; scenario: F = 25.0, $P < 0.0001$) and surviving percentage to the first summer (habitat: F = 58.9, $P < 0.0001$; scenario: F = 41.4, $P < 0.0001$). A similar number of species emerged and survived during the three year in which climatic simulations were applied (Fig. 3). In all cases, forest was the habitat with higher number of species, whereas open showed the minimum. Among scenarios, we found the same pattern that in the case of seedling density, with higher number of species in the wet summer a lower under the dry summer scenario. This pattern was also found in the Shannon *H'* evenness index, being higher in forest and under wet summer scenario (Figs. 1d and 2d). No significant inter-annual differences appeared in the rank-abundance pattern within climatic scenarios for both emerged ($F_{2,41} = 2.25$, $P = 0.12$) and surviving ($F_{2,39} = 1.64$, $P = 0.21$) seedlings. The number of species found is a representative portion of the woody community in the study area, as we tagged 15 seedling species *vs*.16 seed species caught in seed traps in the same plots (2003-2005 data, Mendoza *et al*. *unpublished*).

Results from the survival analysis with the 2007 and 2008 cohorts until the end of the experiment showed differences between climatic scenarios (2007 cohort: $\chi^2 = 44.90$, $P < 0.0001$; 2008 cohort: $\chi^2 = 42.92$, $P < 0.0001$; Fig. 4). Seedlings growing under the wet summer scenario were more likely to survive from emergence onwards, whereas seedlings growing under dry summer scenario reduced their survival respect to the control treatment.

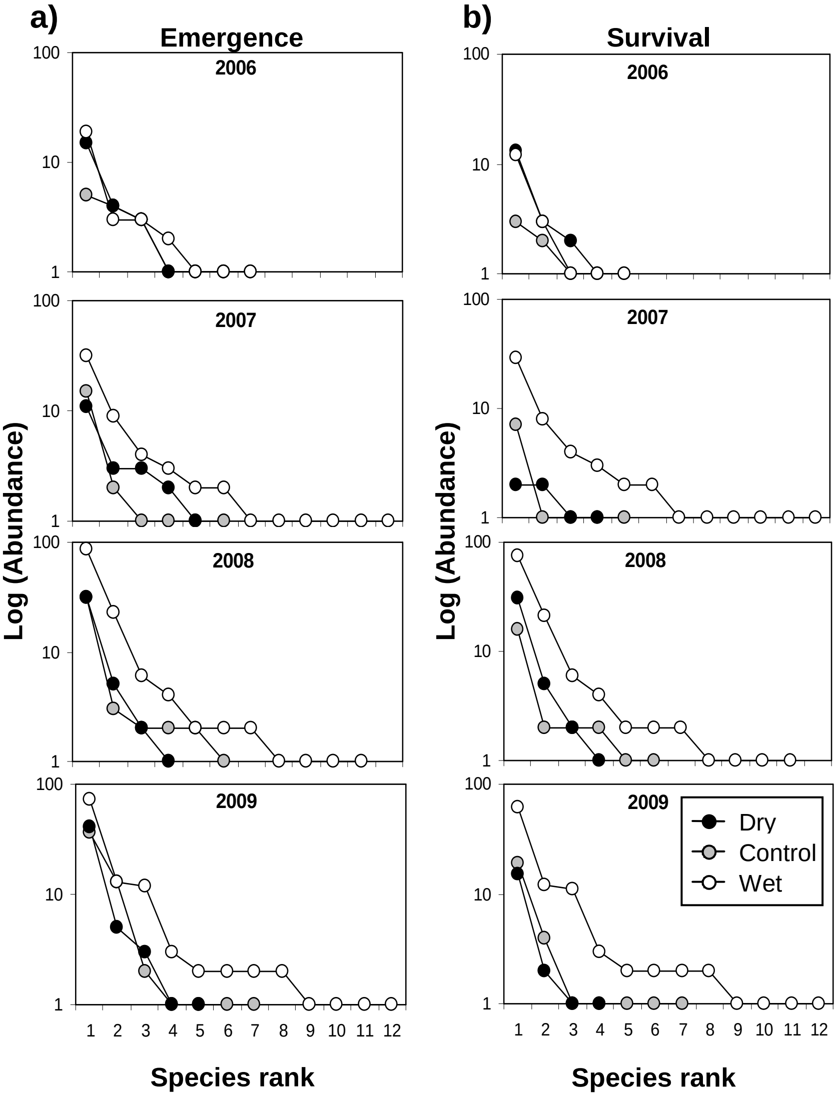


Figure 3: *Rank/abundance plot under the different climatic scenarios: dry summer (black dot), wet summer (white dot), and current climatic conditions (control, grey dot) during the 2006-2009 period. Figure a represents number of individuals and species emerged, and figure b represents the number of individuals and species surviving after the first summer.*

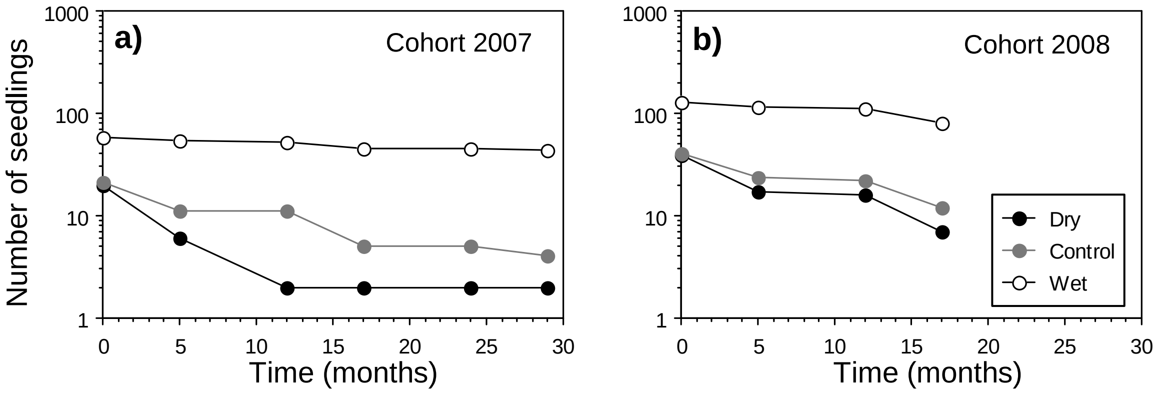


Figure 4: *Seedling survival (logarithmic scale) of the 2007 (a), and 2008 (b) cohorts under the dry (black dots), control (grey dots), and wet (white dots) summer scenarios. Seedlings were monitored from emergence to the end September 2009. Data from the three habitats have been pooled.*

DISCUSSION

By simultaneously assessing climatic and habitat effects on recruitment probabilities of natural seedling bank, the current study allows us to evaluate the importance of the predicted climatic scenarios for population dynamics in a community context. We found strong evidence that the water availability under different climatic scenarios is able to control the expression of the recruitment potential of the different habitats by the number of seedling and species successfully recruited after the first summer.

HABITAT VARIATIONS

Habitat variations in the number of seedlings and species established after the first summer

Results presented in this paper showed a strong habitat effect in the number of seedlings and species emerged. Theses differences have been already detected in the same study area (Castro *et al*. 2005; Mendoza *et al*. 2009a,b) and are principally due to differences in seed production and dispersal (Mendoza *et al*. 2009b; Matías *et al*. 2010; Zamora *et* *al*. 2010) and post-dispersal seed predation (Gómez 2004; Matías *et al*. 2009). The lowest number of seedlings and species (less than 1% of total seedlings and only 2 species) as well as the null survival probability in open are indicating a strong seed and establishment limitation (*sensu* Nathan & Muller-Landau 2000). This is a consequence of the low attractiveness of this habitat for seed dispersers (Jordano & Herrera 1995; Schupp & Fuentes 1995; García *et al*. 2005; Gómez *et al*. 2008) and the highly stressful abiotic conditions for establishment (Mendoza *et al*. 2009a). Most part of seedlings emerged in shrubland, the habitat with the better light and soil conditions (Gómez-Aparicio *et al*. 2004), sowing these seedlings also high survival rate. Forest was the habitat where higher number of species emerged, which joint to the highest survival, confer to this habitat the highest diversity. This diversity increment in forest with respect to the other habitats may be explained by the higher frugivorous bird activity within forest patches (Hódar & Zamora, *unpublished*), which are acting as mobile link organisms (Lundberg & Moberg 2003; Zamora *et al*. 2010) and are introducing a great amount of seeds into this habitat. A large number of Mediterranean fleshy-fruited woody species are late-successional shade-tolerant species (Herrera 1995). Therefore, fleshy-fruited species dispersed by frugivorous birds will have a comparatively higher probability of persisting in the understorey of forest, benefiting from the mild microclimate generated by the canopy (Mendoza *et al*. 2009b, Gómez-Aparicio *et al*. 2010). According to these results, forest would act as a species reservoir from where seeds should be dispersed to the other habitats.

Different climatic scenarios also presented differences in seedling density and number of species. Water is the principal limiting factor for establishment in Mediterranean ecosystems (Herrera *et al*. 1994). Thus, climatic scenarios differing in water availability are expected to alter in a significative way the recruitment pattern in the community. Dry summer scenario reduced total number of emerged and established seedlings as well as the total number of species. Lower precipitation, and thus lower soil moisture, has been proved to reduce seed germination (Borchert *et al*. 1989; Herrera *et al*. 1994), but more importantly is the most important death cause on seedlings during the first summer (Castro *et al*. 2005). This decrease in the total number of emerged and surviving seedlings has as ultimate consequence a species loss. According to Lloret *et al*. (2004), species loss due to climatic alterations is related to species abundance, being less abundant species more prone to disappear under drier conditions, and reducing therefore community diversity. By contrast, wet summer scenario boosted seedling emergence and survival in a significative way in shrubland and forest. Community growing under wet scenario presented the highest number of species, being also the most diverse in the different habitats. From this result, we could conclude that community recruitment is already drought-limited under natural conditions (Mendoza *et al*. 2009b). Is only in the mildest years when community is able to recruit new individuals from the most drought-sensitive species (Mendoza *et al*. 2009a) and express the potential recruitment of the community. This pattern was consistent during the three years in which climatic simulations were applied, appearing the same effect over the three cohorts both in emergence and first summer survival. This result is indicating a strong effect of the different scenarios on the community recruitment capacity. The effect on the successive year's survival was also maintained, seedlings growing under the wet summer scenario survived in a high proportion on the next years. However, for seedlings growing under the dry summer scenario, first year was the most important one in terms of survival, since mortality was lower during the second and especially during the third consecutive dry summer (as found by Castro *et al*. 2005; Mendoza *et al*. 2009a).

If mild summers are less frequent in coming decades and dryer summers are the norm as expected by the forecasting models (Houghton *et al*. 2001; IPCC 2007), a community structure alteration and diversity loss would be expected with a high probability. There are many studies modelling species distribution under future climatic conditions at regional (Erasmus *et al*. 2002; Huntley *et al*. 1995; Peterson et al. 2002; Thuiller *et al*. 2004) and local scales (Gottfried *et al.* 1995; Guisan & Theurillat 2000), and most of them predict species extinction for the next century (Thomas *et al*. 2004; Thuiller *et al*. 2005). But these models need to be based on empirical data taking into account seedling requirements under future climatic scenarios, since this is the most sensitive vital stage to environmental constrictions (Castro *et al*. 2005; Mendoza *et al*. 2009a), the only stage able to move and to colonize new areas, and often with non-coupled requirements with the other vital stages (Schupp 1995; Houle 1998; Quero *et al*. 2008).

Adults' responses to climate alterations in terms of survival are slower than in seedlings (Lloret *et al*. 2009), and mortality is usually the consequence of successive dry periods and/or extreme drought events (Bigler *et al*. 2006; Allen *et al*. 2010), but may suddenly appear as a massive forest die-back (Allen & Breshears. 1998; Breshears *et al*. 2005). With drier summers, a healthy and diverse sapling bank able to compensate tree mortality is less probable to persist, and thus community is prone to drastically change its structure and composition. A sporadic wet summer would be able to compensate some years of low recruitment (García *et al*. 2000), although these episodes are less likely to occur under a global change scenario.

Our results sowed that sampling bank density and diversity is highly dependent to extreme climatic events. Although a sporadic mild summer is able to greatly enhance seedling recruitment, the most probable drier summers drastically reduce both number of individuals and species. This pattern is consistent among cohorts and the different habitats in the community, even in forest, the most diverse one. This individual and species loss in the sapling bank under drier conditions has as ultimate consequence a community structure and dominance alteration for the coming decades in Mediterranean mountains.

ACKNOWLEDGEMENTS

We thank the Consejería de Medio Ambiente (Andalusian Government) and the Direction of the Sierra Nevada National Park for facilities and support to carry out the experiment. We also thank Nacho Villegas for invaluable field assistance. This study was supported by the coordinated Spanish MEC Project DINAMED (CGL2005-05830-C03) and GESBOME (P06-RNM-1890) from the Excellence Research Programme of the Andalusian Government, and by a grant FPI-MEC (BES-2006-13562) to L.M. This research is part of the GLOBIMED network on forest research (www.globimed.net/).

REFERENCES

Allen, C.D., Breshears, D.P. 1998. Drought-induced shift of a forest-woodland ecotone: Rapid landscape response to climate variation. *Proceedings of the National Academy of Sciences* 95: 14839-14842.

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H., Gonzalez, P., Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J.H., Allard, G., Running, S.W., Semerci, A., Cobb, N. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* 259: 660-684.

Agrawal, A.A., Ackerly, D.D., Adler, F., Arnold, A.E., Caceres, C., Doak, D.F., Post, E., Hudson, P.J., Maron, J., Mooney, K.A., Power, M., Schemske, D., Stachowicz, J., Strauss, S., Turner, M.G., Werner, E. 2007. Filling key gaps in population and community ecology. *Frontiers in Ecology and the Environment* 5: 145-152.

Bigler, C., Bräker, O.U., Bugmann, H., Dobbertin, M., Rigling, A. 2006. Drought as an inciting mortality factor in Scots pine stands of the Valais, Switzerland. *Ecosystems* 9: 330-343.

Borchert. M.I., Davis, F.W., Michaelsen, J., Oyler, L.D. 1989. Interaction of factors affecting seedling recruitment of blue oak (*Quercus douglasii*) in California. *Ecology* 70: 389-404.

Breshears, D.P., Cobbs, N.S., Rich, P.M., Priece, K.P., Allen, C.D., Balice, R.G., Romme, W.H., Kastens, J.H., Floyd, M.L., Belnap, J., Anderson, J.J., Myers, O.B., Meyer, C.W. 2005. Regional vegetation die-off in response to global-change-type drought. *Proceedings of the National Academy of Sciences* 102: 15144-15148.

Castro, J., Zamora, R., Hódar, J.A., Gómez, J.M. 2005. Alleviation of summer drought boosts establishment success of *Pinus sylvestris* in a Mediterranean mountain: an experimental approach. *Plant Ecology* 181: 191-202.

Chapin III, F.S., Sala, O.E., Huber-Sannwald, E. 2001. *Global Biodiversity in a Changing Environment*. Springer, New York.

Colwell, R.K. 2005. *EstimateS*: Statistical estimation of species richness and shared species from samples. Version 7.5. Persistent URL <purl.oclc.org/estimates>.

Erasmus, B.F.N., Van Jaarsweld, A.S., Chown, S.L., Kshatriya, M., Wessels, K.J. 2002. Vulnerability of South African animal taxa to climate change. *Global Change Biology* 8: 679-693.

García, D., Obeso, J.R. Martínez, I. 2005 b. Spatial concordance between seed rain and seedling establishment in bird-dispersed trees: does scale matter? *Journal of Ecology* 93: 693-704.

Gómez-Aparicio, L., Zavala, M.A., Bonet, F., Zamora, R. 2010. Are pine plantations valid tools for restoring Mediterranean forests? An assessment along gradients of climatic conditions, stand density and distance to seed sources. *Ecological Applications* (In press).

Gómez, J.M. 2004. Bigger is not always better: conflicting selective pressures on seed size on Quercus ilex. *Evolution* 58:71-80.

Gómez, J.M., Puerta-Piñero, C., Schupp, E.W. 2008. Efectiveness of rodents as local seed dispersers of Holm oaks. *Oecologia* 155: 529-537

Guisan, A., Theurillat, J.P. 2000. Equilibrium modelling of alpine plant distribution: How far can we go? *Phytocoenologia* 30: 353-384.

Herrera, C.M., Jordano, P., López-Soria, L., Amat, J.A. 1994. Recruitment of a mast-fruiting bird-dispersed tree: bridging frugivory activity and seedling establishment. *Ecological Monographs* 64: 314-344.

Herrera, C. M. 1995. Plant-vertebrate seed dispersal systems in the Mediterranean: ecological, evolutionary and historical determinants. *Annual Review of Ecology and Systematics* 26: 705-727

Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Xiaosu, D. 2001. Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change (IPCC), Cambridge, England

Houle, G. 1998. Seed dispersal and seedling recruitment of *Betula alleghaniensis*: spatial inconsistency in time. *Ecology* 79: 807-818.

Hughes, L. 2000. Biological consequences of global warming: Is the signal already apparent? *Trends in Ecology and Evolution* 15: 56-61.

Huntley, B., Berry, P.M., Cramer, W., McDonald, A.P. 1995. Modelling present and potential future ranges of some European higher plants using climate response surfaces. *Journal of Biogeography* 22: 967-1001.

IPCC. 2007. Climate Change, 2007. The Physical Science Basis: Working Group I Contribution to the Fourth Assessment Report of the IPCC. Cambridge University Press, Cambridge.

Jentsch, A., Beierkuhnlein, C. 2008. Research frontiers in climate change: Effects of extreme meteorological events on ecosystems. *Geoscience* 340: 621-628.

Jordano, P. & Herrera, C.M. 1995. Shuffling the offspring: Uncoupling and spatial discordance of multiple stages in vertebrate seed dispersal. *Ecoscience* 2: 230-237.

Kalbfleisch, J.D. & Prentice, R.L. 1980. The statistical analysis of failure time data. Willey, New York.

Lloret, F., Peñuelas, J., Estiarte, M. 2004. Experimental evidence of reduced diversity of seedlings due to climate modification in a Mediterranean-type community. G*lobal Change Biology* 10: 248-258.

Lloret, F., Peñuelas, J., Prieto, P., Llorens, L., Estiarte, M. 2009. Plant community changes induced by experimental climate change: Seedling and adult species composition. *Perspectives in Plant Ecology, Evolution and Systematics* 11: 53-63.

Lundberg, J. and F. Moberg. 2003. Mobile links organims and Ecosystem functioning: implications for ecosystem resilience and management. *Ecosystem* 6: 87-98.

Matías, L., Mendoza, I., Zamora, R. 2009b. Consistent pattern of habitat and species selection by post-dispersal seed predators in a Mediterranean mosaic landscape. *Plant Ecology* 203: 137-147.

Matías, L., Zamora, R., Mendoza, I., Hódar, J.A. 2009a. Seed dispersal pattern by large frugivorous mammals in a degraded mosaic landscape. *Restoration Ecology* doi: 10.1111/j.1526-100X.2008.00475.x

Mendoza, I., Gómez-Aparicio, L., Zamora, R., Matías, L. 2009b. Recruitment limitation of forest communities in a degraded Mediterranean landscape. *Journal of Vegetation Science* 20: 367-376.

Mendoza, I., Zamora, R., Castro, J. 2009a. A seeding experiment for testing tree-community recruitment under variable environments: Implication for forest regeneration and conservation in Mediterranean habitats. *Biological Conservation* 142: 1491-1499.

Nathan, R., Muller-Landau, H. C. 2000. Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends in Ecology & Evolution* 15: 278-285.

Ogaya, R., Peñuelas, J., Martínez-Vilalta, J., Manguirón, M. 2003. Effect of drought on diameter increment of *Quercus ilex*, *Phylirea latifoia* and *Arbutus unedo* in a holm oak forest of NE Spain. *Forest Ecology and Management* 180: 175-184.

Peterson, A.T., Ortega-Huerta, M.A., Bartley, J., Sánchez-Cordero, V., Soberon, J., Buddemeier, R.H., Stockwell, D.R.B. 2002. Future projections for Mexican faunas under global climate change scenarios. *Nature* 416: 626-629.

Quero, J.L., Gómez-Aparicio, L., Zamora, R., Maestre, F.T. 2008. Shifts in the regeneration niche of an endangered tree (*Acer opalus ssp. granatense*) during ontogeny: using an ecological concept for application. *Basic and Applied Ecology* 9: 635-644.

Schupp, E.W. 1995. Deed-seeding conflicts, habitat choice, and patterns of plant recruitment. *American Journal of Botany* 82: 399-409.

Schupp, E.W. & Fuentes, M. 1995. Spatial patterns of seed dispersal and the unification of plant population ecology. *Écoscience* 2: 267-275.

Tilman, D. 1998. Species composition, species diversity, and ecosystem processes: understanding the impacts of global change. In: *Success, Limitations, and Frontiers in Ecosystems Science* (eds. Pace, M.L., Groffman, P.M.) pp. 452-472. Springer, New York.

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., Erasmus, B.F.N., Ferreira de Siquiera, M., Grainger, A., Hannah, L., Huges, L., Huntley, B., van Jaarsveld, A.S., Midgley, G.F., Miles, L., Ortega-Huerta, M.A., Peterson, A.T., Phillips, O.L., Williamns, S.E. 2004. Extinction risk from climate change. *Nature* 427: 145-148.

Thuiller, W. 2004. Patterns and uncertainties of species' range shifts under climate change. *Global Change Biology* 10: 2020-2027.

Thuiller, W., Lavorel, S., Araújo, M., Sykes, M.T., Prentice, C. 2005. Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Science* 102: 8245-8250.

Valladares, F., Guzmán, B. 2006. Canopy structure and spatial heterogeneity of understory light in abandoned Holm oak woodland. *Annals of Forest Science* 63: 749-761.

Valladares, F., Sáchez-Gómez, D. 2006. Ecophysiological traits associated with drought in Mediterranean tree seedlings: individual responses versus interspecific trends in eleven species. *Plant Biology* 8: 688-697.

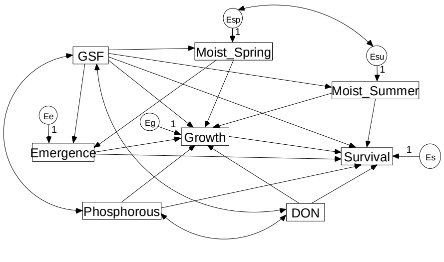
Yahdjian, L., Sala, O. 2002. A rainout shelter design for intercepting different amounts of rainfall. *Oecologia* 133: 95-101.

Zamora, R., Hódar, J.A., Matías, L., Mendoza, I. 2010. Positive adjacency effects mediated by seed disperser birds in pine plantations. *Ecological Applications* (In press).

CHAPTER 5:

EFFECTS OF RESOURCE AVAILABILITY ON PLANT RECRUITMENT AT COMMUNITY LEVEL: AN INTEGRATED ANALYSIS USING STRUCTURAL EQUATION MODELLING

Luis Matías, Lorena Gómez-Aparicio, Regino Zamora, and Jorge Castro



ABSTRACT

Coexisting plant species usually present differences in resource requirements, which may also vary within species among the successive demographic stages. These differences become extremely important during the early life-stages, since are the most critical phases in woody species recruitment, are highly dependent on abiotic resources and may determine future community composition. Under a global-change scenario, where climatic conditions, nutrient availability, and habitat characteristics via land-use change are expected to be altered, is difficult to understand the way in which plant recruitment will be affected. To understand the effect of the main global change drivers on community recruitment, we sowed a set of species representatives of the different successional groups of a whole Mediterranean woody community under field conditions, and studied their emergence, growth and survival along main resource gradients of light, water and nutrients. Light and nutrient gradient followed the natural range of conditions in the study area, but water availability was altered to simulate three contrasted climatic scenarios: wetter, drier and current conditions. Structural equation modelling was used to provide a comprehensive analysis of the factors and relations directly and indirectly affecting plant recruitment. Overall, seedling emergence was directly determined by light conditions, growth by light and summer soil moisture, and survival by summer soil moisture. Light was the main factor indirectly affecting demographic stages of all species. However, the magnitude of the direct and indirect relationships varied among species. Particularly, species differed in their direct response to the expected drier climatic conditions, some of them (*e.g*. *Pinus sylvestris*, deciduous trees) being much more vulnerable than others (*e.g*. pioneer shrubs, *Quercus ilex*). These differential responses could translate into important changes in the structure of the whole plant community. Our results support that the analysis of complex relations among essential resources is critical to better forecast the impact of climate change on community dynamics.

Keywords: climate change, light, nitrogen, phosphorus, structural equation modelling, water.

INTRODUCTION

Understanding plant foraging (*sensu* McNickle *et al*. 2009) is a key topic to understand plant relations with their immediate environment. Coexisting species usually differ in their tolerance to resource availability (Grubb 1998; Schreeg *et al*. 2005), and these differences are even more important if we consider species with different life forms (*e.g*. trees or shrubs) or from different successional stages (*e.g*. pioneers, mid-successional or late-successional). Specific resource requirements determine their relative abundance in the community, as well as the probability to success under the future climatic conditions predicted for forecasting models (Houghton *et al*. 2001). Therefore, community approaches moving beyond the study of particular species are crucial to increase our ability for predicting changes in the recruitment pattern along abiotic gradients, and to precisely determine their relevance as a driver of community dynamic.

Seedling establishment is the most sensitive and critical stage for woody species recruitment (Houle 1994; Hampe & Arroyo 2002; Castro *et al*. 2005; Mendoza *et al*. 2009a). Recruitment in plant populations is the result of success probabilities in a series of consecutive demographical stages, from seed germination to seedling survival (Reid 1989; Jordano & Herrera 1995; Clark *et al*. 1998; Garrido *et al*. 2005). These early life-stages are highly dependents on specific environmental conditions and resource availabilities, mainly light, water, and nutrients (Harper 1977; Kitajima & Fenner 2000; Gómez-Aparicio *et al*. 2008). But resource requirements not only differ among species, it may also vary within species among the successive demographic stages on the life cycle (Chase & Leibold 2003; Miriti 2006; Quero *et al*. 2008), experiencing distinct differences in tolerances to extrinsic factors at different developmental stages (Turner *at al*. 1969; Parrish & Bazzaz 1985; Lyons & Barnes 1998), which would result in an "uncoupling" among stages (*sensu* Jordano & Herrera 1995).

Resource availability is subjected to a high spatial and temporal heterogeneity (Maestre *et al*. 2003; Quero *et al*. 2010), and is expected to be even more heterogeneous under a global change scenario (Houghton *et al*. 2001). Predicted conditions by global change forecasting models for the coming decades may alter the principal resources availabilities by changes in the precipitation pattern (Giorgi & Lionello 2007), alterations of habitat structure (Peñuelas & Boada 2003), or in nutrient cycling (Jonasson *et al*., 1999; Jensen *et al*., 2003; Sardans & Peñuelas 2007), altering therefore their role in plant regeneration. Thus, a complete overview of the response of different species at early life-stages to a resource availability gradient is extremely important to accurately address the regeneration ability of the community under a global change scenario.

The complex relationships among resources and early recruitment in plants could be explored in an integrated way by the use of structural equation modelling (SEM). SEM is a statistical method that helps provide insights into complex theoretical issues. These techniques are typically used to confirm or disprove an a priori hypothesized model, that is, to test the statistical adequacy of a proposed causal model (Browne 1982; Hayduk 1987; Shipley 2000; Iriondo *et al*. 2003). Situations observed when analyzing the processes that affects community recruitment (seedling emergence, growth, and survival) can be placed in the form of a hypothesis that incorporates all the variables that are a priori relevant according to existing knowledge (Iriondo *et al*. 2003), giving an overall view of the factors and relations directly and indirectly affecting plant recruitment.

The objective of this work was to determine the effect and the relative importance of abiotic conditions on the recruitment probabilities of the whole woody community conforming Mediterranean mountains under a global change scenario. For this, we sowed seeds from eight tree and shrubby species representatives of the different successional stages of the community under field conditions, and studied their emergence, growth and survival along a light, water and nutrient gradient. The light and nutrient gradient followed the whole range of conditions in the study area, but natural water availability was altered by adding or excluding rainfall during summer to simulate three contrasted climatic scenarios: milder, drier and current conditions. Although there are studies that have analyzed the response of few species (Laskurain *et al*. 2004; Gómez-Aparicio *et al*. 2008) or demographic stages (Jordano & Herrera 1995; Escudero *et al*. 2000; Garrido *et al*. 2005) to few levels of a determined resource (Carson & Pickett 1990; Stevens *et al*. 2006), to our knowledge, this is the first study experimentally analyzing the effects of abiotic resources availabilities, both current and predicted, on Mediterranean woody-species recruitment and the relationships among the different demographic stages at a community level (but see Luzuriaga & Escudero 2008 for an herbaceous community). Specifically, we used SEM in order to answer the following questions: 1) what are the main resources affecting seedling recruitment both direct and indirectly? 2) How do the sign and the magnitude of the effects vary among demographic stages? 3) Are resources affecting in a similar way to all species and functional groups of the community? 4) What are the community recruitment projections under a global change scenario? Answering these questions we would be able to better understand recruitment dynamics, and predict their consequences under a global change scenario.

METHODS

STUDY AREA AND SPECIES

The experiment was carried out at La Cortijuela area, within the limits of Sierra Nevada National Park (37º05’ N, 3º28’ W, Granada, SE Spain), with an elevation range around 1650 m a.s.l. This mountain area has a continental Mediterranean climate, with cold winters and hot dry summers. Rainfall is 811 mm yr^{-1}, accumulated mostly during spring and autumn (means 1990-2008). The experiment was conducted inside a natural 12.4 ha fenced area with ungulates exclosure since 1986, with areas covered by trees, shrubs, and open areas without woody cover. As study species, we selected the two dominant tree species (*Quercus ilex* L.

and *Pinus sylvestris* L.) in the study area and the principal functional groups of the community accompanying them: deciduous trees, mid-successional shrubs, and pioneer shrubs. Each group was conformed by two representative species (deciduous trees: *Acer opalus* Mill. and *Sorbus aria* L.; mid-successional shrubs: *Berberis vulgaris* L. and *Crataegus monogyna* Jacq.; pioneer shrubs: *Cytisus scoparius* L. and *Salvia lavandulifolia* Vahl.). All of these species are naturally present in the study area.

EXPERIMENTAL DESIGN

To test the effect of different abiotic factors on woody recruitment, we sowed the eight studied species under different conditions of light and water. To do this, we selected three different habitats where seedling emergence naturally occurs (Castro *et al*. 2005; Mendoza *et al*. 2009a), covering most part of the natural light availability in Mediterranean areas: open areas, under shrub canopy, and under tree canopy. Natural water availability gradient was enhanced by experimentally adding or reducing water input respect to control plots to simulate milder (equivalent to the mean summer rainfall of the five milder summers of the 1902-2006 series on the study area) or drier summers (as predicted by SRES-A2 scenario from IPCC; IPCC 2001), as well as current climatic conditions (no water manipulations). Additional methodological details can be found in Matías *et al*. 2023, chapter two of this thesis.

72 plots of 2 × 2 m were placed, randomly assigning eight of them to each one of the habitat and climatic scenario combinations (3 habitats × 3 climatic scenarios × 8 replicates). In each plot, the eight studied species were sown. The number of seeds sown was different on each species depending on seed mass and germination rates, checked on a previous experiment in the same study area (Mendoza *et al*. 2009b), ranging from 5 to 15. The sowing was carried out on December 2006, and seeds stayed on the soil during all winter, suffering a natural cold stratification period on the field. Emergence was recorded on 2007 spring

period, noting the proportion of emerged seedlings from the number of sowed seeds per plot. Survival was monitored after two complete growing seasons, in September 2008. The proportion of surviving seedlings in September 2008 from the number of emerged in 2007 spring in each plot was used as survival indicator. Once the experiment have finished, all surviving seedlings were harvested. The whole plants were extracted by excavating the whole root system with a pneumatic hummer. Roots were carefully washed to remove soil rests. Then, plants were oven dried at 70 ºC for at least 72 h, and weighted for total biomass. Mean biomass of seedlings growing in the same plot was used as indicator of total growth during experiment.

ABIOTIC ENVIRONMENTAL CHARACTERIZATION

Three principal indicators of abiotic environment were selected: light, water, and nutrients availabilities. Light availability was measured by hemispherical photography (Valladares & Guzmán 2006), estimating the global site factor (GSF), which integrate the total amount of light during the whole year, and range from 0 (light absence) to 1 (100% light availability). Soil water content was monthly measured during growing seasons (May to September) by the time domain reflectometry method (TDR-100, Spectrum Technologies Inc., USA). Study plots were sampled by two perpendiculars transects recording the volumetric water content every 0.5m. From these data, we used two variables, soil moisture at emergence (moist spring) as the mean volumetric water content in May, and soil moisture during growing period (moist summer) as the mean volumetric water content from June to September.

Soil nutrient availability was also analyzed for the most important resources: nitrogen (N) and phosphorus (P). In the same plots where seedlings were growing, samples were taken from the upper soil layer (0 to 8 cm) on May and August of 2007 and 2008, coinciding with the moments of higher soil biological activity and higher drought. 15 and 7.5 g of soil samples were extracted for 1 h in agitation with 75ml of 0.5M K_2SO_4 and 0.5M $NaHCO_3$

respectively, and filtered through Whatman GF-D filter. From K_2SO_4 extracts we determined dissolved organic N (DON) with a Shimadzu TOC-V CSH analyzer. Inorganic P was determined in $NaHCO_3$ extracts by the Olsen method (Watanabe & Olsen 1965). Mean values of DON and inorganic P per plot among years and seasons were used as indicators of N and P availability respectively.

DATA ANALYSIS

We used SEM to test the relevance of different abiotic factors on emergence, growth, and survival of woody seedlings (Browne 1982; Hayduk 1987; Shipley 2000). As the sample size is relatively small (71 data in the best case), we followed the recommendations of Tanaka (1987) when designing the model to test. Thus, the number of variables used was limited as much as possible. Our working model proposed that the recruitment probability of each seedling is the result of the probabilities to success in three consecutive processes: seed emergence, seedling growth, and summer survival (Jordano & Herrera 1995). We hypothesized that these processes are controlled by resource availability: light, water, and nutrients, although in a different way (Fig. 1a). Emergence is mainly affected by light conditions and by soil moisture in spring. Growth depends on light and spring soil moisture too, but also on summer soil moisture, and nutrient availability. Finally, survival is controlled by light, summer soil moisture, and nutrient availability. Furthermore, successive processes may be affecting each other, hence, growth is affected by emergence, and survival by emergence and growth. Because soil moisture availability is highly dependent on irradiance, we also include the effect of light on spring and summer soil moisture. Additionally, the correlations among abiotic variables pairs were included in the model: moist spring and moist summer, N and P, light and N, and light and P. The model was calculated separately for the different species and functional groups analyzed: *Q. ilex*, *P. sylvestris*, deciduous tress, mid-successional shrubs, and pioneer shrubs. Due to high mortality in *P. sylvestris* during experiment, growth and related variables were excluded from the model in this case. The relationships included in our model come from a priori knowledge, based in other studies in the same area and with this community species (Castro *et al.* 2005; Gómez-Aparicio *et al.* 2008; Quero *et al.* 2008a; Mendoza *et al.* 2009a; Mendoza *et al.* 2009b).

All variables were assessed for normality prior to statistical analyses. Suitable transformations (log, arcsin) were performed when necessary to improve normality according to Zar (1984). The maximum likelihood method was used to estimate the standardized path coefficients in our model (Shipley 2000). The degree of fit between the covariance in the observed data with that expected if the working model is true was first examined by a goodness-of-fit χ^2. Non-significant χ^2 indicates that the pattern of covariance predicted by the hypothesis is no different from observed data, and thus model could be accepted (Iriondo *et al.* 2003). However, it is generally accepted that the χ^2 test should be interpreted with caution and supplemented with other goodness-of-fit indices (Bollen 1989; Lohelin 1992). Therefore, the Bentler-Bonnet Normed Fit Index (NFI) was used as it gives a measure of the practical fit of the model ignoring the number of subjects (Bentler & Bonnet 1980; Mitchell 1992; Escudero *et al.* 2000). Tanaka (1987) also recommended the simultaneous use of the Goodness-of-Fit Index (GFI) because it is not affected by the methods of estimation (Tanaka & Huba 1985). NFI and GFI range between 0 and 1, and values > 0.9 indicate a good fit of the model to the data (Tanaka 1987; Mulaik *et al.* 1989; Mitchell 1993). Analyses were conducted using AMOS 5.0 (Arbuckle 2003).

RESULTS

Our experiment captured a wide range of the abiotic conditions that affected seedling emergence, growth, and survival (Table 1). The SEM provides a good overall fit for all species and functional groups (Fig. 1). The five path models presented an excellent fit with the observed data, as indicated by their non-significant χ^2 ($P > 0.05$), and by goodness-of-fit indices (NFI and GFI > 0.9 in all cases). Squared multiple correlation estimates for emergence was generally low, ranging from 0.06 for pioneer shrubs to 0.34 for deciduous trees (Fig. 1). Overall, growth was the best fitted demographic stage, with a squared multiple correlation estimates from 0.35 in the case of *Q. ilex* to 0.49 for pioneer shrubs. Finally, survival squared multiple correlation estimates varied from 0.25 in the case of pioneer shrubs to 0.51 for mid-successional shrubs.

Table 1: *Summary statistic of the different abiotic predictors and biotic factors used in the path analysis. Moist in spring and summer are expressed as volumetric water content (%), soil nitrogen and phosphorus concentrations in µg* g^{-1}*, and light availability, measured as GSF, is unitless. Means are given ± SE; N = 72.*

	Mean	Range
Light	0.51 ± 0.03	0.10 - 0.91
Moist spring	23.46 ± 0.41	16.70 - 31.65
Moist summer	8.53 ± 0.28	4.91 - 13.59
Phosphorus	3.01 ± 0.16	1.57 - 11.69
Nitrogen	4.31 ± 0.14	2.52 - 7.30

Overall, seedling emergence was directly affected by light availability and spring soil moisture (Table 2). Light availability negatively affected emergence of all species but *Q. ilex* and pioneer shrubs. Spring soil moisture negatively affected emergence of *Q. ilex* but positively affected that of deciduous trees (Fig. 1b,c). Seedling growth was mainly determined by light availability and summer soil moisture, although other factors such as spring soil moisture or soil nutrients also affected the growth of some species or groups. The effect of light availability and summer soil moisture on growth was positive for all species and groups. Spring soil moisture negatively affected growth of mid-successional shrubs (Fig. 1e), P availability increased deciduous trees growth, whereas N negatively affected the growth of pioneer shrubs. Finally, seedling survival of all species and groups was mainly determined by summer soil moisture. The effect of light availability was positive for all groups but for mid-successional shrubs. Light was the main resource affecting in an indirect way emergence and especially survival, being it effect so strong in some cases to change the sign of direct effect (Table 2). The larger indirect effect of light occurs for emergence of *Q. ilex* and deciduous trees, and for survival of all species. The positive indirect effect of summer soil moisture on mid-successional shrubs survival was reinforcing its positive direct effect.

Successive processes involved in recruitment were not related in the case of tree species, but this was not the case for shrubs. A positive effect of growth on survival was detected for mid-successional shrubs, as well as a positive effect of emergence on growth and survival for pioneers. Among abiotic variables, light was negatively affecting soil moisture, especially during spring, but also in summer for *Q. ilex* and pioneer shrubs. Correlations among abiotic variables were positive in all cases: moist spring and moist summer, N and P, light and N, and light and P.

Table 2: *Standardized direct (DE), indirect (IE), and total effects (TE) of the different variables on seedling emergence, growth, and survival among the different species and functional groups. Significant direct effects are shown in bold type. Dashes indicate that relationships were not included on the model.*

	Emergence			Growth			Survival		
	DE	IE	TE	DE	IE	TE	DE	IE	TE
Quercus ilex									
Moist Spring	**-0.37**	-	-0.37	-0.21	0.04	-0.17	-	0.06	0.06
Moist Summer	-	-	-	**0.24**	-	0.24	**0.46**	-0.05	0.41
Light	-0.07	0.23	0.16	**0.55**	0.03	0.58	0.03	-0.28	-0.25
Phosphorus	-	-	-	-0.07	-	-0.07	0.01	0.01	0.02
Nitrogen	-	-	-	-0.04	-	-0.04	0.06	0.01	0.07
Emergence	-	-	-	-0.11	-	-0.11	-0.06	0.02	-0.04
Growth	-	-	-	-	-	-	-0.20	-	-0.20
Pinus sylvestris									
Moist Spring	-	-	-	-	-	-	-	-	-
Moist Summer	-	-	-	-	-	-	**0.57**	-	0.57
Light	**-0.32**	-	-0.32	-	-	-	0.15	-0.22	-0.07
Phosphorus	-	-	-	-	-	-	-0.13	-	-0.13
Nitrogen	-	-	-	-	-	-	-	-	-
Emergence	-	-	-	-	-	-	0.19	-	0.19
Growth	-	-	-	-	-	-	-	-	-
Deciduous trees									
Moist Spring	**0.26**	-	0.26	-0.09	-0.02	-0.11	-	0.04	0.04
Moist Summer	-	-	-	**0.35**	-	0.35	**0.46**	-0.01	0.45
Light	**-0.41**	-0.13	-0.54	**0.34**	0.06	0.40	0.23	-0.12	0.11
Phosphorus	-	-	-	**0.28**	-	0.28	-0.13	-0.01	-0.14
Nitrogen	-	-	-	-0.12	-	-0.12	-0.11	0.01	-0.10
Emergence	-	-	-	-0.09	-	-0.09	0.15	-	0.15
Growth	-	-	-	-	-	-	-0.01	-	-0.01
Mid-successional shrubs									
Moist Spring	0.05	-	0.05	**-0.29**	-0.01	-0.30	-	-0.11	-0.11
Moist Summer	-	-	-	**0.42**	-	0.42	**0.41**	0.17	0.58
Light	**-0.46**	-0.03	-0.49	**0.49**	0.09	0.58	**-0.48**	0.11	-0.37
Phosphorus	-	-	-	0.08	-	0.08	0.04	0.03	0.07
Nitrogen	-	-	-	-0.03	-	-0.03	-0.09	-0.01	-0.10
Emergence	-	-	-	-0.06	-	-0.06	0.09	-0.02	0.07
Growth	-	-	-	-	-	-	**0.40**	-	0.40
Pioneer shrubs									
Moist Spring	0.13	-	0.13	-0.22	0.03	-0.19	-	-0.01	-0.01
Moist Summer	-	-	-	**0.37**	-	0.37	**0.31**	0.07	0.38
Light	-0.13	-0.08	-0.21	**0.61**	-0.05	0.56	-0.08	-0.06	-0.15
Phosphorus	-	-	-	0.14	-	0.14	-0.02	0.03	0.01
Nitrogen	-	-	-	**-0.23**	-	-0.23	0.03	-0.05	-0.02
Emergence	-	-	-	**0.25**	-	0.25	**0.25**	0.05	0.30
Growth	-	-	-	-	-	-	0.19	-	0.19

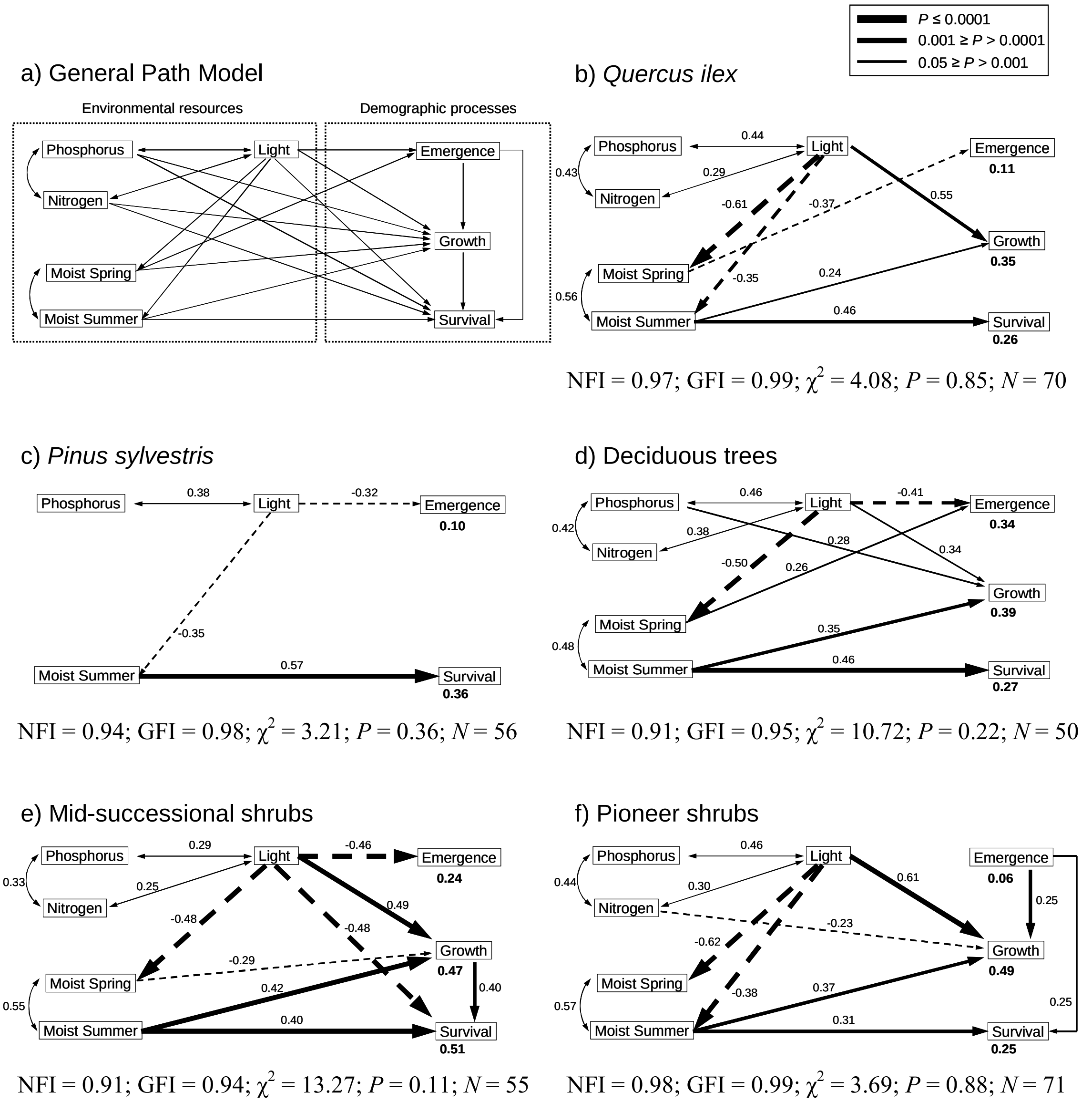


Figure 1: *Path diagram representing hypothesized causal relationships among abiotic predictors and seedling recruitment processes for the different species and functional groups. Positive effects are represented by solid lines, and negatives are indicated by dashed lines, both with standardized regression weights indicated. Squared multiple correlation estimates for demographic stages are indicated below them. Arrow widths are proportional to P-values. Path coefficients non-significantly different from zero are omitted for simplicity. Fit statistics (normal fit index, NFI; goodness of fit index, GFI; χ^2; P-value) and sample size (N) are given at the bottom of each path.*

DISCUSSION

SEM allowed us to disentangle the complex relations among abiotic resources and the early demographic stages that determine plant recruitment. The relative importance of the abiotic factors varied strongly among demographic stages: emergence was mainly determined by a direct light effect, growth by a combination of direct effects of light, summer soil moisture and, in some cases, nutrients, and survival by a direct effect of summer water availability and an indirect light effect. Although this was the general pattern, there were some differences on the intensity of the effect among the species and functional groups that conform the community, which may imply important consequences for the recruitment pattern of the community. These consequences may become even stronger under a global change scenario, where the resource availability pattern is expected to be altered (Houghton *et al*. 2001).

SEEDLING EMERGENCE

At emergence time, light was the main direct factor affecting most groups and species, always in a negative way. The higher emergence in low-light plots with high plant cover is common in dry areas as Mediterranean (Rey & Alcántara 2000; Gómez 2004; Jurado *et al*. 2006; Mendoza *et al*. 2009b), and is likely related to muffled and constant temperatures and soil moisture. However, this relation was non-significant for *Q. ilex* and pioneer shrubs. The heavier mass of *Q. ilex* acorn probably confer some independence from abiotic conditions to emerge (Quero *et al*. 2007), whereas in the case of pioneer shrubs, the lack of a light effect on emergence could be a characteristic of their pioneer status, allowing them to emerge under a wide gradient of light conditions (Gaudio *et al*. 2008). Besides light effect, spring soil moisture was the most important factor determining seedling emergence, but only for some species. We found a contrasted pattern in the case of *Q. ilex* and deciduous trees, where a negative and positive effect of spring soil moisture appeared respectively. The negative effect exerted by soil moisture in *Q. ilex* emergence has been related to waterlogging limitations in other Mediterranean areas with wetter springs (Pérez-Ramos & Marañón 2009), whereas the high water requirements of these deciduous trees (Gómez-Aparicio *et al.* 2006; Quero *et al*. 2008a; Mendoza *et al*. 2009b) may be expressing even during this early life stage. The lack of effect for the rest of species or groups is probably indicating that water is not a limiting resource at emergence time.

SEEDLING GROWTH

Light and summer soil moisture were the main factors directly influencing seedling growth across species or groups. It is commonly accepted that higher light irradiance allows plants to increase net photosynthetic rate (Quero *et al*. 2006) and thus boost total seedling growth (Castro *et al*. 2005; Quero *et al*. 2008b; Gómez-Aparicio *et al*. 2008). Although light was in general an important resource for growth, the magnitude of its effect varied among groups. Pioneer shrubs was the group with the strongest light dependence in terms of growth, being able to greatly increase their biomass in the less covered plots. Deciduous trees, on the other hand, where the group with the weakest response to light. Probably, the higher light requirements and faster growth rate of pioneers (Gaudio *et al*. 2008) allow them to quickly grow when light is not limiting, whereas the lower growth rate of deciduous tree species make them less able to take advantage of higher light availability. The positive relation of summer soil moisture and seedling growth was consistent across species or groups. Water availability during summer is a very important resource, often limiting seedling growth in Mediterranean areas (Castro *et al*. 2005; Quero *et al*. 2008b). In our case, all species gained higher biomass (both aboveground and belowground) under wetter conditions (author’s unpublished data, chapter two of this thesis), increasing both photosynthetic tissue and root mass. Thus, allowing seedlings to increase carbon acquisition and to explore higher volume of soil for water and nutrients in the successive growing seasons (Canadell & Zedler 1995; Lloret *et al*. 1999). Surprisingly, spring soil moisture had no effect on seedling growth. This result suggests that water is not a limiting resource during spring, and it is only in summer when higher water availability translates into higher growth. What is more, spring soil moisture had negative effects on growth of some species such as mid-successional shrubs. Probably, the negative effect of light on spring soil moisture is indirectly beyond this correlation. The effect of nutrients on seedling growth was no as generalized as that of light or water, but they also influenced the growth of some species. Thus, P availability enhanced the growth of deciduous trees, whereas a negative causal relationship was found between N levels and pioneers growth. P has been already identified as one of the most limiting nutrients in Mediterranean ecosystems (Sardans *et al*. 2004) but, at least at this early life stage, only deciduous trees were growth-limited by P. The unexpected negative effect of N on pioneers' growth could be explained by two different reasons: in one hand, a reduction in rhizobial nodulation and nitrogenase activity has been described under experimentally N-enriched soils (Valladares *et al*. 2002), reducing in this way growth of leguminous species (as *C. scoparius*). The other possibility is that seedlings with higher growth demand take higher N from soil, and thus the lower N availability in those plots is the consequence and not the cause.

SEEDLING SURVIVAL

Soil water availability during summer was by far the most important direct factor determining seedling survival during the early life stages. Summer drought has been identified as the main factor constraining woody seedling recruitment on Mediterranean (Rey & Alcántara 2000; Castro *et al*. 2005; Quero *et al*. 2008; Mendoza *et al*. 2009a,b), and our results are in concordance. Although the survival of all species depended on water availability, *P. sylvestris* was the species with the stronger relation, identifying it as the species with higher water requirements from the studied set (Castro *et al*. 2005; Mendoza *et al*. 2009b; chapter 3 of this thesis). On the other side, pioneer shrubs were the less affected group, being the most resistant to dryer conditions in terms of survival. Overall, light did not exert an important direct effect on seedling survival, except for mid-successional shrubs. We could obtain two different conclusions from this result: first, survival is not limited by light on the shadiest plots, because deep shade is not part of the natural gradient of light variation on the study area (10% of full sunlight was the lowest value). Second, higher light availability in open plots did not increased mortality, at least in a direct way. These results provide more arguments to the facilitation/competition theory, since the benefits on seedling survival of the higher soil moisture available under nurse plants (Maestre *et al*. 2001, 2003; Gómez-Aparicio *et al*. 2004) are stronger than the possible competence for light. The lack of relationship between soil nutrients and survival found in this study might be due to the effect of seed reserves, able to support seedling survival during the first growing seasons (Quero *et al*. 2007), whereas higher nutrient availability is necessary to gain biomass on the successive years (Gómez-Aparicio *et al*. 2008; Sardans *et al*. 2008).

Although direct effects were overall more important than indirect ones, in some cases, the indirect influence (especially light) was so relevant to change the sign of the total effect. This was the case of *Q. ilex* emergence, where the negative direct effect of light was compensated by the indirect (and with higher magnitude) positive effect. Despite light was directly affecting in a positive way tree species survival, it was partially cancelled by the negative indirect effect, especially in the case of *Q. ilex* and *P. sylvestris*, where the total effect was negative. These results highlight the importance to explicitly take into account both direct and indirect effects, which may be acting in an opposite way in some cases.

INTER-PROCESSES RELATIONS

Overall, there were no strong relations among the successive processes implied in recruitment. In the case of tree species, successive demographic stages were independent each other, showing no relations among them, although this was not the case for shrubby species. A positive relation between growth and survival was detected for mid-successional shrubs, indicating that seedlings with higher growth have also higher probabilities to survive (Zavala *et al*. 2000; Zavala & Zea 2004). Also in the case of pioneer shrubs positive emergence-growth and emergence-survival relationships appeared, indicating that the best places for emergence are also the best for growth and survival. Thus, no direct indicators of "uncoupling" (*sensu* Jordano & Herrera 1995) among demographic stages appeared for the species conforming this community. However, light exerted an opposite effect among stages. Whereas it had a positive effect on growth, was negatively influencing emergence (deciduous trees and mid-successional shrubs) and survival (mid-successional shrubs). This different requirement along ontogeny (Quero *et al*. 2008a) is only expressed by deciduous species (both trees and shrubs), evidencing the specific light requirements of this life-spam.

CONSEQUENCES UNDER A CLIMATE-CHANGE SCENARIO

Whereas the main factors determining seedling emergence were species-specific (light for some groups or species, spring water availability for others, or none of them for others), there was a common resource for all species with strong implications in growth and survival. We have experimentally demonstrated that water availability during summer is the main factor directly determining seedling growth and survival of the whole woody community. Thus, under a global change scenario, where summer drought is predicted to increase in Mediterranean areas (Houghton *et al*. 2001; Giorgi & Lionello 2007), woody community recruitment would be seriously constrained by both a reduction in seedling growth and survival probability. Overall, all species in the community are prone to reduce their recruitment under drier conditions, although not all of them are affected with the same intensity. A gradient of water dependence is established in the community, indicating *P. sylvestris* as the most drought-intolerant species in terms of recruitment, followed by deciduous trees. On the contrary, shrub species (especially pioneers) are less affected by drier conditions, which together with the strong benefits of light on growth might allow the expansion of their populations to open areas and forest gaps. This alteration on recruitment probabilities of the species from the different functional groups may imply strong consequences for community structure and functioning. A decrease in the recruitment of one of the dominant tree species might induce an important shift in community dominance, favouring the expansion of the other tree species with a higher resistance to drought (as *Q. ilex*) or shrubs. These results strongly support the shrub encroachment trends described for many Mediterranean ecosystems (Scarascia-Mugnozza *et al*. 2000; Acácio *et al*. 2009; Costa *et al*. 2010) by means of the progressive loss of tree species with higher water requirements. This alteration in community structure mediated by abiotic resources may induce changes in specific interactions that need to be explored.

Our experimental approach under field conditions, combined with the robust analytical method using SEM allowed us to identify the most important factors that would be limiting recruitment of the woody community, and how species-specific responses vary along a resource gradient. The results discussed in this study represent another necessary step towards the development of a mechanistic model of Mediterranean woody community dynamics that integrates species-specific responses for the different demographic stages implied in recruitment and that allow the prediction of ecological consequences of resource availability alterations under a global change scenario.

ACKNOWLEDGMENTS

We thank the Consejería de Medio Ambiente (Andalusian Government) and the Direction of the Sierra Nevada National Park for facilities and support to carry out the experiment. We also thank Nacho Villegas, Ramón Ruiz, and Susana Hitos for invaluable field and lab assistance. This study was supported by the coordinated Spanish MEC Project DINAMED (CGL2005-05830-C03) and GESBOME (P06-RNM-1890), and by a grant FPI-MEC (BES-

2006-13562) to L.M. This research is part of the GLOBIMED (www.globimed.net) network in forest ecology.

REFERENCES

Acácio, V., Holmgren, M., Rego, F., Moreira, F., Mohren, G.M.J. 2009. Are drought and wildfires turning Mediterranean cork oak forests into persistent shrublands? *Agroforestry Systems* 76: 389-400.

Allen, C.D., Breshears, D.P. 1998. Drought-induced shift of a forest-woodland ecotone: Rapid landscape response to climate variation. *Proceedings of the National Academy of Sciences* 95: 14839-14842.

Bentler, P.M., Bonnet, D.G. 1980. Significance tests and goodness-of-fit in the analysis of covariance structures. *Psychological Bulletin* 88: 588-606.

Bollen, K.A. 1989. Structural Equations with latent Variables. John Wiley & Sons, New York, USA.

Breshears, D.P., Cobbs, N.S., Rich, P.M., Priece, K.P., Allen, C.D., Balice, R.G., Romme, W.H., Kastens, J.H., Floyd, M.L., Belnap, J., Anderson, J.J., Myers, O.B., Meyer, C.W. 2005. Regional vegetation die-off in response to global-change-type drought. *Proceedings of the National Academy of Sciences* 102: 15144-15148.

Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.L., Cavieres, L.A., Kunstler, G., Liancourt, P., Tielbörger, K., Travis, J.M.J., Anthelme, F., Armas, C., Coll, L., Corcket, E., Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F., Quiroz, C.L., Saccone, P., Schiffers, K., Seifan, M., Touzard, B., Michalet, R. 2008. Facilitation in plant communities: the past, the present and the future. *Journal of Ecology*78: 1958-1965.

Browne, M.W. 1982. Covariance structures. In: D.M. Hawkins (ed.), Topics in applied multivariate analysis. Pp. 72-141. Cambridge University Press, Cambridge, UK.

Canadell, J., Zedler P.H. 1995. Underground structures of woody plants in Mediterranean ecosystems of Australia, California and Chile. In: Arroyo, M.T.K., Zedler, P.H. & Fox, M.D. (eds.), Ecology and Biogeography of Mediterranean ecosystems in Chile, California and Australia. Springer-Verlag, Berlin, pp 177-210.

Carson, W.P., Pickett, S.T.A. 1990. Role of resources and disturbance in the organization of an old field plant community. *Ecology* 71: 226-238.

Castro, J., Zamora, R., Hódar, J.A., Gómez, J.M. 2005. Alleviation of summer drought boosts establishment success of *Pinus sylvestris* in a Mediterranean mountain: an experimental approach. *Plant Ecology* 181: 191-202.

Chase JM & Leibold MA. 2003. Ecological niches: linking classical and contemporary approaches. The University of Chicago Press. Chicago, Estados Unidos.

Clark, J.S., Macklin, E., Wood, L. 1998. Stages and spatial scales of recruitment limitation in southern Appalachian forests. *Ecological Monographs* 68: 213-235.

Costa, A., Pereira, H., Madeira, M. 2010. Analysis of spatial patterns of oak decline in cork oak woodlands in Mediterranean conditions. *Annales of Forest Science* 67: 204p1-204p10.

Escudero, A., Iriondo, J.M., Olano, J.M., Rubio, A., Somolinos, R.C. 2000. Factors affecting establishment of a gypsophyte: the case of *Lepidium subulatum* (Brassicaceae). *American Journal of Botany* 87: 861-871.

Farley, R.A. & Fitter, A.H. 1999. Temporal and spatial variation in soil resources in a deciduous woodland. *Journal of Ecology* 87: 688–696.

Garrido, J.L., Rey, P.J., Herrera, C.M. 2005. Pre- and post-germination determinants of spatial variation in recruitment in the perennial herb *Helleborus foetidus* L. (Ranunculaceae). *Journal of Ecology* 93: 60-66.

Gaudio, N., Balandier, P., Marquier, A. 2008. Light-dependent development of two competitive species (*Rubus idaeus*, *Cytisus scoparius*) colonizing gaps in temperate forest. *Annals of Forest Science* 65: 104p1-104-p5.

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

Gómez-Aparicio, L., Zamora, R., Gómez, J.M. 2005. The regeneration status of the endangered *Acer opalus* subsp. *granatense* throughout its geographical distribution in the Iberian Peninsula. *Biological Conservation* 121: 195-206.

Gómez-Aparicio, L., Pérez-Ramos, I.M., Mendoza, I., Matías, L., Quero, J.L., Castro, J., Zamora, R., Marañón, T. 2008. Oak seedling survival and growth along resource gradients in Mediterranean forests: implications for regeneration under current and future environmental scenarios. *Oikos* 117: 1683-1699.

Grubb, P.J. 1998. A reassessment of the strategies of plants which cope with shortages of resources. *Perspectives in Plant Ecology, Evolution and Systematics* 1: 3-31.

Hampe, A., Arroyo, J. 2002. Recruitment and regeneration in populations of an endangered South Iberian Tertiary relict tree. *Biological Conservation* 107: 236-271.

Harper, J.L. 1977. Population biology of plants. Academic Press.

Houle, G. 1992. Spatial relationships between seed and seedling abundance in a deciduous forest of north-eastern North America. *Journal of Ecology* 80: 99-108.

Houle, G. 1994. Spatiotemporal patterns in the components of regeneration of four sympatric tree spacies, *Acer rubrum*, *A. saccharum*, *Betula alleghaniensis* and *Fagus grandifolia*. *Jounal of Ecology* 82: 39-53.

Hayduck, L.A. 1987. Structural equation modelling with LISREL: essential and advances. John Hopkins University Press, Baltimore, Maryland, USA.

Iriondo, J.M., Albert, M.J., Escudero, A. 2003. Structural equation modelling: an alternative for assessing causal relationships in threatened plant populations. *Biological Conservation* 113: 367-377.

Jensen, K.D., Beier, C., Michelsen, A., Eemmett, B.A. 2003. Effects of experimental drought on microbial processes in two temperate heathlands at contrasting water conditions. *Applied Soil Ecology* 24: 165-176.

Jonasson, S., Michelsen, A., Schmidt, I.K., Nielsen, E.V. 1999. Responses in microbes and plants to changed temperature, nutrient, and light regimes in the arctic. *Ecology* 80: 1828-1843.

Jordano, P., Herrera, C.M. 1995. Shuffling the offspring: Uncoupling and spatial discordante of multiple stages in vertebrate seed dispersal. *Ecoscience* 2: 230-237.

Kitajima, K., Fenner, M. 2000. Ecology of seedling regeneration. In: Fenner, M. (ed.), Seeds: the ecology of regeneration in plant communities. CAB Int., pp.331-359.

Liancourt, P., Callaway, R.M., Michalet, R. 2005. Stress tolerance and competitive-response ability determine the outcome of biotic interactions. *Ecology* 86: 1611-1618.

Lloret, F., Casanovas, C., Peñuelas, J. 1999. Seedling survival of Mediterranean shrubland species in relation to root:shoot ratio, seed size and water and nitrogen use. *Functional Ecology* 13: 210-216.

Lohelin, J.C. 1992. Latent Variable Models. An Introduction to Factor, Path, and Structural Analysis. Lawrence Erlbaum, Hillsdale, New Jersey, USA.

Luzuriaga, A.L., Escudero, A. 2008. What determines emergence and net recruitment in an early succession plant community? Disentangling biotic and abiotic effects. *Journal of Vegetation Science* 19: 445-456.

Lyons, T.M., Barnes, J.D. 1998. Influence of plant age on ozone resistance in *Plantago major*. *New Phytologist* 138: 83-89.

McNickle, G.G., St. Clair, C.C., Cahill, J.F. 2009. Focusing the metaphor: plant root foraging behaviour. *Trends in Ecology and Evolution* 24: 419-426.

Mendoza, I., Gómez-Aparicio, L., Zamora, R., Matías, L. 2009a. Recruitment limitation in forest communities in a degraded Mediterranean landscape. *Journal of Vegetation Science* 20: 367-376.

Mendoza, I., Zamora, R., Castro, J. 2009b. A seeding experiment for testing tree-community recruitment under variable environments: Implication for forest regeneration and conservation in Mediterranean habitats. *Biological Conservation* 142: 1491-1499.

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

Mitchell, R.J. 1992. Testing evolutionary and ecological hypotheses using path analysis and structural equation modeling. *Functional Ecology* 6: 123-129.

Mitchell, R.J. 1993. Path analysis: Pollination. In: S.M. Scheiner & J. Gurevitch (eds.), Design and analysis of ecological experiments, 211-231. Chapman & Hall, New York, USA.

Mulaik, S.A., James, L.R., Van Alstine, J., Bennet, N., Lind, S., Stilwell, C.D. 1989. evaluation of goodness-of-fit indices for structural equation models. *Psychological Bulletin* 105: 430-445.

Parrish, J.A.D., Bazzaz, F.A. 1985. Ontogenetic niche shifts in old-field annuals. *Ecology* 66: 1296-1302.

Peñuelas, J., Boada, M. 2003. A global-change induced biome shift in the Montseny mountains (NE Spain).*Global Change Biology* 9: 131-140.

Pérez-Ramos, I.M., Marañón, T. 2009. Effects of waterlogging on seed germination of three Mediterranean oak species: Ecological implications. *Acta Oecologica* 35: 422-428.

Quero, J.L., Villar, R., Marañón, T., Zamora, R. 2006.Interactions of drought and shade effects on seedlings of four *Quercus* species: physiological and structural leaf responses. *New Phytologist* 170: 819-834.

Quero, J.L., Villar, R.; Marañón, T.; Zamora, R.; Poorter, L. 2007. Seed mass effect in four Mediterranean *Quercus* species (Fagaceae) growing in contrasting light environments. *American Journal of Botany* 94: 1795-1803.

Quero, J.L., Gómez-Aparicio, L., Zamora, R., Maestre, F.T. 2008a. Shifts in the regeneration niche of an endangered tree (*Acer opalus ssp. granatense*) during ontogeny: using an ecological concept for application. *Basic and Applied Ecology* 9: 635-644.

Quero, J.L., Villar, R., Marañón, T., Zamora, R., Vega, D., Sack, L. 2008b .Relating leaf photosynthetic rate to whole-plant growth: drought and shade effects on seedlings of four *Quercus* species. *Functional Plant Biology* 35: 725-737.

Reid, N. 1989. Dispersal of mistletoes by honeyeaters and flowerpeckers: components of seed dispersal quality. *Ecology* 70: 137-145.

Sardans, J., Peñuelas, J. 2007. Drought changes phosphorus and potassium accumulation patterns in an evergreen Mediterranean forest. *Functional Ecology,* 21: 191-201.

Sardans, J., Peñuelas, J., Estiarte, M., Prieto, P. 2008. Warming and drought alter C and N concentration, allocation and accumulation in a Mediterranean shrubland. *Global Change Biology* 14: 2304-2316.

Scarascia-Mugnozza, G., Oswald, H., Piussi, P. 2000. Forests of the Mediterranean region: gaps in knowledge and research needs. *Forest Ecology and Management* 132: 97-109.

Schreeg, L., Kobe, R.K., Walters, M.B. 2005. Tree seedling growth, survival and morphology in response to landscape-level variation in soil resource availability in northern Michigan. *Canadian Journal of Forest Research* 35: 263-273.

Schupp, E.W. 1995. Seed-seedling conflicts, habitat choice, and patterns of plant recruitment. *American Journal of Botany* 82: 399-409.

Shipley, B. 2000. Cause and Correlation in Biology: A User's guide to Path Analysis, Structural Equations, and Causal Inference. Cambridge University Press, Cambridge, UK.

Silvertown, J.W. 1981. Micro-spatial heterogeneity and seedling demography in species-rich grassland. *New Phytologist* 88: 117–128.

Stevens, M.H.H., Shirk, R., Steiner, C.E. 2006. Water and fertilizer have opposite effects on plant species richness in a mesic early successional habitat. *Plant Ecology* 183: 27-34.

Tanaka, J.S. 1987. How big is enough?: sample size and goodness of fit in structural equation models with latent variables. *Child Development* 58: 134-146.

Tanaka, J.S., Huba, G.J. 1985. A fit index for covariance structure models Ander arbitrary GLS estimation. *British Journal of Mathematical and Statistical Psychology* 38: 197-201.

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., Erasmus, B.F.N., Ferreira de Siquiera, M., Grainger, A., Hannah, L., Huges, L., Huntley, B., van Jaarsveld, A.S., Midgley, G.F., Miles, L., Ortega-Huerta, M.A., Peterson, A.T., Phillips, O.L., Williamns, S.E. 2004. Extinction risk from climate change. *Nature* 427: 145-148.

Thuiller, W. 2004. Patterns and uncertainties of species' range shifts under climate change. *Global Change Biology* 10: 2020-2027.

Turner, R.M., Alcorn, S.M., Olin, G. 1969. Mortality of transplanted Saguaro seedlings. *Ecology* 50: 835-844.

Valiente-Vanuet, A., Verdú, M. 2008. Temporal shifts from facilitation to competition occur between closely related taxa. *Journal of Ecology* 96: 489-494.

Valladares, F., Villar-Salvador, P., Domínguez, S., Fernández-Pascual, M., Peñuelas, J.L., Pugnaire, F.I. 2002. Enhancing the early performance of the leguminous shrub *Retama sphaerocarpa* (L.) Boiss.: Fertilisation versus *Rhizobium* inoculation. *Plant and Soil* 240: 253-262.

Watanabe, S., Olsen, R.S. 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. *Soil Sci. Soc. Am. Process* 29: 677-678.

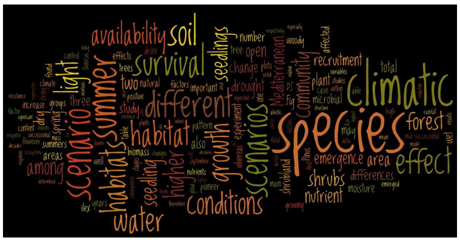
Watkinson, A.R. 1982. Factors affecting the density response of *Vulpia fasciculata*. *Journal of Ecology* 70: 149–161.

Zar, J.H. 1984. Biostatistical analysis, 2nd ed. Prentice-Hall, London, UK.

Zavala, M.A., Espelta, J.M., Retana, J. 2000. Constraints and trade-offs in Mediterranean plant communities: the case of Holm Oak-Aleppo pine forests. *The Botanical Review* 66: 119-149.

Zavala, M.A., Zea, G.E. 2004. Mechanisms maintaining biodiversity in Mediterranean pine-oak forests: insights from a spatial simulation model. *Plant Ecology* 171: 197-207.

DISCUSIÓN GENERAL



DISCUSIÓN GENERAL

A lo largo de la presente memoria de tesis hemos analizado los efectos que los cambios en el patrón de precipitación pueden inducir a diferentes niveles, determinando unas alteraciones en la matiz ambiental, que desencadenan una serie de respuestas en la comunidad vegetal, afectando en última instancia la dinámica y funcionamiento del ecosistema. Estudios previos en la misma zona de estudio sobre los mecanismos y procesos implicados en el reclutamiento indican que tanto la depredación predispersiva de semillas (Castro *et al*. 1999; García *et al*. 2002), como los patrones de dispersión (Mendoza *et al*. 2009a; Matías *et al*. 2010; Zamora *et al*. 2010) o de depredación postdispersiva (Gómez 2004; Matías *et al*. 2009) tienen una elevada estructuración espacial, estando determinados principalmente por el tipo de hábitat. Una vez que la semilla ha sido dispersada con éxito y ha sido capaz de sobrevivir a la depredación, entran en juego otra serie de factores, como la cantidad de precipitación, que pueden interactuar con la estructura del hábitat, determinando el éxito en el establecimiento de nuevos reclutas. Es en estos efectos interactivos entre los diferentes escenarios climáticos y la estructura de hábitat una vez las semillas han sido dispersadas donde nos centramos en esta tesis y sobre lo que a continuación discutiremos.

EFECTOS A NIVEL INDIVIDUAL

El primer proceso que podría verse afectado es la germinación de las semillas. Como en nuestro estudio hemos usado tanto semillas naturales como siembras experimentales en condiciones naturales (*Capítulos 2, 3 y 4*) la tasa de germinación es difícil de evaluar, por lo que usamos la tasa de emergencia de plántulas como un estimador indirecto de la germinación. El hábitat fue el principal factor influyendo en la emergencia, tanto en la densidad de plántulas naturales como en la proporción de plántulas experimentales.

Estos resultados han sido ampliamente discutidos en otros trabajos, y pueden ser explicados por las diferencias en lluvia de semillas (Gómez-Aparicio *et al*. 2007; Mendoza *et al*. 2009a; Matías *et al*. 2010; Zamora *et al*. 2010) y en las condiciones microambientales favorables para la germinación (Callaway 1995; Rey y Alcántara 2000; Castro *et al*. 2002, 2004; Gómez-Aparicio *et al*. 2004; Mendoza *et al*. 2009b), estando determinada principalmente por la luz (*Capítulo 5*) y aumentando en los hábitats más cubiertos. Pero aparte de estas importantes variaciones entre hábitats, en este trabajo hemos demostrado que los diferentes escenarios climáticos pueden tener además un efecto muy importante. Aunque un cambio en el patrón de precipitación no tuvo efectos relevantes sobre la tasa de emergencia de semillas experimentales (*Capítulo 2*), un aumento en la precipitación sí que incrementó la densidad de plántulas naturales emergidas (*Capítulo 4*). Una mayor humedad en el suelo normalmente aumenta la tasa de germinación de las semillas (Borchet *et al*. 1989; Herrera *et al*. 1994). Si además de un año lluvioso contamos con un abundante banco de semillas (Díaz-Villa *et al*. 2003), se puede aumentar de forma importante el banco de plántulas emergidas. Sin embargo, hemos podido comprobar que una disminución de la precipitación no tuvo importantes efectos ni sobre la tasa de emergencia ni sobre la densidad de plántulas emergidas.

El segundo proceso demográfico susceptible de ser alterado por un cambio en el patrón de precipitación es la tasa de crecimiento. Una vez la plántula ha emergido, es necesario ganar biomasa rápidamente para poder desarrollar correctamente el aparato radicular y fotosintético para una correcta adquisición de recursos. La luz y el agua son los dos factores más importantes implicados en el crecimiento de las plantas (Hastwell & Facelli 2003; *Capítulo 5*), por lo que es esperable que varíen a lo largo de nuestra combinación experimental de estos dos factores (hábitat y escenarios climáticos). De forma general, una mayor disponibilidad hídrica supone una mayor biomasa final (tanto aérea como subterránea) para la mayoría de las especies, mientras que las plántulas creciendo bajo un escenario más seco no disminuyen su biomasa de forma significativa respecto a las plantas control (*Capítulo 2*). Estos resultados ponen de manifiesto el elevado estrés hídrico al que están sometidas la mayoría de especies leñosas de la comunidad estudiada, estando limitado su crecimiento por la sequía estival incluso con las condiciones climáticas actuales. Sin embargo, el efecto de los escenarios climáticos está de nuevo modulado por el grado de cobertura vegetal del hábitat. Las zonas más abiertas permiten sacar la máxima ventaja del escenario más húmedo, mientras que las diferencias entre escenarios climáticos en términos de crecimiento se ven amortiguadas al aumentar la cobertura, ya que la falta de luz puede estar limitando parcialmente el crecimiento.

Los veranos excepcionalmente húmedos son un evento de especial importancia implicado en el reclutamiento de las especies leñosas de las montañas mediterráneas. Como hemos visto a lo largo de esta memoria, un aumento en la precipitación durante los meses de verano es capaz de incrementar en gran medida la supervivencia de las plántulas, tanto naturales (*Capítulo 4*) como experimentales (*Capítulo 2*). De hecho, la humedad en el suelo durante los meses de verano es constituye el principal factor determinando la supervivencia de las plántulas de especies leñosas (*Capítulo 5*). Sin embargo, una reducción en la precipitación estival no tiene un efecto tan marcado en la supervivencia de las plántulas experimentales, aunque encontramos una clara tendencia a la reducción en el banco de plántulas naturales, siendo significativo en uno de los años de estudio (*Capítulo 4*). Estos resultados de nuevo ponen de manifiesto las condiciones limitantes en términos de supervivencia de los veranos actuales en la región Mediterránea (García-Fayos *et al*. 2000; Villar-Salvador *et al*. 2004; Castro *et al*. 2005), aunque aún es posible que se vea agravada en las próximas décadas. Como ocurre en el caso del crecimiento, el efecto de los diferentes escenarios climáticos es modulado por las diferencias en cobertura vegetal de los distintos hábitats. Las zonas más abiertas reciben una mayor radiación, lo que además de los problemas de fotoinhibición y sobrecalentamiento (Valladares 2003), supone una mayor evapotranspiración, agravándose los efectos negativos de la sequía. Sin embargo, la supervivencia incrementa en gran medida en los hábitats cubiertos, ya sea por matorrales o por árboles, incluso en el escenario más seco. Por tanto, se pone de manifiesto la importancia del proceso de facilitación (Callaway y Pugnaire 1999; Shumway 2000; Gómez-Aparicio *et al*. 2004) bajo las condiciones climáticas previstas para las próximas décadas.

Ya que la luz es un recurso esencial para el crecimiento (*Capítulo 5*), y que la supervivencia se ve aumentada en las zonas más cubiertas debido al aumento en la humedad del suelo (Quero *et al*. 2010; *Capítulos 2 y 4*), se ha aceptado la existencia un "compromiso[*]" entre crecimiento y supervivencia (Kitajima 1994; Kobe 1999; Sánchez-Gómez *et al*. 2008). Sin embargo, en nuestra aproximación experimental no hemos encontrado indicios de este compromiso en condiciones naturales. Al contrario, en los casos en los que crecimiento y supervivencia han estado relacionados, hemos encontrado una relación positiva (*Capítulo 3*), reforzándose este patrón con el efecto positivo ejercido por el crecimiento sobre la supervivencia de las especies de matorral sucesional (*Capítulo 5*). Según estos resultados, no podemos confirmar esta teoría para las especies mediterráneas (Zavala *et al.* 2000). El vínculo entre crecimiento y supervivencia también de hace patente en la relación entre la resistencia a la sequía en términos de supervivencia (D_S) y algunas variables morfológicas o de distribución de

---

[*] Traducción libre del inglés "*trade-off*"

biomasa (*Capítulo 3*). Así, un mayor crecimiento (biomasa final) o una mayor biomasa foliar por peso de planta (LMR[†]) pueden incrementar la resistencia a la sequía, siendo esta relación especialmente importante en los hábitats menos cubiertos. Además, también encontramos que las especies con una mayor inversión en los órganos de captación de luz respecto a las estructuras para la captación de agua y nutrientes (LARMR[‡]) están menos preparadas para resistir la sequía estival. Como indican estos resultados, una distribución de biomasa que favorezca la captación de agua puede suponer una ventaja competitiva en aquellos ambientes en los que la luz no es un factor limitante para el crecimiento o la supervivencia (Walters y Reich 1996).

Además de los efectos que los cambios en el patrón de precipitación pueden tener sobre la emergencia, crecimiento y supervivencia de las especies leñosas de forma directa, también pueden verse afectadas de forma indirecta a través de las alteraciones en las dinámicas de mineralización y disponibilidad de nutrientes en suelo (Nadelhoffer *et al*. 1991; Jonasson *et al*. 1999; Jensen *et al*. 2003). La comunidad microbiana del suelo es capaz de responder rápidamente a las variaciones de humedad (*Capítulo 1*), lo que tiene consecuencias inmediatas en la disponibilidad de nutrientes para las plantas. Bajo un escenario climático más húmedo, la comunidad de microorganismos del suelo aumenta su biomasa y la captación de nitrógeno y fósforo, aumentándose de este modo la tasa de mineralización y el reciclado de nutrientes, que a medio plazo estarán disponibles para las plantas (Michelsen *et al*. 1999). Por el contrario, un aumento en la aridez reduce la asimilación microbiana de nutrientes, incrementándose su disponibilidad en el suelo. Pero no solo la captación o disponibilidad de nutrientes se ven alterados por las variaciones en la precipitación, la comunidad microbiana también puede ver alterada su estructura. Así, en unas condiciones más húmedas las bacterias

---

[†] Del inglés "*Leaf Mass Ratio*"
[‡] Del inglés "*Leaf Area Root Mass Ratio*"

son las responsables de la mayor parte de la actividad biológica del suelo (determinadas por una baja relación C:N microbiano), mientras que la comunidad de hongos es la dominante en los escenarios más secos (alta relación C:N microbiano; Ross y Sparling 1993; Schiemel *et al*. 2007).

A pesar de estas variaciones en la dinámica del suelo, las plántulas de las especies leñosas no alteran su concentración de nutrientes bajo los diferentes escenarios climáticos. Sin embargo, el mayor crecimiento vegetal bajo el escenario más húmedo supone un mayor contenido total de nutrientes en las plantas. De forma general, la disponibilidad de nutrientes en suelo no juega un papel demasiado importante en el reclutamiento de las especies leñosas, al menos durante los primeros estadíos del ciclo vital (*Capítulo 5*). Sólo la concentración de fósforo tiene un efecto positivo sobre el crecimiento de las especies arbóreas de hoja caduca (*Acer opalus* y *Sorbus aria*). La importancia de este nutriente en los ecosistemas mediterráneos ya ha sido previamente demostrada (Gómez-Aparicio *et al*. 2008; Sardans *et al*. 2008), y esta falta de relación en las primeras fases del ciclo vital puede ser debida al efecto de reserva de las semillas (Quero *et al*. 2007), mientras que es en las fases de juvenil o adulto cuando hay mayores demandas de nutrientes para poder ganar biomasa en los sucesivos períodos de crecimiento.

CONSECUENCIAS A NIVEL DE COMUNIDAD

A pesar de que los efectos anteriormente comentados actuaron de forma general para toda la comunidad de plántulas leñosas, las diferentes especies pueden responder de forma dispar a los cambios en la precipitación o la estructura del hábitat, especialmente las pertenecientes a distintos estadíos sucesionales. Un cambio reclutamiento diferencial entre las especies que cohabitan en un determinado lugar puede implicar una alteración en la abundancia relativa de esas especies, induciendo a acorto-medio plazo variaciones en la estructura de la comunidad.

De forma general, las especies pioneras son las que presentan una mayor resistencia a la sequía, siendo capaces de establecerse con éxito en la mayor parte de los posibles escenarios testados en nuestro experimento, aunque la falta de luz puede llegar a limitar el crecimiento en algunos casos (*Capítulos 2 y 3*). Sin embargo, la elevada respuesta en términos de crecimiento y supervivencia que presentan incluso en las condiciones más extremas (zonas abiertas y veranos secos) puede hacer pensar que este grupo de especies sea capaz de expandir sus poblaciones durante las próximas décadas.

Los matorrales caducos presentan en general una buena resistencia a la sequía, siendo capaces de reclutar nuevos individuos a la población siempre que esté presente algún tipo de cobertura vegetal, ya sea arbórea o arbustiva, que pueda reducir la radiación recibida. Sin embargo, el reclutamiento en las zonas más abiertas se ve seriamente limitado, restringiéndose sólo a los veranos más húmedos. Por tanto, bajo un escenario de cambio climático, podríamos esperar un buen reclutamiento de estas especies tanto en las zonas dominadas por matorrales como en el sotobosque. Sin embargo, para la colonización de nuevas zonas, es necesario el establecimiento previo de matorrales pioneros que puedan crear unas condiciones microclimáticas adecuadas para la supervivencia de las plántulas, es decir un proceso de facilitación por parte de los matorrales pioneros (Gómez-Aparicio *et al*. 2004; Gómez-Aparicio 2009).

Entre las especies arbóreas encontramos una serie de respuestas dispares ante los cambios en el patrón de precipitación. Las dos especies arbóreas dominantes en nuestra comunidad son las que presentan las respuestas más contrastadas. Mientras que *Pinus sylvestris* es la especie con menor resistencia a la sequía y es únicamente capaz de reclutar nuevos individuos durante los veranos más húmedos, *Quercus ilex* es capaz de reclutar en cualquiera de las combinaciones de hábitats y escenarios estudiadas. Estas respuestas tan diferentes pueden ser explicadas por el origen biogeográfico tan dispar de estas dos especies. Mientras que *P. sylvestris* es una especie de origen boreal que quedó acantonada en las montañas del sur de la Península Ibérica durante las glaciaciones del pleistoceno (Castro *et al*. 2004), *Q. ilex* es una especie con un origen típicamente mediterráneo que ha evolucionado en una región donde la sequía estival es un fenómeno frecuente. Estas diferencias en el origen hacen que estas dos especies tengan unos requerimientos ecológicos muy distintos y puedan soportar mejor o peor las condiciones climáticas esperadas para las próximas décadas. De acuerdo a los resultados presentados en la presente memoria, cabría esperar un aumento en la dominancia de *Q. ilex* durante las próximas décadas, que a largo plazo sería capaz de sustituir las zonas actualmente ocupadas por *P. sylvestris*. El reclutamiento de esta última especie podría quedar relegado sólo a aquellos lugares que presenten unas especiales condiciones de humedad, como pueden ser las vaguadas y zonas con menor exposición solar o durante los años excepcionalmente húmedos. Las especies arbóreas de hoja caduca (*A. opalus* y *S. aria*) presentan un comportamiento intermedio respecto a las otras dos especies arbóreas. Aunque se ven enormemente beneficiadas por los veranos excepcionalmente húmedos, son capaces de reclutar nuevos individuos incluso en los veranos más secos, siempre que estén protegidas por cobertura vegetal, especialmente si es bajo matorrales. Es en este hábitat donde estas especies presentan una mayor resistencia a la sequía (*Capítulo 3*) y donde tienen la suficiente luz para el crecimiento (*Capítulo 5*), poniendo nuevamente de manifiesto la importancia de los matorrales como facilitadores para la regeneración de especies arbóreas (Shumway 2000; Gómez-Aparicio *et al*. 2004; Gómez-Aparicio 2009).

Estas diferentes respuestas a la combinación de escenarios climáticos y estructura de hábitat entre las especies leñosas pueden tener como consecuencias finales una alteración en las propiedades de la comunidad, como pueden ser la diversidad, la dominancia de especies o la estructura del hábitat. Aunque la diversidad de las plántulas que emergen de forma natural no se ve reducida bajo un escenario con veranos más secos, tanto la proporción de plántulas que sobreviven al primer verano como la riqueza y diversidad de especies sí que tiende a reducirse. Al contrario, un verano excepcionalmente húmedo es capaz de generar un banco de reclutas abundante y diverso (*Capítulo 4*). La supervivencia durante el primer verano representa el principal cuello de botella para el reclutamiento de las especies leñosas (Castro *et al*. 2005; Mendoza *et al*. 2009b; *Capítulo 4*), por lo que un aumento en la supervivencia durante los primeros estadíos de vida puede suponer una ventana de reclutamiento capaz de mantener las poblaciones, especialmente importante para las especies de vida larga (García *et al*. 2000). Como hemos comentado anteriormente, una de las especies dominantes de la comunidad, *P. sylvestris*, es la más vulnerable al incremento de sequía previsto para las próximas décadas, por lo que una reducción en su reclutamiento podría inducir fácilmente un cambio en la dominancia. Los resultados aportados a lo largo de la presente memoria parecen indicar a *Q. ilex* como la especie que podría sustituir muchas de las zonas actualmente ocupadas por *P. sylvestris*, especialmente aquellas con una mayor insolación o de menor altitud. Las especies de matorral (tanto pioneros como sucesionales) se han presentado como unos buenos competidores ante las condiciones de sequía, por lo que cabría esperar que amplíen sus poblaciones durante los próximos años, es decir, se prevé un proceso de matorralización. En el caso de que sean las zonas actualmente ocupadas por especies arbóreas las que sean sustituidas por especies de porte arbustivo, podríamos hablar de un proceso de matorralización regresiva.

REFERENCIAS

Borchert. M.I., Davis, F.W., Michaelsen, J., Oyler, L.D. 1989. Interaction of factors affecting seedling recruitment of blue oak (*Quercus douglasii*) in California. *Ecology* 70: 389-404.

Callaway, R.M. 1995. Positive interactions among plants. *Botanical Review* 61: 306-349.

Callaway, R. M., and F. I. Pugnaire. 1999. Facilitation in plant communities. Pages 623-648. In: F. I. Pugnaire and F. Valladares, editors. Handbook of functional plant ecology. Marcel Dekker, New York.

Castro, J., Gómez, J.M. García, D., Zamora, R. Hódar, J.A. 1999. Seed predation and dispersal in relict Scots pine forests in southern Spain. *Plant Ecology* 145: 115-123.

Castro, J., Zamora, J., Hódar, J.A. 2002. Use of shrubs as nurse plants: a new technique for reforestation in Mediterranean mountains. *Restoration Ecology* 10: 297-305.

Castro, J., Zamora, J., Hódar, J.A., Gómez, J.M. 2004. Seedling establishment of a boreal tree species (*Pinus sylvestris*) at its southernmost distribution limit: consequences of being in a marginal Mediterranean habitat. *Journal of Ecology* 92: 266-277.

Castro, J., Zamora, R., Hódar, J.A., Gómez, J.M. 2005. Alleviation of summer drought boosts establishment success of *Pinus sylvestris* in a Mediterranean mountain: an experimental approach. *Plant Ecology* 181: 191-202.

Díaz-Villa, M.D., Marañón, T., Arroyo, J., Garrido, B. 2003. Soil seed bank and floristic diversity in a forest-grassland mosaic in southern Spain. *Journal of Vegetation Science* 14: 701-709.

García, D., Zamora, R., Hódar, J.A., Gómez, J.M., Castro, J. 2000. Yew (*Taxus baccata* L.) regeneration is facilitated by fleshy-fruited shrubs in Mediterranean environments. *Biological Conservation* 95: 31-38.

García, D., Zamora, R., Gómez, J.M., Hódar, J.A. 2002. Annual variability in reproduction of *Juniperus communis* L. in a Mediterranean mountain: Relationship to seed predation and weather. *Ecoscience* 9: 251-255.

García-Fayos, P., García-Ventoso, B., Cerdá, A. 2000. Limitations to plant establishment on eroded slopes in south-eastern Spain. *Journal of Vegetation Science* 11: 77-86.

Gómez, J.M. 2004. Bigger is not always better: conflicting selective pressures on seed size on Quercus ilex. *Evolution* 58:71-80.

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

Gómez-Aparicio, L., Gómez, J.M., Zamora, R. 2007. Spatiotemporal patterns of seed dispersal in a wind-dispersed Mediterranean tree (*Acer opalus* subsp. *granatense*): implications for regeneration. *Ecography* 30: 13-22.

Gómez-Aparicio, L., Pérez-Ramos, I.M., Mendoza, I., Matías, L., Quero, J.L., Castro, J., Zamora, R., Marañón, T. 2008. Oak seedling survival and growth along resource gradients in Mediterranean forests: implications for regeneration under current and future environmental scenarios. *Oikos* 117: 1683-1699.

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

Hasstwell, G.T., Facelli, J.M. 2003. Differing effects of shade-induced facilitation on growth and survival during the establishment of a chenopod shrub. *Journal of Ecology* 91: 941-950.

Herrera, C.M., Jordano, P., López-Soria, L., Amat, J.A. 1994. Recruitment of a mast-fruiting bird-dispersed tree: bridging frugivory activity and seedling establishment. *Ecological Monographs* 64: 314-344.

Jensen, K.D., Beier, C., Michelsen, A., Eemmett, B.A. 2003. Effects of experimental drought on microbial processes in two temperate heathlands at contrasting water conditions. *Applied Soil Ecology* 24**:** 165-176.

Jonasson, S., Michelsen, A., Schmidt, I.K., Nielsen, E.V. 1999. Responses in microbes and plants to changed temperature, nutrient, and light regimes in the arctic. *Ecology* 80: 1828-1843.

Kitajima, K. 1994. Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. *Oecologia* 98: 419-428.

Kobe, R.K. 1999. Light gradient partitioning among tropical tree species through differential seedling mortality and growth. *Ecology* 80:187-201.

Matías, L., Mendoza, I., Zamora, R. 2009. Consistent pattern of habitat and species selection by post-dispersal seed predators in a Mediterranean mosaic landscape. *Plant Ecology* 203: 137-147.

Matías, L., Zamora, R., Mendoza, I., Hódar, J.A. 2010. Seed dispersal pattern by large frugivorous mammals in a degraded mosaic landscape. *Restoration Ecology* doi: 10.1111/j.1526-100X.2008.00475.x

Mendoza, I., Gómez-Aparicio, L., Zamora, R., Matías, L. 2009a. Recruitment limitation of forest communities in a degraded Mediterranean landscape. *Journal of Vegetation Science* 20: 367-376.

Mendoza, I., Zamora, R., Castro, J. 2009b. A seeding experiment for testing tree-community recruitment under variable environments: Implication for forest regeneration and conservation in Mediterranean habitats. *Biological Conservation* 142: 1491-1499.

Michelsen, A., Graglia, E., Schimdt, I.K., Jonasson, S., Sleep, D., Quarmby, C. 1999. Differential responses of grass and dwarf shrub to long-term changes in soil microbial biomass C, N and P following factorial addition of NPK fertilizer, fungicide and labile carbon to a heath. *New Phytologist* 143: 523-538.

Nadelhoffer, K.J., Giblin, A.E., Shaver, G.R., Laundre, J.A. 1991. Effects of temperature and substrate quality on element mineralization in six arctic soils. *Ecology* 72: 242-253.

Quero, J.L., Villar, R., Marañón, T., Zamora, R., Poorter, L. 2007. Seed mass effect in four Mediterranean *Quercus* species: physiological and structural leaf responses. *New Phytologist* 170: 819-834.

Quero, J.L., Herrero, A., Zamora, R. 2010. Plant survival in a mosaic landscape: is it determined by spatial or temporal variability? *Basic and Applied Ecology* (En prensa).

Rey, P.J., Alcántara, J.M. 2000. Recruitment dynamics of a fleshy-fruited plant (*Olea europaea*): Connecting patterns of seed dispersal to seedling establishment. *Journal of Ecology* 88: 622-633.

Ross, D.J., Sparling, G.P. 1993. Comparisions of methods to estimate microbial C and N in litter and soil under *Pinus radiata* on coastal sand. *Soil Biology and Biochemistry* 25: 1591-1599.

Sánchez-Gómez, D., Valladares, F., Zavala, M.A. 2006. Performance of seedlings of Mediterranean woody species under experimental gradients of irradiance and water availability: trade-offs and evidence for niche differentiation. *New Phytologist* 170: 795-806.

Sardans, J., Peñuelas, J., Estiarte, M., Prieto, P. 2008. Warming and drought alter C and N concentration, allocation and accumulation in a Mediterranean shrubland. *Global Change Biology* 14: 2304-2316.

Schimel, J., Balser, T. C., Wallenstein, M. 2007. Microbial stress-response physiology and its implications for ecosystem function. *Ecology* 88: 1386-1394.

Shumway, S. W. 2000. Facilitative effects of a sand dune shrub on species growing beneath the shrub canopy. *Oecologia* 124:138-148.

Valladares, F. 2003. Light heterogeneity and plants: from ecophisiology to species coexistence and biodiversity. In: Beyschlag, W. (ed.). Progress in Botany. 64: 439-471. Springer Verlag, Heidelberg.

Villar-Salvador, P., Planelles, R., Oliet, J., Peñuelas-Rubira, J.L. Jacobs, D.F., González, M. 2004. Drought tolerance and transplanting performance of holm oak (*Quercus ilex*) seedlings alter drought hardening in the nursery. *Tree Physiology* 24: 1147-1155.

Walters, M.B., Reich, P.B. 1996. Are shade tolerance, survival and growth linked? Low light and nitrogen effects on hardwood seedlings. *Ecology* 77: 841-853.

Zamora, R., Hódar, J.A., Matías, L., Mendoza, I. 2010. Positive adjacency effects mediated by seed disperser birds in pine plantations. *Ecological Applications* (En prensa).

Zavala, M.A., Espelta, J.M., Retana, J. 2000. Constraints and trade-offs in Mediterranean plant communities: the case of Holm Oak-Aleppo pine forests. *The Botanical Review* 66: 119-149.

La Ciencia tiene las raíces amargas,
pero los frutos muy dulces.
Aristóteles (384 a.C.-322 a.C.)

CONCLUSIONES

CONCLUSIONES

1. Los cambios en la precipitación tienen unas importantes implicaciones en la dinámica de nutrientes. Una mayor humedad en el suelo favorece la captación microbiana, acelerándose el reciclado de nutrientes. Al contrario, una mayor aridez aumenta la disponibilidad de nutrientes en suelo, incrementándose el riesgo de pérdida por lavado o escorrentía.
2. Las plántulas de *Q. ilex* no alteran su concentración de nutrientes bajo los diferentes escenarios climáticos, lo que supone que no pueden aprovechar la mayor disponibilidad de nutrientes del escenario más seco, ni están compitiendo con los microorganismos.
3. Los veranos excepcionalmente lluviosos representan un evento de suma importancia para el reclutamiento de las especies leñosas de las montañas mediterráneas en términos de crecimiento y de supervivencia. Las previsiones de reducción en la frecuencia de aparición de estos veranos húmedos, así como de la mayor frecuencia de veranos secos, puede inducir una pérdida de especies en las montañas mediterráneas, reduciéndose por tanto la diversidad y alterándose la dominancia de la comunidad.
4. Los veranos húmedos suponen además una importante ventaja en términos de crecimiento, aumentando tanto la biomasa aérea como subterránea de las plántulas después de dos estaciones de crecimiento. La falta de diferencias entre el escenario más seco y las condiciones climáticas actuales nos indica que el crecimiento de las especies leñosas durante los primeros estadíos de su ciclo vital está limitado por la sequía estival.

5. Las especies que conforman la comunidad de plantas leñosas del ecosistema estudiado presentan diferente tolerancia a la sequía, siendo los matorrales (especialmente los pioneros) y la encina las especies más tolerantes, es decir, aquellas especies con un origen típicamente mediterráneo.
6. La resistencia a la sequía se ve incrementada en aquellas especies que son capaces de desarrollar una mayor biomasa, o en las que invierten una mayor proporción de biomasa en el aparato radicular que en los órganos fotosintéticos, aunque estas relaciones están moduladas por la estructura del hábitat.
7. No encontramos indicios de un compromiso entre crecimiento y supervivencia en las plántulas de especies leñosas mediterráneas. Las condiciones ambientales que son más propicias para el crecimiento lo son también para la supervivencia.
8. La densidad y diversidad del banco natural de reclutas presenta una elevada dependencia de los eventos climáticos extremos. Aunque los veranos excepcionalmente húmedos son capaces de incrementar en gran medida el reclutamiento de las especies leñosas, los veranos secos que se espera que sean más frecuentes reducen tanto la densidad como la diversidad del banco de reclutas. Este patrón es además consistente entre años y entre los diferentes hábitats.
9. La disponibilidad hídrica durante los meses de verano es el principal factor que controla el crecimiento y la supervivencia de la comunidad de especies leñosas mediterráneas, mientras que la luz tiene un efecto directo positivo sobre el crecimiento. La disponibilidad de nutrientes sin embargo no presenta una importancia relevante durante los primeros estadíos vegetales.

10. Bajo las condiciones climáticas previstas para las próximas décadas, las especies de matorral podrían expandirse y colonizar nuevas áreas, mientras que las zonas ocupadas por *P. sylvestris* podrían reducirse a favor de *Q. ilex* y matorrales.
11. Las diferentes respuestas entre las especies y grupos funcionales a los escenarios climáticos implican un reclutamiento diferencial, lo que puede conllevar a una alteración de la dominancia y la estructura de la comunidad.