Efecto de las sequías extremas en especies arbóreas mediterráneas y su modulación por el microclima y la fragmentación

Extreme drought effects on Mediterranean tree species and its modulation by microclimate and fragmentation

Alicia Forner Sales

Universidad Rey Juan Carlos

Departamento de Biología y Geología, Física y Química Inorgánica

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Dr. Fernando Valladares Ros Profesor de Investigación del Museo Nacional de Ciencias Naturales del Consejo Superior de Investigaciones Científicas y el Dr. Ismael Aranda García del Centro de Investigación Forestal del Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria CERTIFICAN:

Que los trabajos de investigación desarrollados en la memoria de tesis doctoral: "Efecto de las sequías extremas en especies arbóreas mediterráneas y su modulación por el microclima y la fragmentación" han sido realizados bajo su supervisión y son aptos para ser presentados por la Lda. Alicia Forner Sales ante el tribunal que en su día se consigne, para aspirar al Grado de Doctor en Conservación de Recursos Naturales por la Universidad Rey Juan Carlos de Madrid.

VºBº Director de Tesis

Fernando Valladares Ros

VºBº Director de Tesis

Ismael Aranda García

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Director:

Dr. Fernando Valladares Ros

Museo Nacional de Ciencias Naturales – CSIC

Co-director:

Dr. Ismael Aranda García

Centro de Investigación Forestal – INIA

No te rindas, por favor no cedas,

aunque el frío queme,

aunque el miedo muerda,

aunque el sol se esconda y se calle el viento.

Aún hay fuego en tu alma,

aún hay vida en tus sueños.

Porque cada día es un comienzo nuevo,

porque esta es la hora y el mejor momento

porque no estás sola, porque yo te quiero.

MARIO BENEDETTI

ÍNDICE

ABSTRACT

Extreme drought frequency has increased over the last decades in semiarid areas, such as the Mediterranean region. Moreover, water scarcity in summer which historically affects Mediterranean ecosystems is expected to intensify over the next few years due to ongoing climate change. Water stress produced in those ecosystems has favoured coexisting species that have several water regulation strategies as well as ecophysiological mechanisms which allow species to adapt and recover from an increase in the frequency and intensity of disturbances. However, information about the efficacy of those different adaptations and responses to frequent and recurrent droughts is lacking. Besides, the relevance of many factors able to modulate species response to drought; amplifying or attenuating its effects, as well as resilience and resistance processes in the short term, are still unknown. The general objectives of this thesis were: (1) understanding the functional response to drought of coexisting Mediterranean forest species and with contrasting strategies, to cope with extremely dry periods; (2) quantifying the modulation in the response of species to extreme droughts by microclimatic conditions, and (3) assessing the role of forest fragmentation on the ecophysiological response of species to drought.

In order to address these objectives, the main ecophysiological variables as well as the timing and intensity of drought were assessed. Moreover, several tracers of the microclimate and habitat fragmentation were considered as possible factors modulating drought responses in Mediterranean forest species. The study was carried out in a

Mediterranean forest ecosystem where coexisting tree species have different evolution histories and drought tolerances (*Quercus ilex* subsp. *ballota* (Desf) Samp., *Quercus faginea* Lam. y *Pinus nigra* J.F. Arnold subsp. *salzmannii* (Dunal) Franco). Holm oak (*Quercus ilex*), one of the most representative tree species in the Mediterranean ecosystems, was selected to address the fragmentation effect on drought modulation the selected species was

Our results point that the response of Mediterranean forest species to drought events is highly complex, and they differ in their potential ability to cope with extremely dry periods according to species' water regulation strategy. Drought-avoiding species such as *P. nigra* had a certain disadvantage in the carbon balance when events are extreme, which could be accentuated under more frequent extreme events. Microclimate and fragmentation may be significant attenuating factors of extreme droughts and modify the severity of their effects in the study species. Moreover, these factors may facilitate tree recovery after drought events. The timing and intensity of drought, as well as its interaction, were factors that significantly aggravated the impact of water stress on the study species. The timing of the drought was equally or even more important than early drought intensity. Very dry springs or early summers degraded tree functional response, especially in *P. nigra*. Both *Quercus* species were more resistant, but not more resilient, in their response to drought than *P. nigra*. Despite the high drought intensity and obvious symptoms that species were near their tolerance limit, the studied tree species neither declined in growth nor died. This

corroborates the high resilience of Mediterranean ecosystems and point out that climatic dryness has not yet reached irreversible levels for species functionality at our study sites. However, there is a real possibility that repeated extreme droughts modify these patterns and initiate the decline and death of individuals of some species, signaling that the limit for irreversible recovery seems to be not so far away. The weakness of Mediterranean forests may cause a long term change in species abundance patterns, as drought-sensitive species are replaced by drought-tolerant species. In turn, species shift may cause changes in water and carbon balance of the whole forest ecosystem.

Key words: extreme drought, water stress, drought tolerance, isohydric species, anisohydric species, microclimate, forest fragmentation, sap flow, growth, gas exchange, adaptation to drought

RESUMEN

En las últimas décadas se ha incrementado la frecuencia de las sequías extremas en zonas semiáridas, como la región mediterránea. Además, se espera que la escasez hídrica estival a la que se ven históricamente sometidos los ecosistemas mediterráneos se intensifique en los próximos años debido a este cambio climático. El estrés hídrico generado en estos ecosistemas ha propiciado que las especies coexistentes desarrollen diferentes estrategias en la gestión del recurso hídrico así como la puesta en funcionamiento de mecanismos ecofisiológicos que les permitan adaptarse y sobreponerse a unas perturbaciones crecientes en frecuencia e intensidad. Sin embargo, todavía no se dispone de una información completa sobre la efectividad de estas distintas adaptaciones y respuestas a sequías extremas y recurrentes. Además, se desconoce la importancia de muchos de los factores que pueden modular la respuesta a las sequías, amplificando o atenuando los efectos, y de los procesos de resistencia a corto plazo y de resiliencia. Los objetivos generales de esta tesis fueron: (1) comprender la respuesta funcional a la sequía de especies forestales mediterráneas coexistentes y con contrastadas estrategias hídricas, sobre todo en periodos extremadamente secos; (2) cuantificar la modulación de la respuesta a las sequias extremas que ejercen las condiciones microclimáticas, y (3) evaluar el papel de la fragmentación de la masa forestal sobre la respuesta ecofisiológica ante condiciones de sequía.

Para abordar estos objetivos se estudiaron las principales variables ecofisiológicas, se evaluó la intensidad de la sequía y el momento del periodo vegetativo en el que tiene lugar, y se consideraron diferentes descriptores del microclima y de la fragmentación del territorio como posibles factores moduladores de las respuestas de las especies forestales mediterráneas a la sequía. El estudio se llevó a cabo en un ecosistema mediterráneo forestal en el que coexisten especies arbóreas con diferentes historias evolutivas y tolerancias a la sequía (*Quercus ilex* subsp. *ballota* (Desf) Samp., *Quercus faginea* Lam. y *Pinus nigra* J.F. Arnold subsp. *salzmannii* (Dunal) Franco). Para abordar el efecto de la fragmentación en la modulación de la sequía se seleccionó la encina (*Quercus ilex*), una de las especies arbóreas más representativas de los ecosistemas mediterráneos.

Nuestros resultados señalan la elevada complejidad de la respuesta de las especies forestales mediterráneas a los eventos de sequía, así como una diferente capacidad potencial de afrontar periodos secos extremos según la estrategia hídrica propia de cada especie. Los resultados muestran una cierta desventaja en el balance de carbono por parte de especies evitadoras de la sequía como *P. nigra* a la hora de afrontar los eventos extremos, lo cual podría acentuarse bajo una mayor frecuencia de estos eventos. Hemos encontrado que el microclima y la fragmentación pueden ser factores atenuantes significativos de las sequías extremas y de la gravedad de sus efectos sobre las especies estudiadas, así como elementos que facilitan la recuperación del arbolado tras los eventos de sequía. El momento del periodo vegetativo

en el que tiene lugar la sequía y su intensidad, así como su interacción, fueron factores que llegaron a agravar significativamente el impacto del estrés hídrico sobre las especies estudiadas, llegando a tener el momento del periodo vegetativo en el que acontece la falta de agua una relevancia análoga o incluso superior a la propia intensidad de la sequía. En este sentido la presencia de primaveras y comienzos de verano muy secos, impactaron de manera muy negativa en la respuesta funcional del arbolado, y de manera especial en *P. nigra*. Las dos especies de *Quercus* se mostraron más resistentes, pero no más resilientes, en sus respuestas a la sequía que *P. nigra*. A pesar de la intensidad de las sequías acaecidas y de los claros síntomas de las especies de encontrarse cerca de su límite de tolerancia, el arbolado no mostró signos de decaimiento general sostenido ni hubo mortandad en ninguna de las tres especies. Esto corrobora la elevada resiliencia de los ecosistemas mediterráneos y señala que la aridificación climática aún no ha alcanzado niveles irreversibles para la funcionalidad de las especies y localidades estudiadas. No obstante, también pone de manifiesto la posibilidad real de que repetidas sequías extremas puedan modificar esta pauta, y dar lugar al inicio del decaimiento y muerte de los ejemplares de alguna de las especies ya que el umbral de no recuperación no parece encontrarse muy lejos de los niveles observados en las sequias extremas que ya han tenido lugar en la zona de estudio. Este debilitamiento del bosque mediterráneo se podría traducir a largo plazo en cambios en los patrones de abundancia de las especies más sensibles frente a la sequía por otras más tolerantes, lo cual podría

derivar a su vez en modificaciones del balance hídrico y de carbono de todo el ecosistema forestal.

Palabras clave: sequía extrema, estrés hídrico, tolerancia a la sequía, especies isohídricas, especies anisohídricas, microclima, fragmentación del bosque, flujo de savia, crecimiento, intercambio gaseoso, adaptación a la sequía

CAPÍTULO 1

Resumen

Imagen de la página anterior: individuos secos de *P. nigra* en el Parque Natural del Alto Tajo.
Author: Alicia Forner.

ANTECEDENTES

Funcionalidad de los ecosistemas y servicios ecosistémicos asociados: el agua como recurso clave en ecosistemas semiáridos

La dinámica de los ecosistemas está compuesta por un conjunto de procesos interrelacionados y continuos que incluyen principalmente la entrada de energía y la circulación tanto de elementos esenciales para vida (ej. carbono o nitrógeno) como de agua (Birot y Vallejo 2011). En los ecosistemas semiáridos, como el mediterráneo, el agua es un recurso limitante, por lo que ésta adquiere una mayor importancia en el mantenimiento de estos procesos, y por lo tanto, en el funcionamiento de los propios ecosistemas, y en los servicios ecosistémicos que prestan. De hecho, el agua tiene múltiples funciones en el ecosistema, como son el sustento de la actividad fotosintética, el transporte de nutrientes y su reciclado, la meteorización de las rocas o la formación del suelo, entre otros. Además, el agua es utilizada por las plantas para cubrir la demanda atmosférica, la cual es elevada en ecosistemas semiáridos, y así permitir el compromiso entre fijación de CO_2 y transpiración de agua a través de los estomas (Birot y Vallejo 2011).

Las funciones de los ecosistemas son todos aquellos aspectos de la estructura y el funcionamiento de los ecosistemas con capacidad de generar servicios que satisfagan necesidades humanas de forma directa o indirecta (de Groot 1992). Las principales funciones de los ecosistemas pueden clasificarse en cinco grupos: funciones de regulación, de hábitat, de reproducción, de información y de sustento (Gómez-Baggethun y de

Groot 2007). Muchas de estas funciones están relacionadas directa o indirectamente con el agua, por lo que su mantenimiento es especialmente relevante para garantizar los servicios que prestan los ecosistemas semiáridos (Tabla 1).

Dada la escasez hídrica de los ecosistemas áridos, principalmente durante el verano, se puede concluir que el agua tiene un papel crucial en el mantenimiento de los servicios ecosistémicos generados por los ecosistemas mediterráneos.

Tabla 1. Funciones, bienes y servicios del ecosistema relacionados directa (negrita) o indirectamente (cursiva) con el agua. Fuente: modificado de Gómez-Baggethun y de Groot 2007, y de Birot y Vallejo 2011.

Grupo de funciones	Funciones	Componentes y procesos de los ecosistemas	Ejemplos de bienes y servicios
Funciones de regulación	**Regulación atmosférica**	Mantenimiento de los ciclos biogeoquímicos (equilibrio CO_2/O_2, capa de ozono, etc.)	- Protección del ozono frente rayos UVA y prevención de enfermedades - Mantenimiento calidad del aire - Influencia del clima
	Regulación climática	Influencia sobre el clima ejercida por coberturas de suelo y procesos biológicos (ej. producción de dimetilsulfato)	- Mantenimiento de un clima adecuado (temperatura, precipitaciones) para la salud, la agricultura, etc.
	Amortiguación de perturbaciones	Influencia de las estructuras ecológicas en la amortiguación de perturbaciones naturales	- Protección frente a tormentas (ej. arrecifes de coral) o inundaciones (ej. bosques y marismas)
	Regulación hídrica	Papel de la cobertura del suelo en la regulación de la escorrentía mediante las cuencas de drenaje	- Drenaje e irrigación natural
	Disponibilidad hídrica	Percolación, filtrado y retención de agua dulce	- Disponibilidad de agua para usos consuntivos (bebida, riego, industria)

Grupo de funciones	Funciones	Componentes y procesos de los ecosistemas	Ejemplos de bienes y servicios
	Sujeción del suelo	Papel de las raíces de la vegetación y fauna edáfica en la retención del suelo	- Mantenimiento de zonas roturadas - Prevención de la erosión - Control del balance sedimentario
	Formación del suelo	Meteorización de la roca madre y acumulación de materia orgánica	- Mantenimiento de la productividad de los cultivos - Mantenimiento de la productividad natural de los suelos
	Regulación de nutrientes	Papel de la biodiversidad en el almacenamiento y reciclado de nutrientes (ej. N, P y S)	- Mantenimiento de la salud del suelo y de los ecosistemas productivos
Funciones de producción	*Comida*	Conversión de energía solar en animales y plantas comestibles	- Caza, recolección, pesca - Acuicultura y agricultura de subsistencia y pequeña escala
	Materias primas	Conversión de energía solar en biomasa para construcción y otros usos	- Material para construcciones y manufacturas - Combustibles y energía - Piensos y fertilizantes naturales
	Recursos genéticos	Material genético y evolución en animales y plantas silvestres	- Mejora de los cultivos frente a pestes y agentes patógenos - Otras aplicaciones (ej. salud)
	Recursos medicinales	Sustancias bio-geoquímicas	- Medicinas y otras drogas - Modelo y herramientas químicas
Funciones de sustrato	Vivienda	Provisión de un sustrato adecuado para el desarrollo de actividades e infraestructuras humanas	- Espacio para vivir, ya sea en pequeños asentamientos o en ciudades
	Agricultura		- Comida y materias primas provenientes de cultivos agrícolas y acuícolas
	Conversión energética	Dependiendo del uso específico del suelo, se requerirán distintas cualidades ambientales (ej. estabilidad del suelo, fertilidad, clima, etc.)	- Energías renovables como la eólica, la solar o la hidráulica

Sequías extremas y efectos observados en especies arbóreas

Desde los años 70 se ha observado en los ecosistemas mediterráneos un incremento en la temperatura y disminución de las precipitaciones asociado al cambio climático (Bladé y Castro-Díez 2010). Este incremento de la aridez ha sido especialmente intenso en los meses de primavera y verano (Christensen et al. 2007). Este hecho es de gran importancia, ya que es en primavera cuando la mayor parte de las especies mediterráneas adquieren los recursos para su crecimiento y reproducción (Mitrakos 1980). De hecho, se espera que en 2100 el cambio climático y sus impactos sean los principales causantes de la pérdida de biodiversidad y del cambio de los servicios ecosistémicos a escala mundial (Valladares et al. 2009).

El cambio climático también implica un aumento de la variabilidad climática, intra e interanual y una mayor frecuencia e intensidad de los eventos climáticos extremos (Christensen y Christensen 2003). Estos eventos son perturbaciones estocásticas que tienen el potencial de alterar la estructura y funcionamiento de los ecosistema, y por lo tanto, de los servicios ecosistémicos que prestan (Beniston 2007; Thibault y Brown 2008). Entre los eventos extremos se encuentran las sequías extremas, las cuales han incrementado en la región mediterránea desde 1880, y especialmente desde las últimas décadas (Della-Marta et al. 2007; Sánchez-Salguero et al. 2015). Además, se espera que aumente su frecuencia, intensidad y duración en las próximas décadas (IPCC 2014). Este incremento no solo va a afectar a la fisiología y desarrollo de los organismos vivos, sino que también va a

limitar su capacidad de respuesta y aclimatación a eventos futuros (Gutschik y BassiriRad 2003).

Los efectos de las sequías extremas son difíciles de cuantificar y explorar debido a su naturaleza estocástica y a la dificultad de llevar a cabo estudios sólidos que requieren una alta repetitividad, control de las variables y replicación (Valladares et al. 2009). La gran cantidad de estudios científicos en los que se evalúa el efecto de las sequías en especies arbóreas, ha permitido tener una base científica para identificar cuáles podrían ser intensificados bajo condiciones extremas en los bosques mediterráneos. Sin embargo, todavía se desconocen muchas de las implicaciones para las especies arbóreas mediterráneas, así como los efectos derivados de su interacción con otros elementos perturbadores de los ecosistemas, como es la fragmentación de los bosques.

Bajo condiciones de sequía extrema, las especies arbóreas ven muy limitada su actividad fotosintética, debido al temprano y, en algunas especies, casi completo cierre estomático para evitar pérdidas de agua. A ello, también contribuye la disminución de la actividad y eficiencia de la enzima rubisco, implicada en la fijación de carbono (Farooq et al. 2009). Es por ello, bajo condiciones de estrés hídrico las plantas disminuyen su crecimiento. Se espera que otros efectos de las sequías observados en las especies arbóreas ocurran también bajo condiciones extremas, pero intensificados. Sin embargo, los estudios donde se han demostrado los efectos de las sequías extremas en árboles mediterráneos en condiciones naturales son escasos. En la Tabla

2 se recogen algunos de éstos efectos observados en los bosques de la Península Ibérica.

Tabla 2. Ejemplos observados de efectos de las sequías extremas en bosques de la Península Ibérica.

Efecto	Especie	Referencia
Disminución del crecimiento Disminución importante de la conductividad hidráulica	*Pinus sylvestris*	Martínez-Vilalta and Piñol 2002
Gran mortalidad	*Pinus sylvestris*	Martínez-Vilalta and Piñol 2002
Pérdida prematura de hojas	*Pinus sylvestris*	Poyatos et al. 2008
Recuperación del grado de daño previo a la sequía más lenta (recuperación completa tras años húmedos en el 67 % de los lugares) Extenso daño	En 24 especies Especies del centro y sur de España	Peñuelas et al. 2001
Defoliación y decaimiento Descenso $\Delta^{13}C$ y $\delta^{15}N$ (recuperación lenta 3 años después)	*Quercus ilex*	Peñuelas et al. 2000
Extensa mortalidad	*Quercus ilex*	Lloret y Siscart 1995
Menor albura Albura con menor concentración de azúcares solubles	*Abies alba*, Pinus *sylvestris, Pinus halepensis*	Camarero et al. 2012
Disminución de la madera tardía Recuperación lenta (2 años después)	*Quercus faginea*, *Quercus ilex*, *Pinus nigra* *Quercus faginea*	Granda et al. 2013
Descenso agudo del crecimiento Incremento de la eficiencia en el uso del agua	*Quercus faginea*, *Pinus nigra*	Granda et al. 2014
Menor crecimiento Decaimiento y mortalidad	*Pinus sylvestris*	Heres et al. 2012
Menor incremento en la eficiencia en el uso del agua intrínseca en árboles con signos de decaimiento	*Pinus sylvestris*	Heres et al. 2012

Como consecuencia de las sequías extremas, algunas especies alcanzan potenciales hídricos muy bajos, los cuales van asociados a pérdidas de la conductancia hidráulica (Martínez-Vilalta et al. 2002). En estas condiciones, las especies se aproximan a su límite de tolerancia (ej. Carevic et al. 2014), llegando incluso a sobrepasarlo y mostrando, como consecuencia, síntomas de fallo hidráulico (Urli et al. 2013) y de cavitación de los tejidos conductores. La cavitación de los tejidos se produce a un determinado umbral de potencial hídrico, el cual depende de la especie y de las condiciones ambientales a las que esté adaptada la especie. Probablemente, esta sea una de las principales causas del incremento de las tasas de defoliación observado en los bosques mediterráneos durante las últimas dos décadas (Carnicer et al. 2011). Las altas tasas de defoliación han generado un incremento de la mortalidad de muchas especies mediterráneas como la encina (*Quercus ilex)* (Tabla 2) (Peñuelas et al. 2000; Camarero et al. 2004). Estos episodios de decaimiento y mortalidad también se han observado en otros ecosistemas, especialmente durante la última década (Breshears et al. 2008, Allen et al. 2010, McDowell 2011). A pesar de estos estudios sobre los intensos impactos derivados de las sequías extremas, hay que destacar que los ecosistemas mediterráneos tienen una alta estabilidad, es decir, una alta resistencia y alta resiliencia a las perturbaciones (Lloret et al. 2012), lo cual les permite recuperarse, en mayor o menor medida, tras la sequía. Aun así, se espera que la respuesta sea específica de cada especie, ya que las éstas presentan diferente grado de tolerancia a las sequías. A largo plazo, la ventaja de unas especies frente a otras a la hora de afrontar las sequías extremas, podría conllevar desequilibrios en

la coexistencia de las especies, y por lo tanto, cambios en su distribución y en su dominancia dentro del bosque. De hecho, un ejemplo sería el reemplazo de *Fagus sylvatica* y *Calluna vulgaris* por *Quercus ilex* en algunas poblaciones de Cataluña (Peñuelas and Boada 2003).

Mecanismos y adaptaciones de las especies arbóreas a la sequía

Las especies de climas semiáridos, como el Mediterráneo, han desarrollado una gran variedad de mecanismos y adaptaciones que les permiten afrontar las sequías. Frente a ello, las especies pueden presentar dos tipos de estrategias, tolerantes o evitadoras. En realidad, esta clasificación es considerada más bien como todo un rango de variación entre la tolerancia y la evitación de la sequía (Franks et al. 2007; McDowell et al. 2013). Las estrategias evitadoras o isohídricas se caracterizan por presentar un control estomático efectivo, el cual les permite cerrar estomas bajo condiciones de sequía. Sin embargo, al evitar las pérdidas de agua también limitan la asimilación de carbono y aumentan la probabilidad de inanición por falta de carbono (Galiano et al. 2011). Por el contrario, las especies tolerantes o anisohídricas pueden mantener los estomas abiertos durante la sequía, soportando potenciales hídricos muy bajos, pero arriesgando la funcionalidad de su sistema hidráulico (Urli et al. 2013). Sin embargo, el mantenimiento de un balance de carbono positivo incrementa el riesgo de pérdida de funcionalidad del sistema hidráulico y de cavitación (McDowell 2011). Hay que destacar, que tanto las especies evitadoras como las tolerantes

pueden presentar fallo hidráulico o inanición por falta de carbono. Sin embargo, las especies evitadoras probablemente mantendrán su potencial por encima del umbral en el que se produce el fallo hidráulico. Si la sequía es muy intensa, es probable se rebase ese umbral antes de llegar a la inanición por falta de carbono (McDowell et al. 2008). En general, las especies tolerantes tienden a ocupar los ecosistemas que son más propensos a la sequía, y presentan un xilema más resistente a potenciales hídricos más negativos (McDowell et al. 2008).

Algunas de las adaptaciones a la condiciones de sequía son el desarrollo de hojas más duras y gruesas, es decir, más esclerófilas (Salleo and Lo Gullo 1990; Salleo et al. 1997), el incremento del ratio raíz/tallo (Peña-Rojas et al. 2005) y el desarrollo de sistemas radiculares más profundos (David et al. 2007). Además, también son adaptaciones a la sequía el incremento en la eficiencia en el uso del agua (Gulías et al. 2003; Medrano et al. 2009) y la osmoregulación, la cual permite mantener la turgencia de las células a través de la acumulación de solutos (Meinzer et al. 2014). Valladares et al. (2004) clasificó los principales mecanismos de adaptación a la sequía según si permitiesen la tolerancia o la evitación del déficit hídrico (Tabla 3).

Tabla 3. Principales mecanismos de respuesta al estrés hídrico: mecanismos de tolerancia (T) y mecanismos de evitación (E). RWC: contenido hídrico relativo. SLW: peso específico foliar. Ψ: potencial hídrico. Fuente: modificado de Valladares et al. 2004.

Rasgo	Estrategia
Hojas	
Mantenimiento de Ψ alto	E
Mantenimiento de Ψ bajo	T
Cierre de estomas a RWC alto	E
Cierre de estomas a RWC bajo	T
Fuerte cierre de estomas al mediodía	E
Baja absorción de la radiación	E
Alta capacidad foliar	E
Baja conductancia cuticular	E
Alto SLW	E
Poca cantidad de hojas	E
Abscisión foliar durante el estrés	E
Raíces	
Raíces profundas	E
Gran densidad de raíces	E
Alta superficie de absorción en relación con baja superficie transpirante	E
Raíces suberificadas	E
Alta relación biomasa radical/biomasa aérea	E
Sistema radical dual	E
Alto peso específico radical	E
Alta conductividad hidráulica	E
Anatomía/Metabolismo	
Tejidos sensibles a deshidratación (RWC letal > 50%)	E
Tejidos resistentes a deshidratación (RWC letal < 25%)	T
Resistente a la cavitación del xilema	T
Pequeño tamaño celular	T

Rasgo	Estrategia
Ajuste osmótico	T
Alta elasticidad pared celular	T
Capacidad de rebrote	T
Resistencia a la disfuncionalidad de membrana celular	T
Acumulación substancias osmoprotectoras (prolina, betaina…)	T
Sistemas antioxidantes	T
Estabilidad pigmentos fotosintéticos	T
Alta fotoinhibición	T

En condiciones de sequía extrema, las especies van a ser más propensas a presentar pérdidas de la conductividad hidráulica y cavitación debido al descenso del potencial hídrico por debajo del umbral de fallo hidráulico. Por otra parte, se espera que el aumento en la duración de las sequías extremas aumente la probabilidad de morir por inanición, especialmente en las especies evitadoras. Esto se debe a que el cierre de los estomas evitaría la fijación de carbono durante un largo periodo de tiempo superior al del consumo de sus propias reservas de carbono. Por lo tanto, es difícil predecir si las especies mediterráneas van a poder superar los efectos de las cada vez más frecuentes e intensas sequías sin verse afectadas por el decaimiento o la mortalidad.

Modulación de la sequía

Los efectos de las sequías en las especies vegetales pueden ser modulados por las propias características de la sequía o por otros

factores a nivel de microescala, es decir, locales. A su vez, los factores locales pueden estar relacionados con el clima o bien con las características físicas intrínsecas del lugar donde se encuentran las especies.

Entre las características de la sequía que pueden ser consideradas como moduladoras de sus efectos en las especies vegetales se encuentran la escala temporal, la intensidad y la duración de la sequía. La escala a la que se producen las sequías es muy relevante, ya que no va tener el mismo efecto una sequía anual (de media un año sea seco), que una sequía estacional (ej. sequías estivales o invernales), o que sequías a escalas más pequeñas de meses o incluso semanas. Una sequía anual implica, generalmente, que la especie se ha sometido durante más tiempo a condiciones de escasez hídrica, por lo que en un principio tendría un efecto mayor en especies vegetales que otra de escala menor como la estacional. Sin embargo, esto es mucho más complejo, ya que una sequía más severa a menor escala, como las observadas en algunos veranos muy secos, puede ejercer un mayor efecto en las especies que una sequía anual moderada. Además, un año puede ser considerado como seco debido a que todos sus meses han sido secos o debido a que solo algunos meses han sido muy secos y han incrementado la media. También hay que tener en cuenta el momento en el que se produce la sequía, ya las especies presentan su mayor actividad fotosintética, y por lo tanto, mayor crecimiento en primavera cuando las condiciones climáticas son óptimas. Por ejemplo, una sequía intensa en primavera podría tener graves consecuencias para las

especies caducifolias, ya que es la época del año en el que desarrollan sus hojas.

En general, sequías más cortas e intensas producirán mayores efectos que sequías prolongadas y suaves. Por lo tanto, a la hora de estudiar los efectos de la sequía es crucial identificar su escala, intensidad y duración.

El clima a nivel local o microclima también puede modular los efectos de una sequía acaecida a una escala más amplia como la regional. Un ejemplo podría ser las especies que se encuentran en laderas de umbría en zonas semiáridas, ya que las condiciones más húmedas de la ladera pueden amortiguar los efectos de un clima más cálido y seco a nivel regional. Por lo tanto, las condiciones intrínsecas del lugar también tienen un papel importante en la modulación de los efectos de la sequía en las especies vegetales. En este sentido, las condiciones del suelo como la cantidad de materia orgánica, la pedregosidad, las características biogeoquímicas o la estructura pueden favorecer la retención de agua y de nutrientes favoreciendo así la disminución del estrés hídrico (Pugnaire et al. 2004; Flores-Rentería et al. 2015).

Otro factor modulador de la sequía puede ser la composición de las especies y la estructura de la masa vegetal. En este sentido, una composición de especies determinada puede favorecer la competencia por los recursos hídricos y por lo tanto, condicionar la disponibilidad de agua del suelo, especialmente durante épocas de sequía (Grossiord et

al. 2014). Así mismo, masas vegetales más densas generarán una mayor competencia por los recursos hídricos, aumentando los efectos de la sequía (Vericat y Piqué 2012).

Dada la cantidad de los factores que pueden modular los efectos de las sequías en las especies vegetales y la complejidad que supone su combinación, es esencial considerar el mayor número posible de factores a la hora de evaluar éstos efectos.

Fragmentación del hábitat

La fragmentación del hábitat es considerada, junto con el incremento de la aridez producida por el cambio climático, uno de los principales motores del cambio global en la región mediterránea (Matesanz y Valladares 2014). La fragmentación del bosque mediterráneo debido a causas humanas ha sido considerada como la más importante debido a su rapidez e intensidad (Alados et al. 2004; Blondel 2010). Además, se espera que incremente en los próximos años (Millennium-Ecosystem-Assessment 2005).

La fragmentación del hábitat ha sido asociada a la reducción de hábitat, al incremento del aislamiento de las poblaciones, a la pérdida de vegetación nativa y a la alteración física del medio (Lienert 2004, Valladares et al. 2014a). Los impactos de la fragmentación del hábitat en la composición y funcionamiento de las comunidades dependerán de las características de los fragmentos resultantes, como el tamaño o la

conectividad con otros fragmentos cercanos, y de las condiciones bióticas y abióticas de los fragmentos resultantes y de la matriz circundante. A menor tamaño de fragmento mayor será el efecto de la matriz circundante y de las condiciones ambientales, ya que aumentará el efecto borde (Fernández et al. 2002). Por ello, las especies de los fragmentos pequeños se verán sometidas a un incremento de la insolación, la variación térmica y la exposición al viento, lo cual tendrá efectos en la humedad y temperatura del aire, incrementando la evapotranspiración de las plantas (Saunders et al. 1991).

Uno de los principales, y más estudiados, efectos de la fragmentación del hábitat es la pérdida de biodiversidad (Fahrig 2003; Fischer and Lindenmayer 2007), la cual a su vez, amenaza la provisión de servicios de los ecosistemas (Meli et al. 2014). Numerosos estudios han observado efectos negativos derivados de la fragmentación del hábitat en la dispersión de semillas, en las interacciones entre plantas y animales, en la diversidad genética y en la estructura genética de las poblaciones (ej. Santos y Tellería 1997; Aparicio et al. 2012; Ortego et al. 2014; Morán-López et al. 2015). Sin embargo, en la sociedad actual existe la idea preconcebida de que la fragmentación es un proceso que solo genera efectos negativos en el hábitat, lo cual no es exactamente cierto. De hecho, se han observado algunos efectos positivos en la producción de semillas, en la disponibilidad de nutrientes en el suelo y en la retención de agua en el suelo (Morán-López et al. 2016; Valladares et al. 2014b). Por lo tanto, el efecto de la fragmentación es difícil de

determinar y va a depender de la especie y de las características climáticas de la región.

Respecto a la interacción entre la fragmentación y el cambio climático, se espera que los hábitats fragmentados sean más vulnerables a las sequías y otros eventos climáticos, reduciendo además su capacidad de resiliencia (Travis 2003; Opdam y Wascher 2004). Bajo frecuentes e intensas sequías, una menor capacidad de resiliencia de las especies puede conllevar a largo plazo su decaimiento y posterior muerte (Lloret et al. 2004; Breda et al. 2006). Se espera que los árboles de bosques fragmentados sean más vulnerables a las sequías estivales debido a un incremento de las pérdidas de agua por evapotranspiración como consecuencia de un aumento del efecto de la radiación solar en la copa. Sin embargo, los bordes de los fragmentos de bosque reducirían la competencia intraespecífica, generando una mayor disponibilidad hídrica y una reducción del estrés hídrico de los árboles.

Esta complejidad en la respuesta de las especies, tanto a las sequías como a la fragmentación y a su interacción, hace especialmente difícil predecir cuáles van a ser los efectos a corto plazo y largo plazo en las especies arbóreas.

Prioridades ante escenarios de cambio global

A pesar de los numerosos estudios llevados a cabo sobre el efecto de las sequías en las especies vegetales de climas semiáridos, todavía se siguen encontrando nuevos resultados. El incremento en la intensidad y frecuencia de las sequías acaecido durante las últimas décadas ha

abierto nuevos nichos de investigación y generado nuevas e interesantes preguntas, pero a la vez muy complejas de responder.

Las nuevas investigaciones a desarrollar requieren de estudios a largo plazo que permitan abordar no solo impactos de la sequía puntuales, sino que también permitan conocer la duración en el tiempo de dichos impactos. Esto implica un seguimiento del estado y nivel de estrés de las especies, que permita identificar tanto procesos de decaimiento o mortalidad como de recuperación. De este modo, podrá observarse la capacidad de resiliencia de las especies mediterráneas y si ésta se ve reducida ante sequías extremas, frecuentes y/o prolongadas. Además, permitirá identificar retroalimentaciones entre los efectos.

Conocer y comprender los efectos de las sequías, y en especial de las extremas, y además todos los factores que los condicionan requiere de estudios llevados a cabo a diferentes escalas temporales. La respuesta de las especies puede ser muy variada y en ocasiones puede no manifestarse justo después del evento de sequía estudiado. Esto dependerá tanto de las características de la sequía como de la especie. Por este motivo, es necesario estudiar su duración, intensidad, frecuencia y momento en el que se produce, así como el modo en el que estas características condicionan los efectos en las especies.

Además de estudios a diferentes escalas temporales, también es necesario abordar estudios a diferentes escalas espaciales. De esta manera, se ampliaría el conocimiento del efecto que los factores locales tienen en la intensidad de la sequía. En concreto, es crucial conocer si

las condiciones locales pueden modular la sequía y si esta modulación varía según su intensidad, es decir, bajo condiciones de sequía extrema.

En este sentido, a la hora de abordar los efectos de la sequía en las especies vegetales, es esencial llevar a cabo los estudios antes y justo después de la sequía (o bien durante el máximo estrés hídrico). Así se puede aislar el efecto de la sequía de las condiciones previas que pudiese presentar la especie.

Además, a la hora de medir los efectos de la sequía en las especies vegetales, es muy importante utilizar diferentes técnicas y aproximaciones que permitan una visión más amplia y completa de todo lo que está pasando, no solo en lo referente a la especie, sino que también en el ambiente que lo rodea. En este sentido, es esencial abordar diferentes escalas dentro de una misma especie, ya que los efectos pueden manifestarse en unas escalas pero no en otras, desde lo que ocurre a nivel molecular a lo que está sucediendo a nivel de todo el árbol o masa forestal. Esto permitiría profundizar en los mecanismos que desarrollan las especies para tolerar la sequía, y si van a seguir siendo efectivos, o bajo qué condiciones, tras las frecuentes e intensas sequías. De este modo, podrían hacerse mejores predicciones en lo referente a las retroalimentaciones entre varios factores que generan estrés en las especies como son las sequías y la fragmentación del hábitat.

Los estudios en especies mediterráneas en los que se ha abordado los efectos derivados de las interacciones entre la

fragmentación y la sequía son muy escasos. Faltan estudios que aborden no solo los efectos que producen dichas interacciones, sino que también los factores que los condicionan, y cómo estos varían según las especies y regiones de estudio.

OBJETIVOS DE LA TESIS

Los objetivos generales de esta tesis fueron: (1) comprender la respuesta funcional a la sequía de especies forestales mediterráneas coexistentes y con contrastadas estrategias hídricas, sobre todo en periodos extremadamente secos, (2) cuantificar la modulación de la respuesta a las sequias extremas que ejercen las condiciones microclimáticas, y (3) evaluar el papel de la fragmentación de la masa forestal sobre la respuesta ecofisiológica ante situaciones de sequía.

Los objetivos específicos fueron:

1) Determinar si la estrategia hídrica de especies arbóreas coexistentes condiciona su respuesta funcional a las sequías extremas (capítulos 2, 3, 4).

2) Evaluar si el descenso en la tasa fotosintética neta de especies arbóreas derivado de una sequía es debido principalmente a un cierre estomático o a daños en la bioquímica de la hoja que afecten a la relación entre la funcionalidad del sistema hidráulico y el intercambio gaseoso (capítulo 2).

3) Estudiar la implicación que tiene el mecanismo de osmoregulación y la elasticidad de los tejidos foliares en la tolerancia a la sequía por parte de dos especies arbóreas del mismo género (capítulo 2).

4) Comparar los efectos que generan el momento en el que se produce una sequía y su intensidad en el crecimiento, la transpiración y la eficiencia en el uso de agua de especies arbóreas coexistentes (capítulo 3).

5) Determinar la capacidad de ajuste de la eficiencia en el uso del agua de especies arbóreas coexistentes dependiendo de su estrategia hídrica (capítulo 3).

6) Evaluar la medida en que el microclima atempera los efectos de las sequías extremas a nivel regional sobre la respuesta ecofisiológica de las especies arbóreas (capítulo 4).

7) Analizar los efectos de la sequía estival en la ecofisiología de bosques fragmentados y estudiar si dichos efectos dependen de las condiciones de aridez del lugar (capítulo 5).

8) Estudiar si la fragmentación de los bosques atenúa el estrés hídrico ante periodos secos extremos: los encinares como modelo de estudio (capítulo 5).

A través del desarrollo de estos objetivos específicos se analizan los efectos que a largo plazo puedan tener las sequías extremas en el funcionamiento de los bosques, y el impacto que puedan tener sobre el balance hídrico y de carbono del ecosistema. Efectos que en último término, y a largo plazo, se podrían traducir en cambios en la composición de los bosques. Además, se pretende contribuir en el conocimiento de todos los factores implicados en la respuesta de los bosques mediterráneos a las cada vez más frecuentes e intensas sequías extremas. Con el fin de alcanzar estos objetivos, esta tesis doctoral incluye cuatro capítulos escritos en inglés, para su publicación en revistas científicas de ámbito internacional. Además, se incluye un apartado de apéndices, donde se resumen otros experimentos en los que se ha participado a lo largo del desarrollo de esta tesis doctoral fruto de colaboraciones con otros investigadores. Todos ellos están íntimamente relacionados con los objetivos planteados en la tesis y suponen un complemento, y en algún caso ampliación, de los estudios y temas abordados.

METODOLOGÍA

Zonas de estudio y especies

Parque Natural del Alto Tajo

Los estudios de la respuesta funcional a la sequía de especies forestales mediterráneas coexistentes y con contrastadas estrategias hídricas, y la identificación de la modulación de la respuesta de estas especies a sequías extremas según el microclima se llevaron a cabo en el Parque Natural del Alto Tajo (Guadalajara, Castilla-La Mancha, España, 40°40'51" N, 02°04'31" O) (Capítulos 2-4) (Fig. 1).

Figura 1. Localización de las zonas de estudio en la Península Ibérica: Parque Natural del Alto Tajo (Guadalajara, Castilla-La Mancha), Lerma (región norte; Burgos, Castilla y León) y Quintanar de la Orden (región sur; Toledo, Castilla-La Mancha). Mapa extraído de Google Earth.

La altitud media es de 1293 m s.n.m, y el clima es mediterráneo continental con veranos muy secos y calurosos e inviernos muy fríos. La precipitación y la temperatura anual media son de 490 mm y 10.3°C, respectivamente, siendo enero el mes más frío y julio-agosto los meses más cálidos (Fig. 2). La temperatura máxima media mensual es de 24.1°C, correspondiendo al mes de julio (Agencia Estatal de Meteorología, estación de Molina de Aragón, 40°50'40" N, 01°53'07" O, 1063 m s.n.m, periodo 1961-2013).

El sustrato que predomina es el calizo y los suelos están poco desarrollados, presentando con frecuencia afloramientos de roca madre procedentes, principalmente, del Jurásico inferior y el Cretácico superior (Ferrero et al. 2006). El Alto Tajo posee una representativa y bien conservada muestra de la flora ibérica, lo cual le confiere una gran diversidad florística en la que destacan los pinares (*Pinus nigra* subsp. *salzmannii*, *Pinus sylvestris, Pinus pinaster, Pinus halepensis*), sabinares (*Juniperus thurifera*, *Juniperus phoenicea*) y masas de quercíneas (*Quercus ilex* subsp. *ballota*, *Quercus faginea*, *Quercus pyrenaica*), así como formaciones mixtas de todas ellas (Ferrero et al. 2006). Los numerosos cañones y hoces albergan poblaciones de rapaces rupícolas como el águila real, el alimoche, el halcón peregrino, el buitre leonado y el búho real. Además, este parque natural también alberga grupos de cabra montés, gatos monteses, tejones, garduñas, comadrejas y ginetas. Los corzos, ciervos y jabalíes aprovechan las masas arboladas como lugares de reposo y refugio (Ferrero et al. 2006).

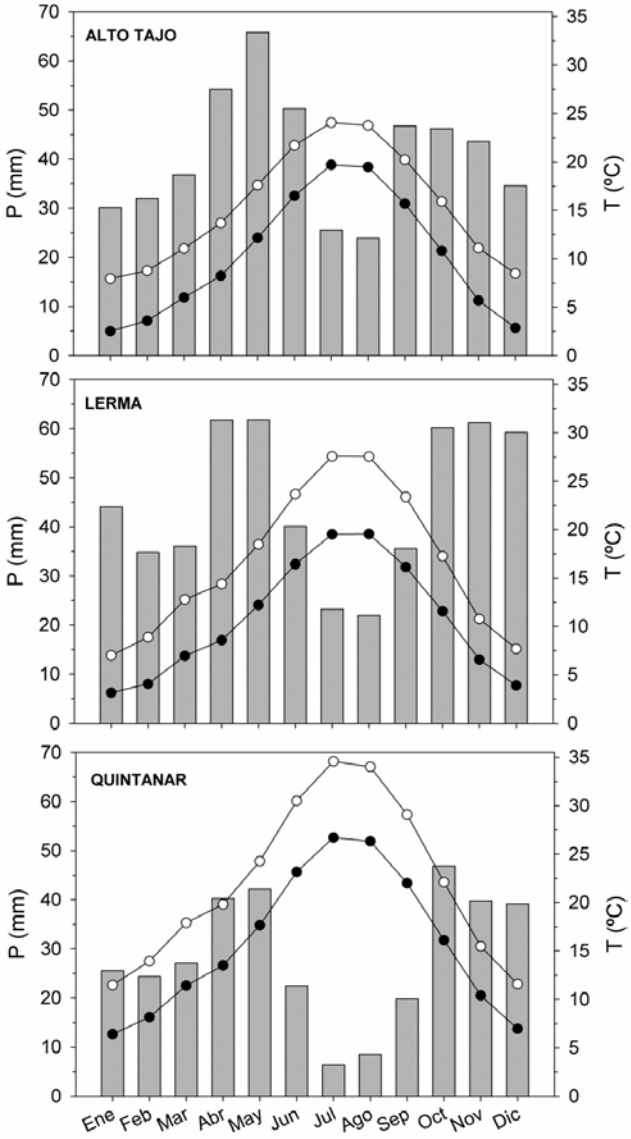

Figura 2. Diagrama ombrotérmico de las estaciones meteorológicas más cercanas a las zonas de estudio (Molina de Aragón para el Parque Natural del Alto Tajo, Villafría para Lerma y Toledo para Quintanar de la Orden). Las barras representan la precipitación total mensual media (mm, eje izquierdo) y las líneas y puntos blancos y negros la temperatura máxima y la media mensual (°C, eje derecho) del período 1961-2013 para Molina y 1982-2013 para Villafría y Toledo. Datos de la Agencia Estatal de Meteorología (AEMET).

Para el estudio se seleccionaron tres especies dominantes y coexistentes en el Parque Natural del Alto Tajo (Fig. 3): la encina (*Quercus ilex* subsp.*ballota* (Desf) Samp.), el quejigo (*Quercus faginea* Lam.) y el pino laricio (*Pinus nigra* subsp. *salzmannii* (Dunal) Franco). Estas especies pueden formar bosques mixtos, aunque suelen dar lugar a formaciones boscosas donde hay dominancia de alguna de ellas. Esto es debido a que sus rasgos funcionales diferenciales les permiten predominar y adaptarse mejor en unas condiciones de suelo y ambientales que en otras (Tabla 4).

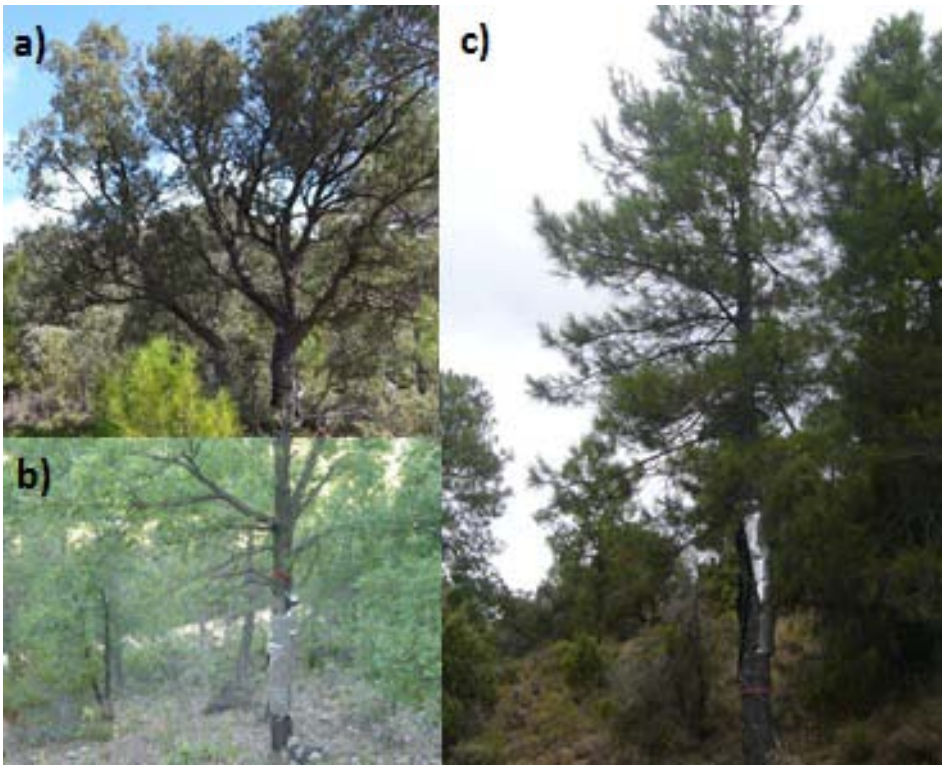

Figura 3. Fotografías de las especies de estudio: (a) *Q. ilex*, (b) *Q. faginea*, (c) *P. nigra*.

Dentro del Parque Natural del Alto Tajo se seleccionaron dos zonas de estudio, Armallones (AR; 40°46’37” N, 02°19’42” O; 1079 m s.n.m.) y Huertapelayo (HP; 40°47’33” N, 02°17’14” O; 907 m s.n.m.). La distancia entre ambas zonas es de aproximadamente 8 km y su orientación es este y norte, respectivamente. AR presenta un 48 % de pendiente y un área basal de 13.7 $m^2.ha^{-1}$, mientras que la pendiente de HP es de 68% y su área basal de 11.4 $m^2.ha^{-1}$. Las principales especies arbóreas en AR son *Q. faginea* (38.7 %), *P. nigra* (35.1 %), *Q. ilex* (25.8 %) y *Juniperus oxycedrus* (0.2 %). En HP domina *P. nigra* (62.4 %), *Q. faginea* (35.1 %), *Juniperus phoenicea* (1.6 %) y *J. oxycedrus* (0.9 %). La precipitación total anual es de 796.2 mm y 598.7 mm en AR y HP respectivamente, mientras que la temperatura media anual es de 11.5 °C y 11.7 °C, respectivamente (Granda et al. 2013).

Tabla 4. Rasgos funcionales característicos de las especies de estudio (*Q. ilex*, *Q. faginea* y *P. nigra*). Tabla simplificada y modificada de la tesis doctoral de Granda (2013).

Tipo de rasgo	Rasgo	*Q. ilex*	*Q. faginea*	*P. nigra*
Preferencias	Clima	Zonas interiores de clima continental o zonas litorales con veranos cálidos y secos	Zonas de clima submediterráneo o mediterráneo continental no muy extremo	Entre mediterráneo semiárido de inviernos frescos y continental templado frío
	Suelos	Sin preferencias	Todo tipo de sustratos, más frescos y profundos que la encina	Todo tipo de terrenos aunque muestra preferencia por los calizos
Rasgos foliares	Hábito foliar	Siempreverde	Decídua, marcescente	Siempreverde
	Tipo de hoja	Hoja ancha	Hoja ancha	Acículas
Rasgos reproductores	Rebrotante	Sí	Sí	No
	Época de floración	Marzo-mayo	Marzo-mayo	Marzo-mayo
	Época de fructificación	Octubre-noviembre	Septiembre-octubre	Las piñas maduran en el otoño del 2º año, disemina en primavera
Rasgos de madera	Conductos	Porosidad difusa o semidifusa	Porosidad en anillo	Tráqueas y traqueidas
Rasgos radiculares	Sistema radicular	Raíz principal potente y penetrante y secundarias de gran vitalidad	Raíz principal potente y penetrante	Raíz principal poco desarrollada y secundarias largas y abundantes, casi superficiales
Tolerancia	Riesgo de embolias	Alto	Alto	Bajo
	Estrategia frente a sequía estival	Tolerante-resistente, anisohídrica	Cierta tolerancia, anisohídrica	Evitadora, isohídrica

Lerma (región norte) y Quintanar de la Orden (región sur)

El estudio de la importancia de la fragmentación del bosque en la respuesta ecofisiológica de las especies a los eventos extremos (Capítulo 5) se llevó a cabo en dos localidades, Lerma (Castilla y León, España, 42°05' N, 03°45' O) y Quintanar de la Orden (Castilla-La Mancha, España, 39°35' N, 03°02' O) (Fig. 1). Estas zonas de estudio albergan encinares representativos de la meseta central (Santos and Tellería 1998). La altitud media es de 930 m y 870 m s.n.m., respectivamente. Ambas se caracterizan por presentar un verano seco pronunciado desde julio a septiembre aunque presentan climatología contrastada, sobre todo en verano (Fig. 2).

La zona de estudio de Lerma es la región norte, una zona bioclimática supramediterránea (Rivas-Martínez 1981) donde la temperatura media anual es de 10.7°C y la precipitación total anual de 540 mm (Agencia Estatal de Meteorología, estación de Villafría, 42°21'56" N, 03°36'59" O, 891 m s.n.m, periodo 1982-2013 (Fig. 2). La especie arbórea dominante es la encina (*Q. ilex*), aunque también se encuentran algunos quejigos (*Q. faginea*) aislados y sabinas (*Juniperus thurifera*). El sotobosque está compuesto por arbustos típicos de zonas supramediterráneas como por ejemplo *Cistus laurifolius*, *Genista scorpius* o *Thymus zygis* (Santos y Tellería 1998; Díaz et al. 1999). Los suelos dominantes son cambisoles (WRB 2007).

Quintanar de la Orden es considerada la región sur, una zona bioclimática mesomediterránea (Rivas-Martínez 1981) cuya

temperatura anual media es de 15.8°C y la precipitación total anual de 342 mm (Agencia Estatal de Meteorología, estación de Toledo, 39°51’29” N, 04°01’21” O, 515 m s.n.m, periodo 1982-2013 (Fig. 2). La especie dominante es la encina, aunque también aparecen coscojas (*Quercus coccifera*) y especies de arbustos típicas del mesomediterráneo xérico como *Rhamnus lycioides*, *R. alaternus*, *Cistus ladanifer* o *Asparagus acutifolius* (Santos y Tellería 1998; Díaz y Alonso 2003). Los suelos que predominan son cambisoles (WRB 2007).

Para la selección de los fragmentos se realizó un estudio de la cartografía de ambas zonas y se seleccionaron fragmentos de tamaños contrastados, grandes (área superior a 10 ha) y pequeños (mínimo de tres individuos). Posteriormente, ya en campo se seleccionaron los fragmentos de estudio mediante los siguientes criterios: que fuesen (1) áreas con baja pendiente, (2) fragmentos rodeados de una matriz de uso agrícola de secano, (3) fragmentos pequeños con un mínimo de tres encinas y un máximo de 0.5 ha, y (4) fragmentos con condiciones análogas dentro de los dos tipos de tamaño de fragmento, con el fin de poder ser consideradas réplicas. En cada una de las dos regiones de estudio se seleccionaron dos fragmentos grandes y entre tres y cinco fragmentos pequeños. Para el estudio de los fragmentos grandes, éstos se dividieron en interior (> 30 metros desde el borde del fragmento) y bordes (Fig. 4).

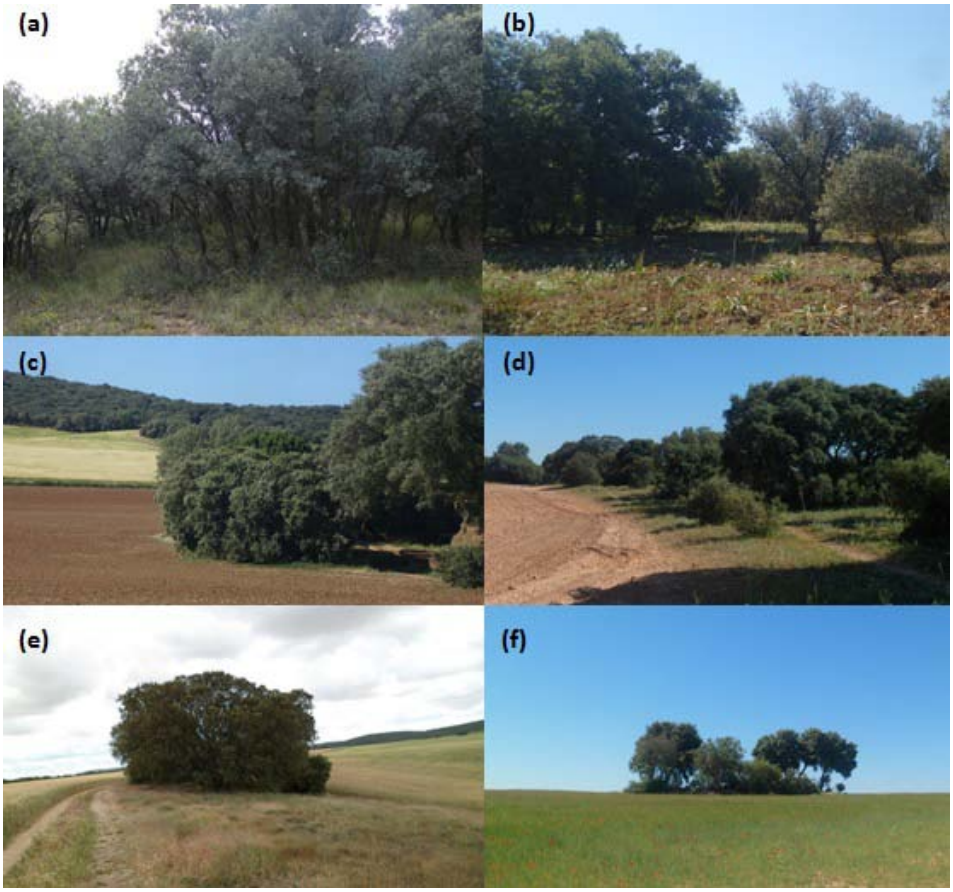

Figura 4. Fotografías de los fragmentos de bosque de *Quercus ilex* objeto de estudio. Interior del bosque de Lerma (a) y de Quintanar de la Orden (b). Borde del bosque de Lerma (c) y de Quintanar de la Orden (d). Fragmentos pequeños de Lerma (e) y de Quintanar de la Orden (f). Fotos tomadas por Dulce Y. Flores y Alicia Forner en junio-julio de 2011-2013.

Metodología por capítulos

Durante el desarrollo de la presente tesis doctoral se han utilizado diferentes técnicas y metodologías. A continuación se resumen los métodos de cada uno de los capítulos (para más detalle consultar el capítulo correspondiente).

Datos climáticos

Los datos climáticos (medias mensuales y anuales de temperatura y precipitación) se obtuvieron a partir de la estación meteorológica de Molina de Aragón, Guadalajara (40°50’40” N, 01°53’07” O, 1063 m s.n.m, periodo 1951-2013) (capítulos 2-4) y de las estaciones meteorológicas de Villafría (42°21’56” N, 03°36’59” O, 891 m s.n.m, periodo 1982-2013) y Toledo (39°51’29” N, 04°01’21” O, 515 m s.n.m, periodo 1982-2013) (capítulo 5), todas procedentes de la Agencia Estatal de Meteorología (AEMET).

Utilizando los datos de temperatura y de precipitación del periodo 1951-2012 y 1970-2012, se calcularon los percentiles (5, 50 y 95 %) para identificar valores inusuales, especialmente durante el periodo de estudio (2009-2012) (capítulo 3-4). Además, con el fin de determinar la intensidad de la sequía para el periodo 1961-2012, se estimó el agua relativa diaria extraíble en el suelo (REW) (Granier et al. 1999) utilizando los datos diarios de precipitación, temperatura del aire, humedad relativa del aire, radiación global y velocidad del viento (capítulo 2-4). En este mismo estudio se determinó el inicio (BEGWS) y duración (NDWS) de la sequía (REW < 0.4) y un índice de sequía (I_S) calculado a partir de los valores de REW (ver capítulos 3-4). Además, se calculó el índice de sequía P/PET dividiendo la precipitación (P) por la evapotranspiración potencial (PET) para el período 1951-2013 (capítulo 3).

También había instaladas estaciones meteorológicas (HOBO® Weather Station, Onset Computer Co., Bourne, MA, USA) en el Parque Natural del

Alto Tajo (capítulos 2-4) cuyos datos fueron usados para describir la temperatura, precipitación, el déficit de presión de vapor (VPD; mediante la ecuación de Campbell), la evapotranspiración potencial (PET; mediante la ecuación de Penman) (Campbell and Norman 1998; Penman 1948).

Estudio del crecimiento y el uso del agua (capítulos 2-4)

Desde septiembre de 2009 a octubre de 2012 se hizo un seguimiento del crecimiento y el uso del agua de individuos de *P. nigra, Q. faginea* y *Q. ilex* mediante el uso de dendrómetros digitales (DRL26, ems Brno, Czech Republic) y medidores de flujo de savia (EMS 51, Brno, Czech Republic), respectivamente, en Armallones y Huertapelayo (Čermák et al. 2004).

En el capítulo 2 se estudió el flujo de savia diario por unidad de área basal (Q_v) medido a lo largo de los tres días en los que se midió el intercambio gaseoso, tanto durante principios (ESu) como finales de verano (LSu) de los años de estudio (2011-2013). También se midió el valor medio diario de esta medida teniendo en cuenta 7 días de medida, entre los cuales se encontraban los días de medida del intercambio gaseoso. En ambos casos se midió en 2 individuos de *Q. ilex*, 4 de *Q. faginea* y 3 de *P. nigra*, siendo éstos parte de los árboles en los que se midió el intercambio gaseoso.

Con el fin de estudiar la transpiración de las especies (*E*, mm), en el capítulo 3 se midió el flujo de savia en 5 individuos de *Q. faginea*, 4 individuos de *P. nigra* y 3 individuos de *Q. ilex*. La transpiración fue calculada tanto para todo el periodo de crecimiento (E_{gs}), como para la primavera (E_{sp}) y el otoño (E_{au}) durante el periodo 2010-2013. Para ello, se dividió el flujo de savia total de cada periodo considerado (dm^3) por el área de la copa proyectada (m^2). En estos mismos individuos se calculó el crecimiento para todo el periodo vegetativo, la primavera y el otoño. Para ello, se calculó el incremento de área basal (BAI, cm^2), que es BAI $=\pi(r^2_t - r^2_{t-1})$, siendo r el radio del individuo y t el tiempo de medida (Hoffmann and Poorter 2002). Además, se calculó la tasa de crecimiento relativo (RGR, $mm^2.d^{-1}$) para los mismos periodos utilizando la fórmula RGR = $(\ln r^2_t - \ln r^2_{t-1})/t$ (Jump et al. 2006). El inicio (Beg) y duración (Len) del crecimiento en primavera y otoño se determinó considerando cambios en la pendiente de la evolución del BAI.

En el capítulo 4, se calculó el flujo de savia por árbol ($L.h^{-1}$) de las especies de estudio durante el periodo 2010-2012. Para ello, se seleccionaron 8 individuos de *P. nigra* (4 en Armallones y 4 en Huertapelayo) y 9 de *Q. faginea* (5 en Armallones y 4 en Huertapelayo). Además, se calculó el (Q_v, $m^3.d^{-1}.m^{-2}$) considerando cinco periodos de transpiración de las especies representativos. El periodo 1 (p1) corresponde a un inicio significativo en el uso del agua durante primavera (REW ~ 1, máxima cantidad de agua extraíble), momento en el cual la especie caducifolia *Q. faginea* está desarrollando sus hojas. El segundo periodo (p2) muestra la máxima transpiración de las especies,

lo cual ocurre a finales de primavera. El tercer periodo (p3) considera el momento del pico del verano en el que el flujo de savia es mínimo y REW < 0.4 (por debajo de este valor el agua del suelo limita la transpiración y se produce el cierre estomático en algunas especies) (Granier et al. 1999). El cuarto periodo (p4) muestra la recuperación del flujo de savia durante las tempranas lluvias de otoño, cuando REW se encuentra entre 0.4 y 1. Por último, el periodo 5 (p5) considera los valores mínimos de flujo de savia medidos a finales de otoño y principios de invierno. En cada uno de estos periodos se seleccionaron días similares con iguales condiciones meteorológicas. El flujo de savia relativo (Q_r) se calculó con el fin de terminar el descenso en el consumo de agua por parte de los árboles durante la sequía de verano y su posterior recuperación durante las lluvias de otoño. Éste fue calculado dividiendo Q_v por el máximo flujo de savia movido por la especie (Q_{max}, p2), estando ambas variables calculadas por unidad de área basal. Además, se obtuvo el día del inicio y del máximo flujo de savia, así como la duración del flujo de savia durante primavera. En estos mismos árboles, y con el fin de hacer un seguimiento del crecimiento, se calculó el BAI anual y la tasa de crecimiento diario en primavera (SGR, $cm^2.d^{-1}$), dividendo el BAI de todo el periodo de crecimiento primaveral por la duración en número de días. Además, se calculó el Len y Beg del crecimiento primaveral de todos los años de estudio (2010-2012).

Todos ellos árboles seleccionados eran árboles sanos, completamente expuestos al sol y con un diámetro a la altura del pecho similar.

Eficiencia en el uso del agua (capítulo 3)

La eficiencia en el uso del agua (WUE) fue estimada mediante dos métodos en el capítulo 3. Por una parte se calculó la WUE ($cm^2.m^{-3}$) para todo el periodo vegetativo (WUE_{gs}), la primavera (WUE_{sp}) y el otoño (WUE_{au}) de los años 2011-2013 dividiendo el BAI por la suma de todo el flujo de savia del periodo considerado. Estos cálculos se llevaron a cabo para 5 individuos de *P. nigra*, 4 de *Q. faginea* y 3 de *Q. ilex*. El otro método utilizado fue la estimación de la eficiencia en el uso del agua intrínseca a partir de la composición isotópica de carbono en hoja ($\delta^{13}C$, ‰) (Dawson et al. 2002; McCarroll and Loader 2004). En este caso se utilizaron dos hojas por cada uno de los 5 individuos de las tres especies de estudio y para primavera y verano de los años 2011-2013. El análisis isotópico de las muestras molidas se llevó a cabo en el Technical Platform of Functional Ecology del INRA, Francia.

Indicadores de estrés hídrico (capítulos 2 y 5) y conductancia hidráulica (capítulo 2)

El estrés hídrico experimentado por las especies durante las sequías se obtuvo a través del potencial hídrico en hoja medido antes del amanecer (Ψ_{pd}, MPa). Estas medidas se llevaron a cabo en dos hojas (capítulo 2) y dos ramitas (capítulo 5) por individuo mediante el uso de la cámara de presión Scholander (PMS 1000, Soil Moisture Equipment Corp., Santa Bárbara, CA, USA). Las muestras se recolectaron antes del amanecer, fueron almacenadas en bolsas zip, donde se saturó el

ambiente de humedad y CO_2 y se mantuvieron en oscuridad y refrigeradas hasta su media (Pérez-Harguindeguy et al. 2013).

En el capítulo 2, Ψ_{pd} se midió en 5 individuos de cada una de las tres especies de estudio, durante ESu y LSu y a lo largo de tres años (2011-2013). En las mismas fechas se midió el potencial hídrico en hoja medido por la tarde (13-15 h) en hojas transpirantes (Ψ_{leaf}) y hojas no transpirantes (Ψ_{xyl}). Estas últimas se obtuvieron cubriendo las hojas con una bolsa zip recubierta de papel de aluminio desde la tarde del día anterior (Begg and Turner 1970; Schultz and Matthews 1993). En estos mismos individuos y para las tres especies, se calcularon para el inicio y final del verano y los años 2011-2013 tres conductancias hidráulicas siguiendo la analogía a la Ley de Ohm (van den Honert 1948). La conductancia hidráulica aparente específica de la hoja en el continuo suelo-planta-atmósfera (K_h, $kg.m^{-2}.s^{-1}.MPa^{-1}$) fue calculada dividiendo la tasa de transpiración (E) medida en la tarde, por la diferencia entre Ψ_{pd} y Ψ_{leaf} y considerando Ψ_{pd} como un proxy del potencial hídrico del suelo cuando el flujo de savia en el árbol es cero. La conductancia hidráulica aparente de las ramas (K_b, $kg.m^{-2}.s^{-1}.MPa^{-1}$) y la conductancia hidráulica aparente de la lámina peciolo-hoja (K_{pl}, $kg.m^{-2}.s^{-1}.MPa^{-1}$) fueron calculadas utilizando la diferencia entre Ψ_{pd} y Ψ_{xyl} y entre Ψ_{leaf} y Ψ_{xyl}, respectivamente (Aranda et al. 2005).

En el capítulo 5, se midió el Ψ_{pd} en un total de 163 encinas (entre 26 y 28 encinas por nivel de fragmentación y lugar) localizadas en fragmentos grandes (interior y borde) y fragmentos pequeños procedentes tanto de la región norte (Lerma) como de la región sur (Quintanar). Estas

medidas se llevaron a cabo tanto durante ESu como LSu en los años 2012 y 2013. Por cada día se tomaron de media 15 medidas, todas tomadas en encinas escogidas aleatoriamente entre el interior y borde de fragmentos grandes y los fragmentos pequeños.

Ajuste osmótico en especies de Quercus (capítulo 2)

El ajuste osmótico de *Q. ilex* y *Q. faginea* en el Parque Natural del Alto tajo, en Armallones, se calculó mediante la construcción de curvas presión volumen en dos ramitas seleccionadas de 5 individuos de cada especie (Dreyer et al. 1990; Aranda et al. 1996; Corcuera et al. 2002). Dichas ramitas fueron cortadas bajo agua e hidratadas con agua destilada durante 12 horas con el fin de alcanzar la máxima turgencia y evitar la sobresaturación. Se estimó el contenido hídrico relativo en el punto de turgencia (RWC_{tlp}, %), el contenido hídrico relativo apoplástico (RWC_a, %), el módulo de elasticidad (ε, MPa), el potencial osmótico en el punto de máxima turgencia (π_0, MPa) y en el punto de pérdida de turgencia (π_{tlp}, MPa) y el ratio entre el peso seco de la hoja y el peso turgente de la hoja (DW/TW).

Medidas fisiológicas: intercambio gaseoso, eficiencia fotoquímica del fotosistema II y rasgos foliares (capítulos 2 y 5)

En el capítulo 2, el intercambio gaseoso y la fluorescencia de la clorofila se midieron en dos hojas sanas completamente desarrolladas en 5

individuos de cada una de las tres especies de estudio de Armallones, utilizando un sistema de medida de intercambio gaseoso LI-Cor 6400 XT con un cabezal donde iba integrada una cámara de medida de la fluorescencia (Li-Cor, Inc., NE). Estas medidas se llevaron a cabo tanto durante ESu como LSu de cada uno de los años de estudio (2011-2013). Las medidas fueron tomadas por la mañana (de 10:30 a 13 h) y por la tarde (13 -15 h). Las variables que se obtuvieron fueron la tasa fotosintética neta en base al área foliar (A_{area}, µmol.m^{-2}.s^{-1}), la conductancia estomática (g_s, mmol.m^{-2}.s^{-1}), la tasa de transpiración (E, mmol H_2O.m^{-2}.s^{-1}), el rendimiento cuántico efectivo (ΔF'/Fm'), la atenuación fotoquímica (qP) y la atenuación no fotoquímica (qN). Éstas fueron medidas a concentración de CO_2 constante (400 µmol CO_2 .mol^{-1}) y con una intensidad lumínica saturante (1200 µmol.m^{-2}.s^{-1} en ESu y 1500 µmol.m^{-2}.s^{-1} en LSu).

Además, para los mismos individuos se calculó la eficiencia en el uso del nitrógeno fotosintético (PNUE, µmol CO_2.mg^{-1}N.s^{-1}) dividiendo A_{area} por el contenido de nitrógeno foliar por unidad de área (N_a), el cual a su vez fue calculado dividendo el contenido de nitrógeno foliar elemental (N_m, %) por el área folia específica (SLA, m^2.kg^{-1}). N_m y el contenido de carbono foliar elemental (C_m, %) se obtuvieron a partir del análisis de dos hojas recogidas de cada uno de los 15 individuos de estudio (5 de cada especie) y para cada periodo de estudio y año. Este análisis, se llevó a cabo en el mismo centro del INRA en el que se analizó la composición isotópica de carbono en la hoja (INRA). Además, se calculó el ratio entre C_m y N_m (C_m/N_m). El SLA se obtuvo midiendo el área de la

hoja fresca y dividiéndola por el peso seco de la hoja después de secarlo en la estufa a 65°C durante 48 h. Las hojas utilizadas para el SLA, la composición isotópica y el contenido de C y N fueron las mismas.

En el capítulo 5, se midió la g_s en dos-tres hojas de cada una de las 163 encinas de estudio seleccionadas entre los fragmentos grandes (interior y borde), y fragmentos pequeños, de la región norte y la región sur. Las medidas fueron llevadas a cabo durante ESu y LSu de los años 2012 y 2013 entre las 10 y 12 h (horas de máxima conductancia) utilizando un porómetro de hoja SC-I (Decagon, Pullman, USA). Además, en los mismos individuos se midió en dos-tres hojas por árbol, la máxima eficiencia fotoquímica del fotosistema II tanto antes del amanecer (Fv/Fm_{pd}) como a mediodía (Fv/Fm_{md}) utilizando un fluorímetro FMS2 (Hansatech, Norfolk, UK). El SLA también fue estimado en dos hojas por individuo durante ESu en ambos años de estudio. Por último, se obtuvo el contenido de materia foliar seca (LDMC, $mg.g^{-1}$) de los mismos individuos en tres hojas por individuo recogidas durante LSu de 2012 y 2013. LDMC se calculó dividiendo el peso seco de la hoja por su peso una vez saturada la hoja durante 12 h.

Los análisis estadísticos aplicados son descritos detalladamente en cada uno de ellos en el apartado *Statistical analysis*.

CONCLUSIONES GENERALES

A continuación se enumeran las principales conclusiones obtenidas en esta tesis doctoral:

1. La estrategia hídrica evitadora, como la observada en *Pinus nigra*, no evitó completamente los efectos negativos derivados de las sequías extremas.

2. Las sequías extremas produjeron mayores descensos en la eficiencia del sistema hidráulico, en la tasa de intercambio gaseoso y en el consumo de agua del árbol en *P. nigra* que en especies más tolerantes a la sequía como *Quercus ilex* y *Quercus faginea*. Esto repercutió en mayores disminuciones en la fijación de carbono a nivel de hoja en esta especie evitadora. Sin embargo, el crecimiento secundario durante los años extremadamente secos se redujo menos en esta especie que en las especies de *Quercus*.

3. Los descensos en el crecimiento secundario anual durante las sequías extremas observados en *P. nigra* fueron menores que en las otras especies y se correlacionaron con su mayor plasticidad en la eficiencia en el uso de agua, tanto anual como estacional. La eficiencia en el uso del agua en *P. nigra* durante sequías extremas incrementó en mayor medida que en las especies de *Quercus.*

4. A pesar de la intensidad de la sequía, los descensos en la tasa fotosintética neta de las especies objeto de estudio se debieron principalmente al cierre estomático y en mucha menor medida a daños en la bioquímica de la hoja.

5. *Quercus ilex* fue más tolerante a la sequía que *Q. faginea*, mostrando menores descensos en la transpiración foliar y de todo el árbol, y en el crecimiento secundario. Además, *Q. ilex* mantuvo una estrategia mucho más derrochadora de agua durante las épocas de máximo estrés hídrico. Esto se debe a que *Q. ilex* combinó varios mecanismos para soportar el estrés hídrico tales como un sistema hidráulico más eficiente y una mayor capacidad de osmoregulación en las hojas, lo cual le permitió mantener una mayor turgencia en las hojas bajo condiciones de sequía intensa.

6. A pesar de la gran correlación entre el crecimiento y el uso de agua o transpiración, ambos procesos estuvieron desacoplados y se vieron afectados en diferente grado por el momento de la sequía y su intensidad, así como por la estrategia de la especie.

7. A pesar de la intensidad de la sequía, las especies estudiadas no mostraron signos de defoliación severa o de mortalidad tanto a escala anual como estacional (primavera y verano). Esto sugiere que las especies no superaron su límite de tolerancia a la sequía en el lugar de estudio, y pone de relieve, además, su gran resiliencia. Sin embargo, no todas las especies presentaron la

misma resiliencia a sequías extremas, siendo mayor en *P. nigra* y menor en *Q. faginea*.

8. Pequeñas diferencias en la humedad del suelo, a escala de micrositio, modularon de forma muy significativa el impacto de la sequía extrema independientemente de la estrategia hídrica de las especies.

9. El momento en el que se produce la sequía, su duración y la escala temporal pueden afectar al comportamiento de los árboles, incluso en mayor medida que la intensidad de la propia sequía.

10. Bajo sequías más frecuentes, duraderas e intensas se espera que las especies evitadoras estén en desventaja frente a las especies más tolerantes. Una menor fijación de carbono durante periodos prolongados derivará en procesos de decaimiento y mortalidad en estas especies evitadoras. Sin embargo, si la frecuencia e intensidad de las sequias continúa aumentando, las especies más tolerantes podrían verse sometidas a niveles de estrés hídrico superiores a sus umbrales de tolerancia, derivando por tanto en fallos en el sistema hidráulico y también en procesos de decaimiento.

11. En general, las encinas de masas forestales continuas acusaron en mayor medida los periodos secos extremos que aquellas localizadas en masas fragmentadas. Esto quedó reflejado en un

mejor estado hídrico al amanecer de los árboles sometidos a una menor competencia intraespecífica. Esto también se observó a partir de una menor disminución en la regulación estomática de las pérdidas de agua y en la actividad fotoquímica foliar al final del verano.

12. En bosques de encinas, la fragmentación atenuó el estrés hídrico experimentado por los árboles individuales incluso en condiciones de sequía extrema y con independencia de la aridez general de la región de estudio.

13. El estudio de la escala, duración e intensidad de las sequías extremas, las condiciones microclimáticas y su interacción con otras fuerzas de cambio global como la fragmentación, son esenciales para determinar la tolerancia real de los árboles mediterráneos al estrés hídrico severo, y por lo tanto, para conocer su potencial adaptativo ante los nuevos escenarios climáticos.

GENERAL CONCLUSIONS

The main conclusions obtained during this PhD are detailed below:

1. Drought-avoiding strategies, such as the observed in *Pinus nigra*, did not completely avoid the negative effects of extreme droughts.

2. Due to the extreme drought, *P. nigra* had the highest decrease in the efficiency of the hydraulic system, gas exchange rates and water use compared to more drought-tolerant species such as *Quercus ilex* and *Quercus faginea*. As a consequence, this drought-avoiding species showed the greatest decrease in leaf carbon fixation. However, its secondary growth in extremely dry years was less limited than for *Quercus* species.

3. The lowest decrease in secondary growth during extreme droughts observed in *P. nigra* was correlated with its higher plasticity in water use efficiency, both annual and seasonal. Water use efficiency during extreme droughts increased more in *P. nigra* than in *Quercus* species.

4. Despite the intensity of the drought, reductions in net photosynthetic rate in the study species were mainly due to stomatal closure and to a lower extent to damages in the leaf biochemistry.

5. *Quercus ilex* was more drought tolerant than *Q. faginea*, having lower decreases in both leaf and whole-tree transpiration, and in

secondary growth. Moreover, *Q. ilex* maintained a more water-spending strategy than *Q. faginea* under maximum water stress conditions. The former species maintained this strategy by combining several mechanisms to cope with water stress such as a more efficient hydraulic system and greater osmoregulation ability in leaves. These mechanisms allowed *Q. ilex* to have higher leaf turgor under intense drought.

6. Despite the high correlation between growth and water use or transpiration, both processes were uncoupled and therefore, were differently affected by the intensity and timing of the drought, as well as by the species' strategy.

7. Despite the intensity of the drought, the study species did not exhibit signs of severe defoliation or mortality, neither at the annual nor seasonal (spring and summer) scale. This suggests that the species did not exceed their drought tolerance threshold in the study site, and also highlights their great resilience. However, not all species had the same resilience to extreme drought, being highest in *P. nigra* and lowest in *Q. faginea*.

8. Slight differences in soil moisture, at microsite scale, significantly modulated the impact of extreme drought regardless of the species' water strategy.

9. Timing, length and the temporal scale of the drought might affect tree performance even more than the intensity of the drought.

10. Drought-avoiding species are expected to be at a disadvantage compared to more drought-tolerant species under more frequent, lasting and intense droughts. A lower carbon fixation during extended periods could lead to the decline and mortality of such species. However, more frequent and intense droughts could also result in the decline of drought-tolerant species if their tolerance threshold to water stress is met.

11. In general, holm oaks from forest continuous stands were more affected by extremely dry periods than those from fragmented stands. This was reflected by better predawn water conditions in trees with lower intraspecific competition. Moreover, this was also observed through lower decreases in their stomatal regulation of water loss and their foliar photochemical activity at the end of the summer.

12. In holm oak forests, fragmentation attenuated the water stress experienced by individual trees even under extreme drought conditions and independently of the aridity of the study region.

13. Addressing the scale, length and intensity of extreme droughts, microclimatic conditions and their interaction with other global change drivers such as fragmentation is key to elucidate the actual tree tolerance to severe water stress, and therefore, their adaptive potential to new climatic scenarios.

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CAPÍTULO 2

Mediterranean trees coping with severe drought: avoidance might not be safe

Alicia Forner, Fernando Valladares, Ismael Aranda

Manuscript in preparation

Images from the front page: leaf water potential measurement in a *Q. faginea* leaf and a sealable plastic bag covering leaves of *Q. faginea* in the Alto Tajo Natural Park.
Author: Alicia Forner.

ABSTRACT

Plants growing in semiarid climates, such as the Mediterranean, have to cope with several stresses associated with drought. Coexisting plants differ widely in their sensitivity to drought and therefore in their ability to maintain carbon assimilation and a functional hydraulic system by avoiding or tolerating stressful periods. We studied the response of three coexisting Mediterranean tree species (*Quercus ilex*, *Quercus faginea* and *Pinus nigra*) under natural conditions during three years to test their functional strategies to deal with extreme dry years. Despite *P. nigra* following a drought-avoiding strategy, it was unable to effectively avoid the hampering effects of a severe drought in the driest year and experienced important drops in the efficiency of the hydraulic system, in gas exchange at the leaf level and in tree water use estimated from daily sap flow. In contrast, the two *Quercus* species showed more drought-tolerant strategies by maintaining a more profligate water use at the leaf and tree level, and with *Q. ilex* having a larger tolerance threshold to severe drought. In spite of the intensity of the drought and the associated stress experienced by the trees studied, especially *P. nigra*, mortality or severe defoliation were not observed after the extreme dry year.

Key words: *Quercus ilex*, *Quercus faginea*, *Pinus nigra*, gas exchange, water status, pressure-volume curves

INTRODUCTION

Tree species growing in semiarid climates, such as the Mediterranean, have to cope with several stresses associated with drought, which involves not only water scarcity but also high temperature and high radiation exposure. Mixed stands are frequent in the Mediterranean, where coexisting tree species differ widely in their sensitivity to drought (Martínez-Vilalta et al. 2003; Quero et al. 2011). Therefore Mediterranean species differ in their ability to maintain carbon assimilation in pace with an efficient control of water loss and function of the hydraulic system during those stressful periods (Nardini et al. 1999; Trifilò et al. 2014). Species in this kind of ecosystem have developed several adaptations following a range between two differentiate strategies: avoidance and tolerance to drought stress (Tardieu and Simonneau 1998; Franks et al. 2007). However, differentiation between both strategies is not always straightforward among closely related species, and classification as drought-avoiding or drought-tolerant species is not so discrete (Franks et al. 2007; Klein 2014; Attia et al. 2015). Drought-avoiding species have developed several functional adaptations such as early stomatal closure in response to soil water scarcity (Rambal 1992; Attia et al. 2015), or even under extreme conditions throwing away leaves (Breda et al. 2006; Peguero-Pina et al. 2015). In general, drought-avoiding species have effective stomatal control which is activated during early drought conditions but limiting carbon assimilation (Galiano et al. 2011). Contrary to drought avoiders, drought-tolerant species can maintain a

positive carbon balance and avoid photosystem damage under drought, even at low leaf and xylem water potentials, but leading to potentially higher risk of functionality lost in the hydraulic system (Urli et al. 2013). But plant water economy cannot be summarized merely by division into isohydric vs. anisohydric strategy considering only the stomatal regulation of plant water status. Species have some functional adaptations to tolerate drought, such as osmoregulation (Meinzer et al. 2014) or the production of sclerophyllous leaves (Salleo and Lo Gullo 1990). These adaptations combined with drought avoiding mechanisms such as increasing root/shoot ratio (Peña-Rojas et al. 2005) and the development of a deeper root system (David et al. 2007), allow very effective mechanisms to cope with the typical summer dry periods of Mediterranean climates (Nardini et al. 1999). Nonetheless, and despite this general classification, switching from an anysohydric to an isohydric strategy could be a common process for some species at levels of water stress endured at specific times of the growing season (Rogiers et al. 2011).

Drought is expected to bring about efficient down regulation of stomatal conductance of water vapor, consequently leading toa reduction in net photosynthetic rate (Misson et al. 2011), and the overall plant carbon assimilation (Rodríguez-Calcerrada et al. 2016). A water saving strategy can be effective during short periods of water stress, and a brief period of water deprivation can even increase plants' tolerance to subsequent stresses, increasing its productivity in the long term. However, this potential improvement does not occur under

severe droughts (Bruce et al. 2007; Fleta-Soriano and Munné-Bosch 2016). Under chronic or extreme water stress, species show evidence of 'cavitation fatigue' (Hacke and Sperry 2001) which increases their vulnerability to cavitation and therefore, to deterioration of the hydraulic system in the long term (Savi et al. 2015). In this way, species show a tradeoff between the avoidance of water lose and maintenance carbon fixation, which represents the safety margin of keeping the hydraulic system functioning well when facing carbon starvation (Mitchell et al. 2013). Decreases in carbon fixation under severe droughts might not only be due to stomatal closure, but also to impairment of the photosynthetic system itself. Damage suffered to PSII causes photo-inhibition, decreases in the effective quantum yield, or even triggering photorespiration (Horton et al. 1996). Conversely, drought-tolerant species can maintain higher photosynthetic rates and stomatal conductances during drought, supporting low water potentials (Rambal et al. 2003). Although this water use strategy can put these species closer to the risk of hydraulic dysfunction (Choat et al. 2012).

Different functional adaptations have allowed Mediterranean species to cope with typical summer droughts after years of evolution, but nevertheless their success under extreme conditions might not be enough to assure their long-term persistence. Different functional mechanisms to cope with water stress are effective under droughts of differing intensity and duration. However, once their thresholds of effectiveness are exceeded, irreversible damages even death to forest trees can occur (Allen et al. 2010). In fact, decline and even mortality of

Mediterranean forest tree species is already occurring as a consequence of severe drought events (McDowell 2011, Klein 2015), which causes hydraulic failure to plants (McDowell et al. 2008). Moreover, more frequent extreme droughts have been predicted under climate change in the Mediterranean region for the future (Della-Marta et al. 2007, IPCC 2014), which may place species closer to their drought- tolerant limits.

Quercus ilex L. is a typical evergreen sclerophyllous tree from the Mediterranean Basin with a distribution spanning over a large geographical and altitudinal range from high altitudes to near-coastal sites, always in semiarid climates (Niinemets 2015). A large number of studies in these climates have focused on this species due to its high tolerance to drought (Niinemets 2015). However, a previous study at *Q. ilex*, suggests that under extreme droughts, i.e. long and intense drought events, *Q. ilex* may reach their safety margin and show symptoms of hydraulic failure (Urli et al. 2013). Another coexisting *Quercus* species is *Q. faginea*, a winter deciduous tree, with a leaf life span limited to one growing season (Mediavilla and Escudero 2003). *Q. faginea* also has a relatively high tolerance to summer drought, given the presence of some soil moisture (Esteso-Martínez et al. 2006). Its ability to adapt to semiarid climates is aided by reducing its leaf area or having high stomatal and mesophyll conductance (Peguero-Pina et al. 2016). However, not all species in semiarid climates are drought-tolerant, and species with different strategies coexist (Pratt et al. 2007; Vilagrosa et al. 2014; Forner et al. 2014). *P. nigra* is a conifer species that coexists with *Quercus* species in some Mediterranean forests and shows

high vulnerability to drought. Under drought, *P. nigra* closes stomata early to avoid leaf water potential decreases and hydraulic cavitation, compromising its carbon assimilation and therefore growth (Martín-Benito et al. 2008; Forner et al. 2014).

We determined differences in response to severe drought of these three coexisting Mediterranean tree species that could represent the extremes from isohydric to anisohydric strategy. Functional performance was analyzed during three years of contrasting intensity according to extent of the soil dry-drown period and duration of effective seasonal drought (Forner et al. 2014). Moreover, we assess whether decrease in net photosynthetic rate under drought conditions was primarily due to stomatal closure or to damage to the leaf biochemistry. We describe the interplay between functionality of the hydraulic systems and gas exchange. In addition, and in particular for the two *Quercus* species, osmoregulation as a drought tolerance mechanism was evaluated together with changes in leaf tissue elasticity from P-V curves. We hypothesized that under acute dry periods, drought-resistant species maintain carbon assimilation and the functionality of their hydraulic system, by an efficient strategy that combines mechanisms of avoidance and tolerance to drought. In this way, *Quercus* species maintain a more profligate use of water, but keep higher leaf carbon assimilation, outperforming *Pinus nigra* in response to drought, especially during the driest periods.

MATERIAL AND METHODS

Study site and meteorological data

The study was carried out in a stand at the Armallones site (40º46'37"N, 2º19'42"W, 1,079 m a.s.l.), located in the Alto Tajo Natural Park, Guadalajara (central Spain). The climate is continental Mediterranean with hot and dry summers and cold winters. The main woody species have similar basal areas and are *Quercus faginea* (38.7%), *Pinus nigra* (35.1%) and *Quercus ilex* (25.8%). For more details of the study site see Granda et al. (2013) and Forner et al. (2014).

We randomly-selected five healthy and fully-sun exposed representative dominant trees with similar DBH (diameter at breast height, 1.3 m) of Holm oak, *Q. ilex* subsp. *ballota*, Portuguese oak, *Q. faginea* and black pine, *P. nigra* subsp. *salzmanii* (total n = 15). Mean DBH of the selected trees were 18.38 ± 0.27 cm, 16.40 ± 0.93 cm and 22.82 ± 2.89 cm for *Q. ilex*, *Q. faginea* and *P. nigra* respectively. The low density of the stand allowed the selection of scattered trees with sun exposed crowns, and little interference from other trees nearby.

Meteorological conditions were recorded continuously from 2011 to 2013 at the study site using a HOBO® (Onset Computer Co., Bourne, MA, USA) weather station. It comprised a rain gauge (RGA-MoXX), temperature and relative air humidity logger (12-Bit Temperature/RH Smart Sensor), solar radiation sensor (S-Lib-MOO3) and wind speed guage (Wind Speed Smart Sensor, S-WA-MOO3). Readings of each sensor were recorded every 30 min with a data logger (HOBO® H21-001;

Onset Computer Co.). Air vapour pressure deficit (VPD, kPa) was calculated according to Campbell and Norman (1998), from the atmospheric pressure, the air temperature and the air relative humidity. The relative extractable water in the soil (REW) was calculated according to Granier et al. (1999). REW was calculated daily as the ratio of plant available soil water to maximum extractable water. REW below the threshold of 0.4 indicates water stress reached levels in which water is not available to tree roots, limiting transpiration, and causing high stomatal closure for most temperate forest tree species (Granier et al. 1999).

During the study period (2011-2013) daily temperature and VPD varied between 27.2-18.5 ºC and 2.9-1.5 kPa in early summer, and 23.5-19.1 ºC and 2.5-1.2 kPa in late summer, respectively. The year 2012 was the driest year: it had the highest temperature and VPD and the lowest REW in both, early and late summer (Fig. 1, Table S1). Moreover, early summer of 2012 was even drier than the late summer of the three studied years. There was no precipitation on the days directly prior to measurements of the trees were made.

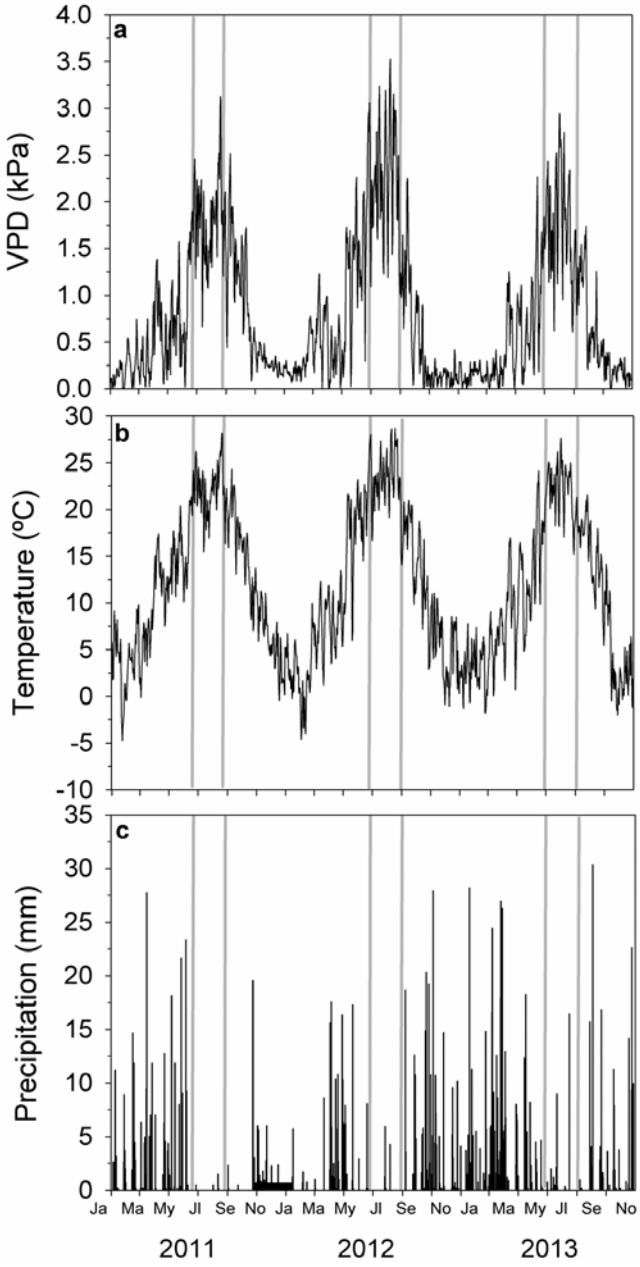

Figure 1. Mean monthly vapour pressure deficit (VPD, kPa) (a) and temperature (T, ºC) (b) and total monthly precipitation (P, mm) (c) for each study year (2011-2013). *Grey lines* divide the two sampling periods, early and late summer.

Water use at the tree level and water status

Water stress endured by trees was assessed from predawn leaf water potential (Ψ_{pd}, MPa) measured in two leaves per tree for each species (five trees per species) at twice through the growing season in all 3 years of the study (early (ESu) and late (LSu) summer respectively; 2011-2013). Excised leaves were collected before predawn and enclosed into sealable plastic bags at saturated relative humidity and CO_2 and kept refrigerated and in dark until measurement was done (Pérez-Harguindeguy et al. 2013). Leaf water potential was measured again in the afternoon (from 1 to 3 p.m.) in transpiring (Ψ_{leaf}, MPa) and non-transpiring leaves as surrogate of xylem water potential (Ψ_{xyl}, MPa). Non-transpiring leaves were attached to the same shoots as transpiring leaves, but enclosed in sealable plastic bags covered with aluminum foil from the afternoon of the previous day until measurements (Begg and Turner 1970; Schultz and Matthews 1993). All leaf water potential measurements were performed using a Scholander pressure chamber (PMS 1000, Soil Moisture Equipment Corp., Santa Barbara, CA, USA).

Water use at tree level was monitored using sap flow sensors EMS 51 (Environmental Measuring Systems, Brno, Czech Republic) over a week of measurements during the two sampling periods, ESu and LSu, all three years (2011-2013). Sensors were installed at breast height on the north side of the trunk of several of those same trees selected for the leaf functional trait measurements (*Q. ilex*: n = 2; *Q. faginea*: n = 4; *P. nigra*: n = 3). The stem heat balance (THB) was used to estimate sap flux (Čermák et al. 2004). Daily sap flow per basal area (Q_v, $m^3.m^{-2}.h^{-1}$)

was measured during three days, including the day of gas exchange measurements for each species at the two sampling periods (ESu and LSu) and for the three years of study (2011-2013). We also calculated the mean of total daily sap flow per basal area (Q_{v7}, $m^3.m^{-2}.h^{-1}$) measured for a full week in the same periods and years, and the percentage reduction of the total daily sap flow per basal area from the maximum recorded during the study period (RM, %).

Three hydraulic conductances were calculated by analogy to the Ohm's law (van den Honert 1948) for the five trees per species and for ESu and LSu in each of the study years (2011-2013). Leaf-specific apparent hydraulic conductance in the soil-plant-atmosphere continuum (K_h, $kg.m^{-2}.s^{-1}.MPa^{-1}$) was calculated by dividing transpiration rate (E, see below) measured in the afternoon by the difference between Ψ_{pd} and Ψ_{leaf}, and considering the predawn leaf water potential as a proxy of the bulk-soil water potential under zero sap flow in the trees. In similar way, the branching system apparent hydraulic conductance (K_b, $kg.m^{-2}.s^{-1}.MPa^{-1}$) and petiole-leaf lamina apparent hydraulic conductance (K_{pl}, $kg.m^{-2}.s^{-1}.MPa^{-1}$) were calculated using the difference between Ψ_{pd} and Ψ_{xyl}, and between Ψ_{leaf} and Ψ_{xyl}, respectively (Aranda et al. 2005).

Leaf functional traits

Functional traits were measured in ESu and LSu in the three years of study. We selected two current-year shoots per tree with fully expanded

and sun leaves without symptoms of herbivore damage. Specific leaf area (SLA, $m^2.kg^{-1}$) was estimated from fresh leaves without their petioles scanned and oven-dried at 65ºC for 48 h. Leaf area was estimated with the software ImageJ from scanned leaves (Rasband 2009). Dry leaves were finely ground (MM300, Retsch), and between 1 and 2 mg of the powdered leaf material from each tree was weighed (Radwag XA 52/2X Semi-Micro Balance, Nevada Weighing, USA) and placed into tin capsules (Sn 98 capsules, Lüdiswiss) to obtain elemental leaf carbon and leaf nitrogen content (C_m and N_m, respectively; %). Analyses were carried out at the Technical Platform for Functional Ecology (OC081) at the INRA Forest Ecology and Ecophysiology Unit, using an EA-GC/IRMS (Delta S, Finnigan MAT, Bremen, Germany; ± 0.2%). We also calculated the ratio between C_m and N_m (C_m/N_m).

Gas exchange and chlorophyll fluorescence measurements were performed on two healthy, fully developed leaves from the same trees of each species selected for all measurements (n = 15) using a LI-Cor 6400 XT (Li-Cor, Inc., NE) gas exchange system with an integrated fluorescence chamber head (Chamber Li-6400-40, Li-Cor, Inc., NE) during ESu and LSu of each of the study years (2011-2013). Measurements were carried out in the morning (M, from 10:30 a.m. to 1 p.m.) and the afternoon (A, from 1 to 3 p.m.). Net photosynthetic rate on a leaf area basis (A_{area}, $\mu mol.m^{-2}.s^{-1}$), stomatal conductance of water vapour (g_s, $mmol.m^{-2}.s^{-1}$), transpiration rate (E, mmol $H_2O.m^{-2}.s^{-1}$), effective quantum yield (ΔF'/Fm'), photochemical quenching (qP) and non-photochemical quenching (qN) were measured at constant CO_2

concentration (400 µmol CO_2.mol^{-1}) using the built-in-Li-6400 XT CO_2 controller, at saturating light intensity (1200 µmol.m^{-2}.s^{-1} in ESu and 1500 µmol.m^{-2}.s^{-1} in LSu), provided by the built-in Li-6400 blue-red light source. Since not all leaves completely covered the Li-6400 photosynthesis chamber, the leaf sections used were traced and removed to estimate their exact area. All areas were calculated using the image analysis software, ImageJ (Rasband 2009). Photosynthetic nitrogen-use efficiency (PNUE, µmol CO_2.mg^{-1} N.s^{-1}) was calculated as the ratio between A_{area} and leaf nitrogen content per area (N_a), which in turn was calculated dividing N_m by SLA.

Pressure-volume curves

Pressure-volume curves were constructed from leaves collected on the same shoots where gas exchange and water stress of trees were recorded. Parameters from P-V curves were only estimated from leaves of two oaks. Two healthy twigs with two or three leaves per tree were cut underwater, and hydrated during 12 h with distilled water in plastic tubes to bring leaves to full turgor but avoiding oversaturation of the tissues. On some cases, this was unavoidable and first points from the Ψ – fresh leaf weight (FLW) relationship were ruled out. Leaf weight at full turgor was estimated from a linear regression of pair of points maintaining the linearity in the Ψ – FLW relationship. The base of each twig was cut under water before the beginning of the curve. Pressure-volume curves were constructed according to the previously well-

established protocol for the free-transpiration method (Dreyer et al. 1990; Aranda et al. 1996; Corcuera et al. 2002). From the pressure-volume curves we estimated: the relative water content at the turgor lost point (RWC_{tlp}, %), relative apoplastic water content (RWC_a, %), modulus of elasticity (ε, MPa), osmotic potential at full turgor (π_o, MPa), osmotic potential at the turgor lost point (π_{tlp}, MPa) and ratio of leaf dry weight to leaf turgid weight (DW/TW).

Statistical analysis

Linear models were used to test differences in meteorological variables (temperature, precipitation and vapor pressure deficit) between years. Linear mixed-effects models (LMMs) were used to assess the effect of year, period (ESu and LSu) and their interaction (fixed factors) on variables related to leaf traits (SLA, C_m, N_m, C_m/N_m, PNUE), gas exchange and chlorophyll fluorescence traits (A_{area}, g_s, ΔF'/Fm', qP, qN), tree water use (Q_v, Q_{v7}, RM), water status (Ψ_{pd}, Ψ_{leaf}, Ψ_{xyl}, K_h, K_b, K_{pl}) and water parameters from pressure-volume curves (RWC_{tlp}, RWC_a, ε, π_o, π_{tlp}) (Zuur et al. 2007). Tree identity was considered as random in the LMMs when considering repeated measures. We performed LMMs by species due to analyze how year, period and meteorological conditions could affect traits differently depending on species. Models were fitted based on a restricted maximum likelihood method using the nlme package from R statistical software (Pinheiro et al. 2007). Differences between species in each of the measured traits according to time of the day,

season and year were tested using a nonparametric Kruskal-Wallis test. Kruskal-Wallis test was used when data were not normal.

Relationships between functional traits were established by fitting linear and non-linear models according to species and period. The instantaneous water-use efficiency (iWUE) was calculated from the slope of the regression between net photosynthetic rate and stomatal conductance to water vapour. The relationship between soil available water and available water for trees was assessed fitting regression curves between relative extractable water and predawn water potential by species. Regressions were performed using SigmaPlot version 11, from Systat Software, Inc., San Jose California USA. Adjusted R-squared (R^2) was indicated in all cases.

Spearman's rank correlation analysis was used to establish relationships among all functional traits. Results were shown using correlograms which were performed using the package corrplot from R statistical software. All analyses were carried out using the R statistical software (version R3.2.3; R Development Core Team 2015, Vienna), if not otherwise indicated.

RESULTS

Water status and water use: differences among species and years

Water stress endured by trees of the three species, as assessed from predawn leaf water potential (Ψ_{pd}) was higher in late (LSu) than in early (ESu) summer. From most of the dates *P. nigra* had a lower Ψ_{pd} than the other species, with the exception of the driest period (LSu of 2012) when *Q. ilex* had the lowest Ψ_{pd} recorded for all the studied years (Fig. 2a,b). In general, *Quercus* species had similar Ψ_{pd} in each of the two sampling periods and years. Reductions in Ψ_{pd} between ESu and LSu were the lomgest in 2011 for the three species, but especially for *Q. ilex*, followed by *P. nigra* and *Q. faginea*. Ψ_{pd} in the three species depended on the interaction between period and year (Table S2). Ψ_{pd} in *P. nigra* was more sensitive to relative extractable water (REW) than Ψ_{pd} of the two *Quercus* species (Fig. S1). At the threshold of REW = 0.4, Ψ_{pd} of *P. nigra* was more than twice as low as the Ψ_{pd} of the two *Quercus* species. At REW = 0.1, the *Quercus* species significantly decreased their Ψ_{pd} and reached a similar (*Q. faginea*) and lower (*Q. ilex*) values compared to *P. nigra*.

Afternoon leaf water potential (Ψ_{leaf}) was higher in *P. nigra* and varied less between Ψ_{pd} and Ψ_{leaf} in both ESu and LSu than for both *Quercus* species (Fig. 2c,d). *P. nigra* was the only species without significant differences in Ψ_{leaf} between years (Table S2). Afternoon leaf water potential in non-transpiring leaves (Ψ_{xyl}) was lowest during the driest

year, 2012, and for *Q. faginea* and *Q. ilex* in ESu and LSu, being significantly lowest in *Q. ilex* in LSu of 2012 (data not shown).

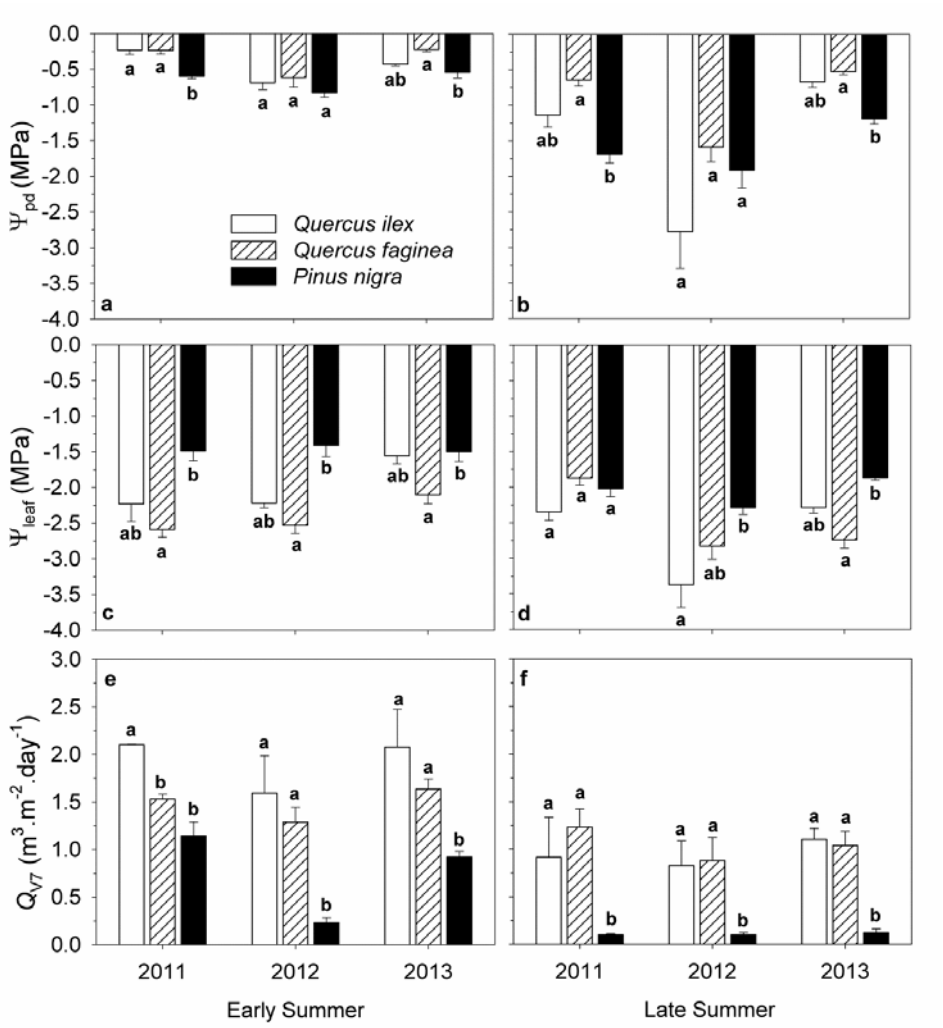

Figure 2. Mean ± SE of predawn leaf water potential (Ψ_{pd}, MPa; a, b), afternoon leaf water potential (Ψ_{leaf}, MPa; c, d) and total daily sap flow per basal area (Q_{v7}, $m^3.m^{-2}.h^{-1}$; e, f) measured for a week at the two sampling periods, early (a, c, e) and late summer (b, d, f) for each species and year (2011-2013). *Small letters* indicate significant differences between species in each sampling period and year at $P < 0.05$.

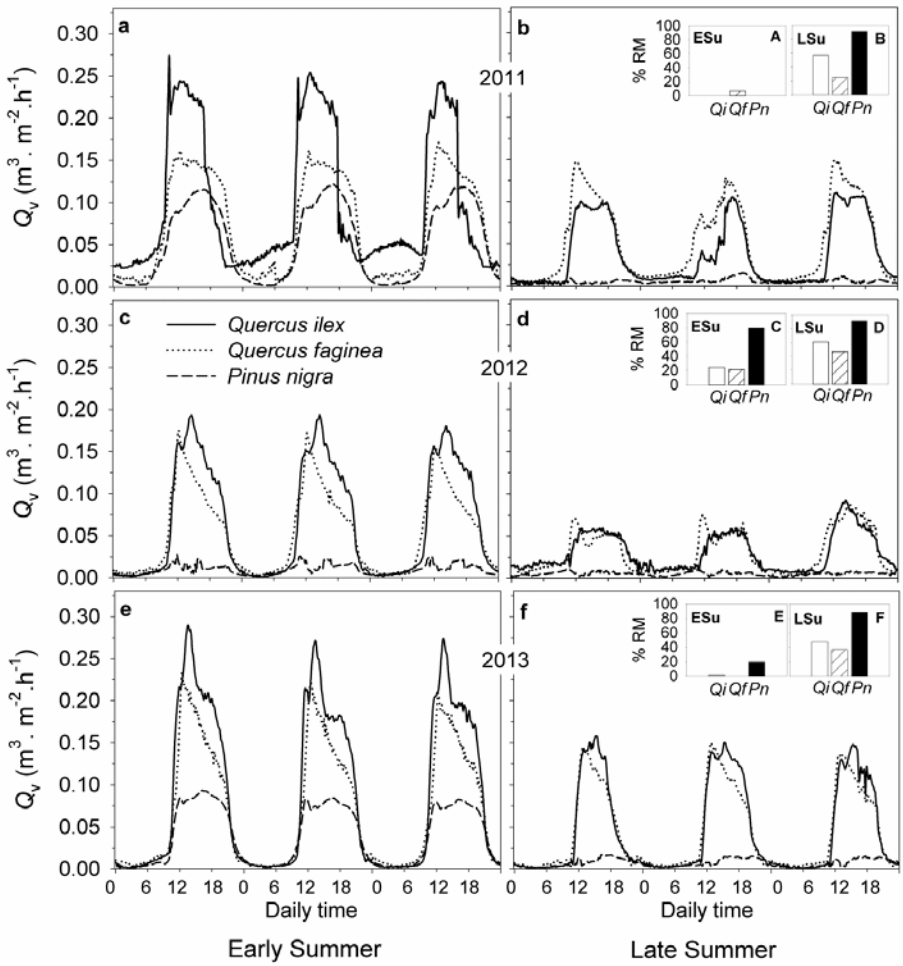

Figure 3. Daily sap flow per basal area (Q_v, $m^3.m^{-2}.h^{-1}$) measured at the two sampling periods, early (a, c, e) and late summer (**b**, **d**, **f**) for each species and year (2011: **a**, **b**; 2012: **c**, **d**; 2013: **e**, **f**). Small inset figures show the percentage reduction of the total daily sap flow per basal area from the maximum, measured for the week of sampling at early (ESu; **A**, **C**, **E**) and late summer (LSu; **B**, **D**, **F**) periods for each species (*Qi*: *Quercus ilex*; *Qf*: *Quercus faginea*; *Pn*: *Pinus nigra*) and year (2011: **A**, **B**; 2012: **C**, **D**; 2013: **E**, **F**).

Water use during the days and week around the gas exchange and water status recording date was estimated from daily sap flow per basal area (Q_v and Q_{v7} respectively). Both Q_v and Q_{v7} were lower in *P. nigra* than the *Quercus* species in both periods and for all study years (Fig. 2e,f;3). *Q. ilex* had higher water use than *Q. faginea* in ESu, but slightly lower in LSu (Fig. 2e,f). Moreover, the differences between both *Quercus* species were higher in 2011. In the driest year, 2012, all species had low water use, although in ESu *P. nigra* already decreased Q_v to similar levels as in LSu. In the three years, the decrease in water consumption from early to late summer was more noticeable in *P. nigra*, with a diminution over the three summers of more than 80 % from maximum measured in ESu of 2011(Fig. 3b,c,d,f, and inlets A-F). Moreover, in ESu of 2012, *Q. faginea* produced an earlier and faster daily decrease in Q_v in the afternoon than *Q. ilex* (Fig. 3c). Water consumption, from analysis of Q_v, depended only on the season in *Q. ilex*, but also on the year for *Q. faginea*, and in the case of *P. nigra* both factors and their interaction influenced the water use of the specie (Table S2).

All three species had lower leaf-specific apparent hydraulic conductance in the soil-plant-air continuum (K_h) in the driest year, 2012, in both ESu and LSu, with the exception of *Q. ilex* in LSu (Fig. 4). Reductions in K_h between 2011 and the driest year, 2012, were higher in *Q. faginea* in both sampling periods, but with similar percentage decrease in *P. nigra* in LSu (ESu: 69 % *Q. ilex*, 77 % *Q. faginea*, 57 % *P. nigra*; LSu: -14 % *Q. ilex* (increase), 79 % *Q. faginea*, 77 % *P. nigra*).

Reductions in K_h due to summer drought were highest in 2013 for *Q. ilex* (60 %) and *Q. faginea* (56 %), but slightly lower in 2013 than in 2012 for *P. nigra* (46 %). The year and its interaction with seasonal period was an important factor determining K_h in all three species, with the exception of *P. nigra*, where K_h did not depend on this interaction (Table S2).

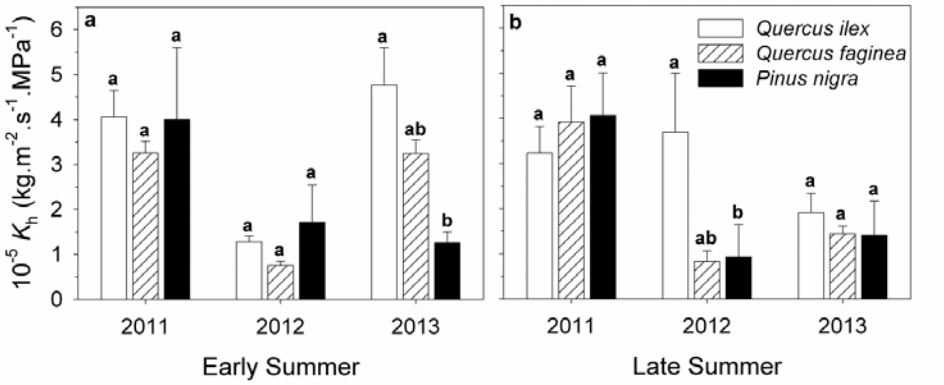

Figure 4. Mean ± SE of leaf-specific apparent hydraulic conductance in the soil-plant-air continuum (K_h, 10^{-5} kg.m^{-2}.s^{-1}.MPa^{-1}; a, b) calculated at the two sampling periods, early (a) and late summer (b), for each study species and study year (2011-2013). *Small letters* indicate significant differences between species in each sampling period and year at $P < 0.05$.

In LSu, only in *Q. ilex* was Ψ_{pd} correlated with any hydraulic conductances, specifically Ψ_{pd} was negatively correlated with branching system apparent hydraulic conductance (K_b) (Fig. S4,S5,S6). In fact, neither Ψ_{leaf} nor Ψ_{xyl} were correlated with any hydraulic conductance in *Q. ilex*, while in *P. nigra* both Ψ_{leaf} and Ψ_{xyl} were correlated with petiole-leaf lamina apparent hydraulic condunctance (K_{pl}). In *Q. faginea* there were correlations between Ψ_{leaf} and all hydraulic conductances, but only

with K_h and K_b in the case of Ψ_{xyl}. All leaf water potentials were correlated with each other in *Q. ilex* and *P. nigra*, but only Ψ_{leaf} and Ψ_{xyl} in *Q. faginea* (Fig. S4,S5,S6).

Species and year differences in leaf functional traits

In general, *Q. faginea* had the highest specific leaf area (SLA), elemental leaf nitrogen content (N_m) and photosynthetic nitrogen-use efficiency (PNUE) (Table 1). *P. nigra* had the lowest values for all those leaf traits with the exception of SLA measured in ESu. Contrary, *Q. faginea* had the lowest ratio between elemental leaf carbon content (C_m) and N_m (C_m/N_m), and *P. nigra* the highest values. No general patterns were evidence for C_m. Period had higher values in ESu for PNUE in *Q. ilex*, N_m in *Q. faginea* and SLA, N_m and PNUE in *P. nigra* (Table S2). Contrary, the ratio C_m/N_m was significantly higher in LSu in *Q. faginea* and *P. nigra*. SLA and PNUE depended on year in all three species, and the driest year (2012) was the year with highest SLA and lowest PNUE (Table 1, S2). In LSu of 2012, *P. nigra* had a reduced PNUE of 96 % and 95 % compared with the same period of 2011 and 2013, respectively. N_m only varied significantly between years in *Q. ilex*, being lower in 2012.

Table 1. Mean ± SE of the specific leaf area (SLA, $m^2.kg^{-1}$), elemental leaf carbon content (C_m, %), elemental leaf nitrogen content (N_m, %), ratio between C_m and N_m (C_m/N_m, unitless) and photosynthetic nitrogen-use efficiency (PNUE, µmol $CO_2.mg^{-1}$ $N.s^{-1}$) during the two sampling periods, early and late summer, for each species and years (2011-2013). For each year and period *different letters* indicate significant differences between species at $P = 0.05$.

Table 1

		2011		2012		2013	
		ESu	LSu	ESu	LSu	ESu	LSu
SLA ($m^2.kg^{-1}$)	*Qi*	3.87 ± 0.11[a]	4.88 ± 0.11[ab]	5.97 ± 0.26[a]	5.43 ± 0.37[a]	5.20 ± 0.25[a]	5.01 ± 0.21[ab]
	Qf	6.78 ± 0.50[b]	8.13 ± 0.20[a]	9.04 ± 0.18[b]	8.74 ± 0.25[b]	9.16 ± 0.43[b]	7.91 ± 0.15[a]
	Pn	4.61 ± 0.31[ab]	4.15 ± 0.10[b]	6.63 ± 0.11[ab]	5.12 ± 0.10[a]	7.46 ± 0.23[ab]	3.76 ± 0.21[b]
C_m (%)	*Qi*	46.52 ± 0.61[ab]	46.09 ± 0.29[a]	43.67 ± 1.63[a]	44.55 ± 1.84[a]	46.50 ± 0.19[a]	44.73 ± 2.49[ab]
	Qf	44.01 ± 0.76[a]	42.98 ± 1.14[a]	45.70 ± 0.43[a]	45.34 ± 1.27[a]	44.25 ± 0.73[a]	45.00 ± 0.35[a]
	Pn	50.64 ± 1.06[b]	42.61 ± 4.00[a]	48.20 ± 1.44[a]	44.96 ± 1.47[a]	43.04 ± 3.00[a]	48.61 ± 0.27[b]
N_m (%)	*Qi*	1.17 ± 0.07[ab]	1.32 ± 0.13[a]	1.06 ± 0.01[a]	1.04 ± 0.04[ab]	1.26 ± 0.05[ab]	1.12 ± 0.08[ab]
	Qf	1.59 ± 0.14[a]	1.44 ± 0.11[a]	1.86 ± 0.21[b]	1.37 ± 0.08[a]	1.83 ± 0.17[a]	1.63 ± 0.14[a]
	Pn	0.99 ± 0.08[b]	0.72 ± 0.08[b]	1.16 ± 0.12[ab]	0.75 ± 0.07[b]	1.13 ± 0.06[b]	0.90 ± 0.03[b]
C_m/N_m	*Qi*	40.42 ± 2.53[ab]	36.16 ± 2.94[a]	41.27 ± 1.73[ab]	43.08 ± 2.02[ab]	37.25 ± 1.47[a]	40.17 ± 1.68[ab]
	Qf	28.47 ± 2.49[a]	30.57 ± 2.66[a]	26.11 ± 3.47[a]	33.68 ± 2.78[a]	24.91 ± 2.08[b]	28.44 ± 2.64[a]
	Pn	52.27 ± 4.42[b]	60.19 ± 2.69[b]	43.31 ± 4.60[b]	61.60 ± 4.73[b]	38.08 ± 0.87[a]	54.53 ± 1.87[b]
PNUE ($\mu mol\ CO_2.mg^{-1}\ N.s^{-1}$)	*Qi*	54.33 ± 1.37[ab]	39.41 ± 4.77[ab]	52.30 ± 8.19[a]	31.21 ± 3.01[ab]	69.94 ± 5.80[ab]	51.16 ± 6.97[ab]
	Qf	84.16 ± 15.78[a]	81.89 ± 17.95[a]	52.83 ± 8.37[a]	46.02 ± 4.01[a]	101.19 ± 10.34[a]	62.68 ± 5.88[a]
	Pn	41.20 ± 5.56[b]	25.72 ± 7.62[b]	35.27 ± 7.99[a]	0.95 ± 4.02[b]	44.34 ± 5.76[b]	18.04 ± 3.58[b]

Regarding to correlations between leaf traits, and leaf water potential and apparent hydraulic conductance, *Q. ilex* only showed correlations between PNUE and Ψ_{pd}, Ψ_{xyl}, K_b and K_{pl} (Fig. S4). In *Q. faginea* SLA was correlated with Ψ_{pd} and Ψ_{xyl} and PNUE with Ψ_{leaf}, Ψ_{xyl}, K_h and K_b (Fig. S5). *P. nigra* was the species with most correlations between these variables (SLA with Ψ_{leaf}, Ψ_{xyl} and K_{pl}; N_m with Ψ_{pd} and Ψ_{leaf}; ratio C_m/N_m with Ψ_{pd}, Ψ_{leaf} and K_{pl}; PNUE with all three leaf water potentials) (Fig. S6).

Net photosynthetic rate on a leaf area basis (A_{area}) and stomatal conductance to water vapor (g_s) followed similar patterns, the highest values in *Q. faginea* were in the morning of the ESu. The lowest values of A_{area} and g_s were measured in *P. nigra*, and according to the year in 2012 for all species (Fig. 5a-d). Differences between g_s measured in ESu of 2011 and of 2012 were higher than the observed for A_{area} in all species. The largest reductions were in g_s between morning and afternoon in all species (> 91 %). However in 2012, in LSu, *P. nigra* A_{area} decreased (mean: 93 %) than g_s (mean: 81%). Reductions in *Q. faginea* and *Q. ilex* for measurements done in the afternoon (A_{area} and g_s) varied between 87-90 % and 72-75 % respectively. Both, morning and afternoon measurements of A_{area} and g_s depended on the year and period (Table S3). A_{area} and g_s were significantly correlated in *Quercus* species but not in *P. nigra*, and in particular in the summer of 2012, when both variables were partially decoupled (Fig. S4,S5,S6). Regressions between A_{area} and g_s measured in morning were significant for all species, and in ESu and LSu, had strong relation was in LSu for all

species (Fig. S2). The slope of the regression can be related with the intrinsic water-use efficiency (iWUE) and this slope increased for all species from ESu to LSu. In fact, *P. nigra* was the species with the largest increase of iWUE through the summer and with the highest iWUE compared with both *Quercus* species. In ESu, all species had similar iWUE. Moreover, in ESu the iWUE of *P. nigra* did not depend on A_{area}, or g_s on Ψ_{pd}, but it did in LSu (Fig. 6a-d). The same trend was observed in *Q. faginea* between g_s and Ψ_{pd} (Fig. 6c,d). In the driest conditions, LSu, and at a same Ψ_{pd} *P. nigra* had the lowest A_{area} and g_s , while both *Quercus* species had similar values within the range of Ψ_{pd} between -1 and -3 MPa. Higher water potentials (> -1 MPa) allowed higher A_{area} and g_s in *Q. faginea* than in *Q. ilex*, while lower values of Ψ_{pd} (< -3 MPa) were only recorded in *Q. ilex*. In fact, both *Quercus* species showed significant correlations between A_{area} and g_s and Ψ_{pd}, Ψ_{leaf} and Ψ_{xyl} but those correlations were not apparent for *P. nigra* (Fig. S4,S5,S6). However, A_{area} and g_s were correlated with K_h, K_b and K_{pl} only in *Q. faginea* and *P. nigra* (no significant correlation between A_{area} and K_{pl} in *P. nigra*) (Fig. S4,S5,S6).

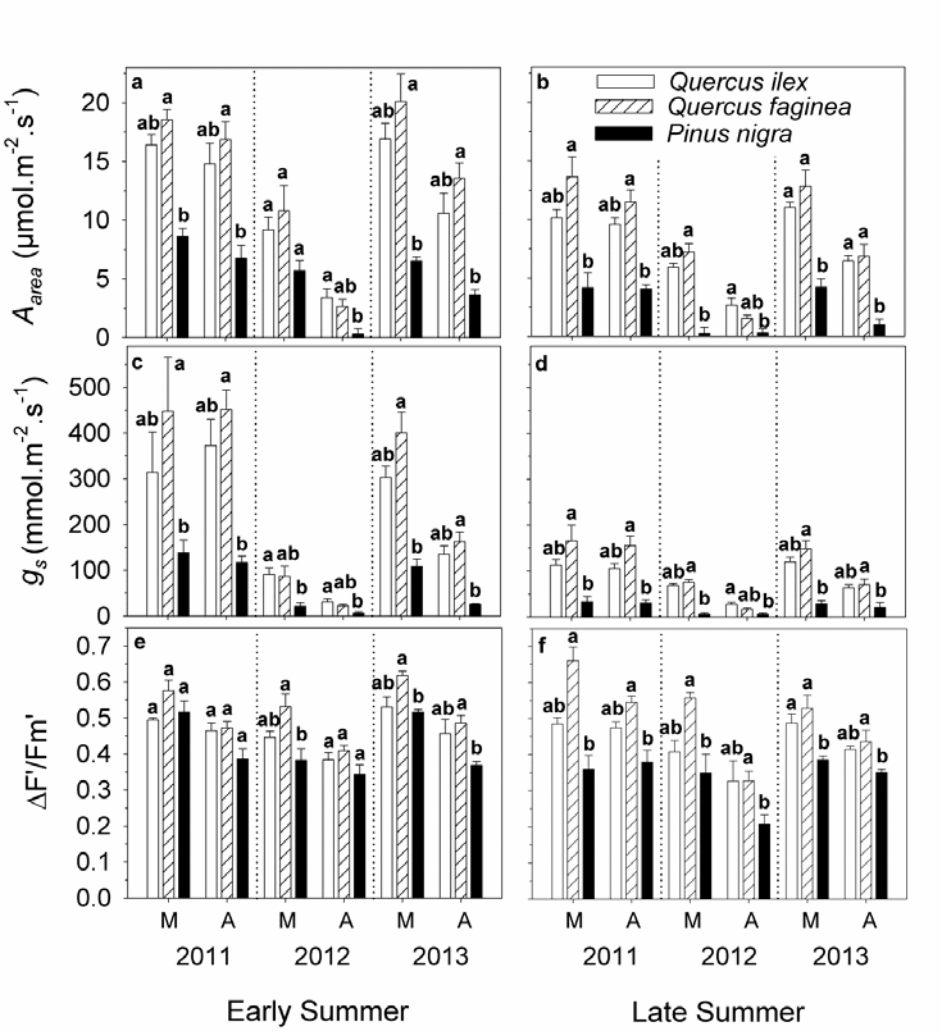

Figure 5. Mean ± SE of net photosynthetic rate on a leaf area basis (A_{area}, µmol.m^{-2}.s^{-1}; **a**, **b**), stomatal conductance of water vapour (g_s, mmol.m^{-2}.s^{-1}; **c**, **d**) and effective quantum yield (ΔF'/Fm'; **e**, **f)** measured in the morning (M) and the afternoon (A) in the two sampling periods, early (**a**, **c**, **e**) and late summer (**b**, **d**, **f**) for each species and year (2011-2013). Dotted lines separate different years. *Small letters* indicate significant differences between species each morning and afternoon for each sampling period and year at $P < 0.05$.

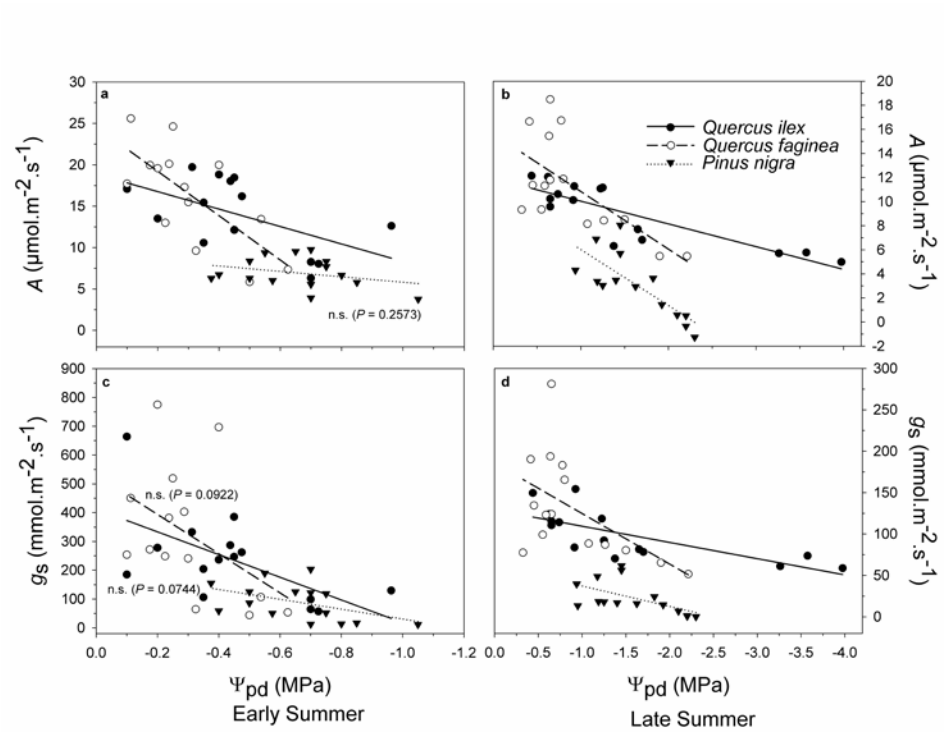

Figure 6. Relationship between predawn leaf water potential (Ψ_{pd}, MPa) and net photosynthetic rate on a leaf area basis (A_{area}, µmol.m^{-2}.s^{-1}; a, b) and stomatal conductance of water vapor (g_s, mmol.m^{-2}.s^{-1}; **c**, **d**) measured in the morning for each species and the two sampling periods, early (**a**, **c**) and late summer (**b**, **d**). Significant regressions for early summer: A_{area} = 18.87 + 10.55*Ψ_{pd}, R^2 = 0.29, P = 0.0215 and g_s = 412.25 + 394.28*Ψ_{pd}, R^2 = 0.33, P = 0.0143 (*Quercus ilex*; **a** and **c** respectively); A_{area} = 24.58 + 25.80*Ψ_{pd}, R^2 = 0.46, P = 0.0045 (*Quercus faginea*; **a**). Significant regressions for late summer: A_{area} = 11.93 + 1.89*Ψ_{pd}, R^2 = 0.70, P < 0.0001 and g_s = 129.08 + 19.61*Ψ_{pd}, R^2 = 0.52, P = 0.0015 (*Quercus ilex*; **b** and **d** respectively); A_{area} = 15.63 + 4.79*Ψ_{pd}, R^2 = 0.40, P = 0.0066 and g_s = 185.78 + 61.04*Ψ_{pd}, R^2 = 0.25, P = 0.0337 (*Quercus faginea*; **b** and **d** respectively); A_{area} = 10.66 + 4.64*Ψ_{pd}, R^2 = 0.56, P = 0.0013 and g_s = 61.83 + 24.63*Ψ_{pd}, R^2 = 0.28, P = 0.0253 (*Pinus nigra*; **b** and **d** respectively). Note that different scales for independent graphs are for clarity.

Effective quantum yield (ΔF'/Fm') was slightly higher in ESu than in LSu, in the morning than in the afternoon and in *Q. faginea* than in the other two species (Fig. 5e,f). *P. nigra* produced the lowest values of ΔF'/Fm' although in general they were not significantly different from *Q. ilex*. The driest year brought about the lowest values recorded in the three species. In *P. nigra*, ΔF'/Fm' depended on year and period, but in both *Quercus* species seasonal ΔF'/Fm' was stable and only depended on year (Table S3). ΔF'/Fm' was significantly correlated with water potentials and hydraulic conductance in *Q. faginea*, but only with the former in *P. nigra* (Fig. S5,S6). Photochemical and non-photochemical quenching (qP and qN, respectively) did not show a clear pattern of differences between species and in response to drier conditions, although lower values of qP were observed in LSu for all species (Fig. S3).

Pressure-volume curves in Quercus species

General patterns indicated that relative water content at the turgor lost point (RWC_{tlp}), osmotic potential at full turgor (π_o) and at the turgor lost point (π_{tlp}), and ratio of leaf dry weight and leaf turgid weight (DW/TW) were higher in current year leaves of *Q. ilex* than in those of *Q. faginea* in ESu (Table 2). In LSu, $RWC_{tlp,}$ and the modulus of elasticity (ε) tend to be higher in *Q.ilex*, and also had larger QW/LW in most periods and years. The most relevant result was that related with π_o and π_{tlp}, that decreased significantly in both species in 2012 with respect to 2011 and

2013. Only in LSu of the driest year, there were significant differences between both species in π_o and marginally non-significant in π_{tlp} (Table 2). The response of ε, π_o and π_{tlp} to Ψ_{pd} was only significant in *Q. ilex* (Fig. 7). Among those three variables, in both species we observed that π_{tlp} and π_o were the variables most determined by Ψ_{pd}. However, in *Q. faginea* none of water related parameters obtained from pressure-volume curves were significantly correlated with water leaf and xylem potentials or hydraulic conductances (Fig. S5). However, in *Q. ilex* there were significant correlations among most of those parameters and Ψ_{pd} , Ψ_{xyl}, Ψ_{leaf} and K_b (Fig. S4).

Table 2. Mean ± SE of the relative water content at the turgor lost point (RWC_{tlp}, %), relative apoplastic water content (RWC_a, %), modulus of elasticity (ε, MPa), osmotic potential at full turgor (π_o, MPa), osmotic potential at the turgor lost point (π_{tlp}, MPa) and ratio of leaf dry weight and leaf turgid weight (DW/TW) during the two sampling periods, early (ESu) and late summer (LSu), for *Quercus* species (*Qi*: *Quercus ilex*; *Qf*: *Quercus faginea*) and study years (2011-2013). *Numbers* in bold indicate significant differences between species at $P = 0.05$.

Table 2

		2011		2012		2013	
		ESu	LSu	ESu	LSu	ESu	LSu
RWC_{tlp} (%)	*Qi*	**90.21 ± 1.24**	**84.35 ± 1.47**	87.38 ± 1.61	83.60 ± 1.15	85.14 ± 1.33	**89.76 ± 0.51**
	Qf	**83.92 ± 1.92**	**77.83 ± 1.39**	83.75 ± 1.33	78.64 ± 2.18	83.48 ± 1.36	**85.56 ± 1.74**
RWC_a (%)	*Qi*	**60.09 ± 8.48**	34.69 ± 7.01	40.56 ± 9.53	34.35 ± 6.33	54.08 ± 5.99	**70.97 ± 2.19**
	Qf	**30.38 ± 3.86**	25.33 ± 7.05	34.72 ± 6.38	27.71 ± 6.37	40.85 ± 5.20	**54.39 ± 4.57**
ε (MPa)	*Qi*	7.27 ±2.28	11.48 ± 1.30	11.19 ± 2.15	12.35 ± 1.14	6.46 ± 0.92	6.15 ± 1.15
	Qf	8.00 ± 0.69	8.80 ± 1.01	11.61 ± 1.43	9.90 ± 1.44	8.16 ± 0.72	6.19 ± 0.69
π_o (MPa)	*Qi*	-1.75 ± 0.27	-2.67 ± 0.13	-2.03 ± 0.11	**-2.96 ± 0.12**	-1.98 ± 0.16	-1.77 ± 0.12
	Qf	-1.91 ± 0.22	-2.37 ± 0.10	-2.25 ± 0.16	**-2.42 ± 0.13**	-2.06 ± 0.12	-1.89 ± 0.15
π_{tlp} (MPa)	*Qi*	-2.54 ± 0.39	-3.41 ± 0.10	-2.64 ± 0.13	-3.91 ± 0.13	-2.97 ± 0.23	-2.76 ± 0.09
	Qf	-2.54 ± 0.24	-3.31 ± 0.13	-3.00 ± 0.18	-3.44 ± 0.19	-2.87 ± 0.13	-2.78 ± 0.12
DW/TW	*Qi*	0.53 ± 0.03	**0.55 ± 0.01**	0.50 ± 0.03	**0.53 ± 0.00**	**0.55 ± 0.00**	**0.55 ± 0.01**
	Qf	0.51 ± 0.03	**0.49 ± 0.01**	0.47 ± 0.01	**0.48 ± 0.00**	**0.46 ± 0.01**	**0.50 ± 0.01**

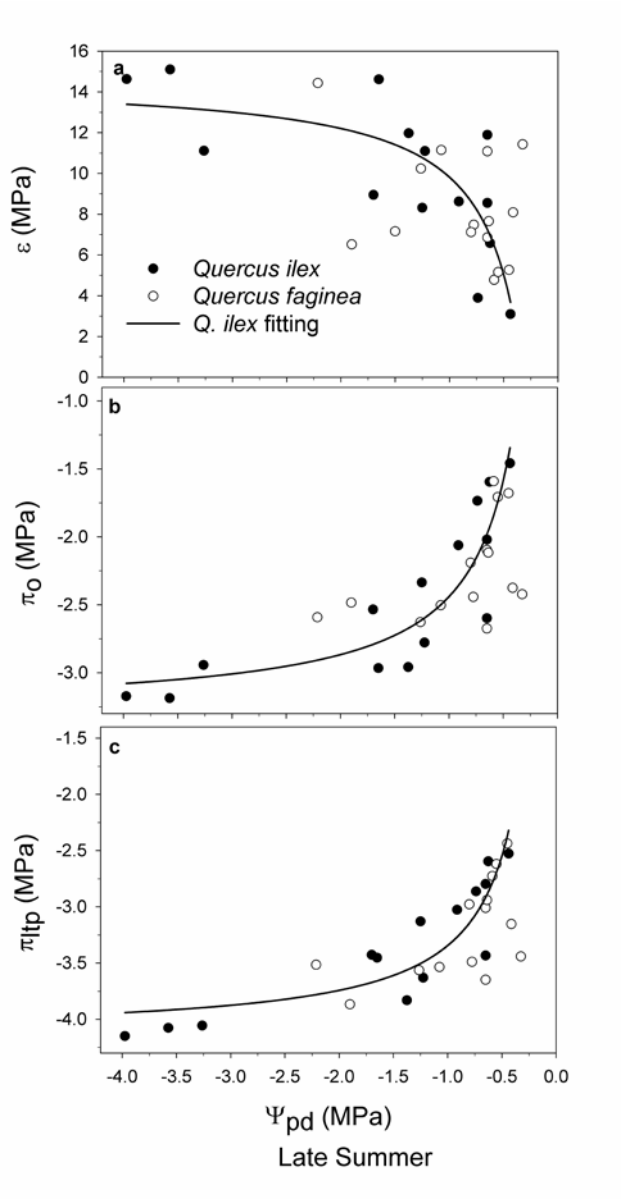

Figure 7. Relationship between predawn leaf water potential (Ψ_{pd}, MPa) and modulus of elasticity (ε, MPa; **a**), osmotic potential at full turgor (π_o, MPa; **b**) and osmotic potential at the turgor lost point (π_{tlp}, MPa; **c**) for *Quercus* species for the late summer period. Significant regressions for *Quercus ilex*: $\varepsilon = 14.59 + (4.77/\Psi_{pd})$, $R^2 = 0.56$, $P = 0.0013$; $\pi_o = -3.29 + (-0.85/\Psi_{pd})$, $R^2 = 0.74$, $P < 0.0001$ and $\pi_{tlp} = -4.14 + (-0.80/\Psi_{pd})$, $R^2 = 0.75$, $P < 0.0001$ (**a**, **b** and **c** respectively). No significant regressions for *Quercus faginea*.

DISCUSSION

The three species followed specific strategies to cope with dry environmental conditions typical of a continental Mediterranean site. But regardless of this, all of them showed the negative impact caused by the extreme drought. Even though *P. nigra* followed a drought-avoiding strategy, the species was not able to cope with the severe drought that occurred in 2012 without substantial effects on its water use and carbon uptake capacity. Contrary, both *Quercus* species adopt more drought-tolerant strategies: *Q. ilex* had wider tolerance threshold to severe drought than *Q. faginea* (Mediavilla and Escudero 2003). This agrees with studies where *Q. ilex* had higher plasticity in response to seasonal changes in temperature and soil water availability than other coexisting Mediterranean species (Sperlich et al. 2015).

All three species leaf net photosynthetic rates declined as drought increased from early to late summer, with very low rates especially during the severe drought of 2012, and in *P. nigra*, which even came to photorespirate in the afternoon. However, main decrease in carbon assimilation was due to stomatal closure to avoid water losses, with a minor effect in the photochemistry of the three species. The drought-avoiding species *P. nigra* had high sensitivity of stomatal conductance to soil moisture scarcity and high evaporative demand, closing stomata more than *Quercus* species even in early summer. In fact, during the severe drought of 2012, *P. nigra* showed almost total stomatal closure during early summer that it was confirmed at the tree level from the very low sap flow measured even from early summer. In

2012 *P. nigra* maintained the lowest gas exchange rates recorded at any time during the full study for all species. This was due to the very high vapour pressure deficit and temperatures recorded during early summer, together with very little rainfall. These were the most stressful meteorological conditions observed during the whole study period. The relationship between net photosynthesis rate and stomatal conductance got stronger in late summer, when drought increased. In fact, water use efficiency, i.e. the slope of this regression, was also highest in late summer for all three species indicating an increase in WUE. The drought-avoiding species, *P. nigra*, had the highest water use efficiency during late summer and also the highest sensitivity to drought. It was able to increase its WUE more than *Quercus* species under varying intensities of summer drought. However, the water saving strategy of *P. nigra* translated into severe reductions in carbon uptake already in the dry early summer of 2012. This highlights the inefficiency of this strategy under severe drought to support continued carbon uptake. In the long term, this strategy could jeopardise the sustainability of *P. nigra*, because of carbon starvation if equally dry periods as in 2012 were to reoccur (Anderegg et al. 2012).

The deciduous *Quercus*, *Q. faginea*, had higher stomatal conductances and net photosynthetic rates than the evergreen *Quercus*, *Q. ilex* (Mediavilla and Escudero 2003). Despite similar trends in regulation of gas exchange by both oaks, differences in whole tree daily stomatal control of water losses between *Quercus* species became apparent when daily sap flow was analysed in detail. *Q. ilex* kept

stomata open at midday even in extremely dry years such as 2012; while *Q. faginea* closed its stomata from early morning onward through the day avoiding water loss and exhibiting a drought-avoidance strategy throughout the day. Therefore, and within the general anysohidric performance of both species, it seems like *Q. ilex* has a wider range of drought tolerance than *Q. faginea* (that showed a tighter control of leaf water loss from early hours in the day). In fact, leaf water relations of both species confirm this claim (see below). The strategy might penalise Q*. faginea* by causing larger decreases in net photosynthetic rate between morning and afternoon, in comparison with *Q. ilex*. This was most apparent in early summer of 2012 under high VPD. All three species only attained lower net photosynthetic rates during the morning and afternoon of 2012 than normally expected, due to their low stomatal conductance, especially in early summer and in *P. nigra*. This could suggest the existence of another factor conditioning net photosynthetic rate, such as damage to the photosynthetic system. However, the decreases in maximum photochemical efficiency of PSII and photochemical quenching we report were not severe and are similar to those expected under drought (Peña-Rojas et al. 2004). We found no significant increases in non-photochemical quenching, which would reinforce the hypothesis the primary role of stomata as the main limitation to photosynthesis in these three species. Though other sources of limitation not studied at present such as mesophyll conductance or biochemical limitations could also have influenced leaf photosynthesis (Cano et al. 2013; reviewed in Flexas et al. 2016)

The biochemical characteristics of *Q. faginea* leaves partially compensated for its higher water stress resulting from its stomatal sensitivity, compared with *Q. ilex*. Thus, leaves of *Q. faginea* were more productive, having a lower SLA, C_m/N_m, DW/TW and higher N_m than *Q. ilex.* Trait characteristic of highly productive species within the worldwide leaf "economics spectrum" (Wright et al. 2004), and conferring higher biochemical capacity and higher stomatal conductance for *Q. faginea* than *Q. ilex* (Juárez-López et al. 2008). These leaf characteristics would also explain the high leaf PNUE in *Q. faginea*, resulting from the evolutionary trade-off between leaf carbon uptake, leaf lifespan and the construction costs of foliage (Villar and Merino 2001; Shipley et al. 2006). Even though the drought was very intense, in 2012, it never reached levels that could have affected tree carbon balance of both *Quercus* by altering significantly their phenology by advancing leaf senescence (Montserrat-Martí et al. 2009), so most changes in the potential for carbon sequestration at the tree level were confined by the specific strategy in regulation of water losses. On the other hand, *P. nigra* was the species with the lowest PNUE, which was due to it being at the opposite end of the range of leaf morphological and biochemical characteristics to *Q. faginea*. Thus, SLA was similar to that of *Q. ilex*, but N_m of pine needles was the lowest of the three species. In this case, leaves with low productivity sum up to the overall higher sensitivity of the species to drought.

Under severe drought, species not only maximize carbon assimilation avoiding damage to their photosynthetic system, but also

limit water losses that could bring about hydraulic failures compromising the survival (McDowell 2011; Anderegg et al. 2012). *P. nigra* had the highest basal water stress during early and late summer, reaching almost -2 MPa of xylem water potential during late summer of 2012. This effective water stress endured by *P. nigra* was near to the threshold which separates safety from damage to the hydraulic system at 50% loss of conductivity (-3.2 MPa: Martínez-Vilalta and Piñol 2003). In fact, it was the species that showed highest drop in K_h, and slowest recovery in the year following the driest one. This could be indicative of slight embolism in *P. nigra*, which could in part limit gas exchange (Sperry et al. 2002) and its ability to resist drought (Hacke and Sperry 2001). *P. nigra* has a narrow safety margin, so during the severe drought it had to close its stomata almost completely to avoid water potential decreasing under its threshold safety at afternoon. However, both *Quercus* species were more drought-tolerant than *P. nigra* and despite their larger drops in predawn water potential than *P. nigra* they kept their stomatas open through the afternoon, allowing continued carbon assimilation and the decrease of leaf and xylem water potentials to their lowest values during the severe drought. The 50% loss of conductivity in *Q. faginea* seedlings occurs at -3.9 MPa (Esteso-Martínez et al. 2006) and in *Q. ilex* adults at -5.6 MPa (Corcuera et al. 2004). Therefore, both *Quercus* species showed a wider range of safety than *P. nigra*. This agrees with the idea that anisohydric species are less prone to hydraulic failure than isohydric species (Garcia-Forner et al. 2016).

The access to available soil water was species-specific, though the largest drop of Ψ_{pd} for the three species was in late summer of 2012. The two oaks seem to have maintained slightly higher values of Ψ_{pd} than the pine in early and late summer. This could be related to a higher capacity to forage for groundwater in both oak species than the pine (Canadell et al. 1999). The exception was the summer of 2012 when unexpectedly low Ψ_{pd} was recorded in *Q. ilex*. We also observed that Ψ_{pd} in the deciduous *Quercus* tended to be higher than that of the evergreen *Quercus*, which was the contrary to the results of Mediavilla and Escudero (2003) observed in the same species. This higher capacity to take water from the soil, together with a high maintenance of the hydraulic function especially in very dry years, allowed a higher resilience of both oak species under extreme events of drought. In this respect, functioning of both species in early 2013 regarding most ecophysiological parameters was in the range of those values recorded in early summer of 2011. However, *P. nigra* recovering was delayed in important parameters such as the K_h. In addition, drought tolerance of both oaks was reinforced by the leaf osmotic capacity adjustment rendering a decrease in the leaf wilting point stated from π_{tlp}. Minimum values of π_o were observed in the late summer of the driest year, but only in *Q. ilex* it the high osmoregulation capacity of the species in response to drought was indicated by the significant relationship between π_o and Ψ_{pd} as a surrogate for the water stress endured by the trees. Relevance of this mechanism to tolerate drought has been stated from long time (Hsiao et al. 1976; Turner and Jones 1980; Bartlett et al. 2012), and in particular is important for understanding the ecological

consequences of drought tolerance within the genus (Abrams 1990; Corcuera et al. 2004; Castro-Díez and Navarro 2007; Aranda et al. 1996). The highest decrease π_{tlp} as consequence of the lowering of π_o would result in higher leaf turgor maintenance in *Q. ilex* than in *Q. faginea*, and the possibility to sustain higher degrees of leaf tissues dehydration as leaf water potentials decreased from early to late summer. This suite of traits explains the high water use strategy maintained during acute drought periods by the evergreen *Q. ilex*.

CONCLUSIONS

As extreme droughts in Mediterranean areas are expected to be more frequent under future warming (IPCC 2014), Mediterranean tree species are expected to face with longer and harsher stressful periods, especially those species not well adapted to drought stress. In our study, extreme droughts impacted much more on *Pinus nigra* than in both *Quercus* sp. Water use was drastically reduced by pines with a direct impact on the capacity to uptake carbon. However, the water use decrease in *Quercus* was smaller, and although both species are considered anisohydric, it was the evergreen *Quercus ilex* that maintained a profligate water use at times of maximum water stress. This strategy was possible because a combination of other mechanisms such as an efficient hydraulic system and the capacity for osmoregulation in leaves conferred the capacity for *Q. ilex* to maintain leaf turgor under stress conditions. Our results suggest that under more frequent extreme droughts, drought-avoiding species will be at a disadvantage compared with drought-tolerant species, and could show signs of decline, probably finally dying of starvation. Although hydraulic failure, with fatal consequences for the health of trees in the long term, cannot be disregarded as a possible future cause of mortality from an increase in recurrence of extreme dry periods. Despite the high water stress endured by trees, hydraulic function and water use at the tree level recovered well during winter and spring from the driest late summer of 2012 to the wet early summer of 2013 in both *Quercus*. In the worst case scenario, changes in forest composition will potentially

affect ecosystem water budgets considering the large differences in water use at the tree level shown by the three species compared.

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SUPPLEMENTARY DATA

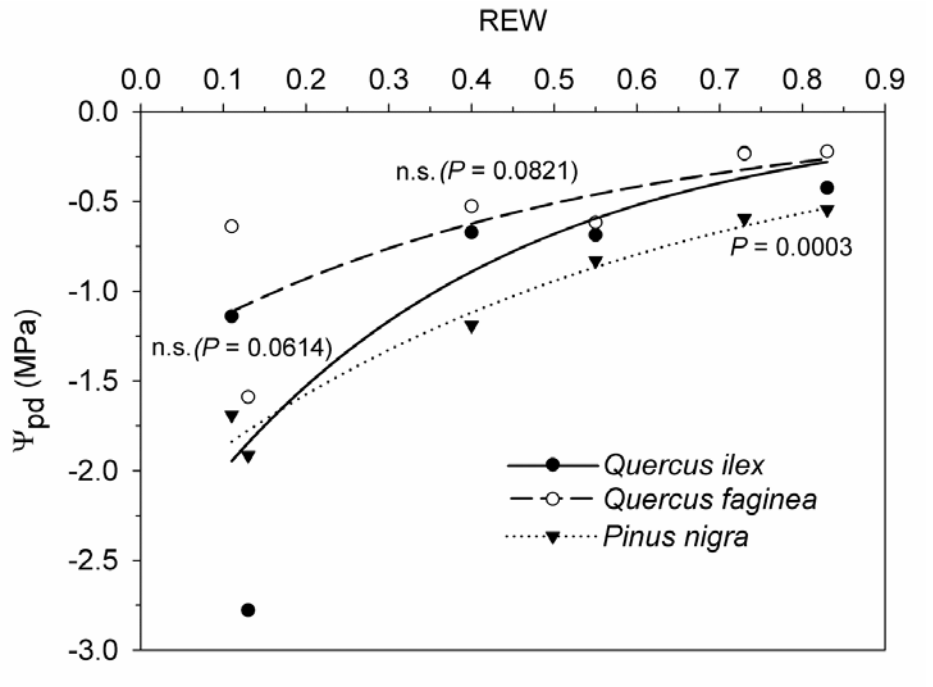


Figure S1. Relationship between relative extractable water (REW) and predawn leaf water potential (Ψ_{pd}, MPa) for each study species. Significative regressions: Ψ_{pd} = -2.22*exp(-1.71*REW), R^2 = 0.96, P = 0.0003 (*Pinus nigra*).

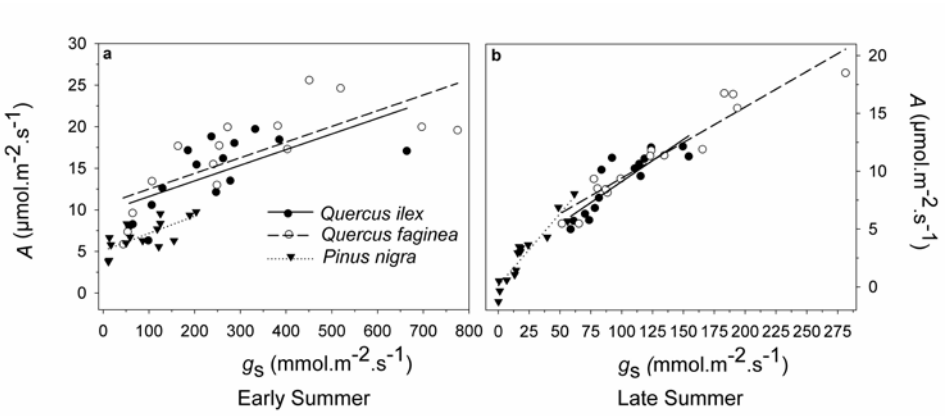


Figure S2. Relationship between stomatal conductance ofwater vapour (g_s, mmol.m^{-2}.s^{-1}) and net photosynthetic rate on a leaf area basis (A_{area}, µmol.m^{-2}.s^{-1}) measured in the morning for each species during the two sampling periods, early (a) and summer (b). Regressions for early summer: A_{area} = 9.72 + 0.02*g_s, R^2 = 0.40, P = 0.0064 (*Quercus ilex*); A_{area} = 10.61 + 0.02*g_s, R^2 = 0.51, P = 0.0017 (*Quercus faginea*); A_{area} = 5.08 + 0.02*g_s, R^2 = 0.48, P = 0.0026 (*Pinus nigra*). Regressions for late summer: A_{area} = 1.83 + 0.07*g_s, R^2 = 0.73, P < 0.0001 (*Quercus ilex*); A_{area} = 3.26 + 0.06*g_s, R^2 = 0.90, P < 0.0001 (*Quercus faginea*); A_{area} = 0.09 + 0.12*g_s, R^2 = 0.88, P < 0.0001 (*Pinus nigra*). Note that different scales for independent graphs are shown to allow better display.

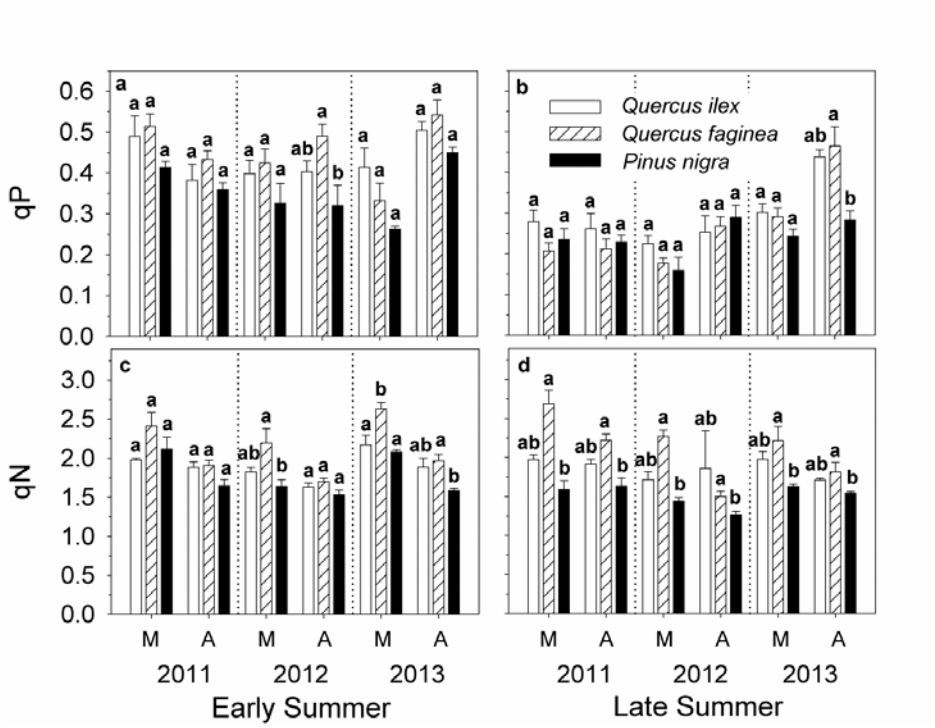


Figure S3. Mean ± SE of photochemical quenching (qP; a, b) and non-photochemical quenching (qN; c, d) measured in the morning (M) and the afternoon (A) at the two sampling periods, early (a, c) and late summer (b, d) for each study species and study year (2011-2013). Dotted lines separate different years. *Small letters* indicate significant differences between species each morning and afternoon for each sampling period and study year at $P < 0.05$.

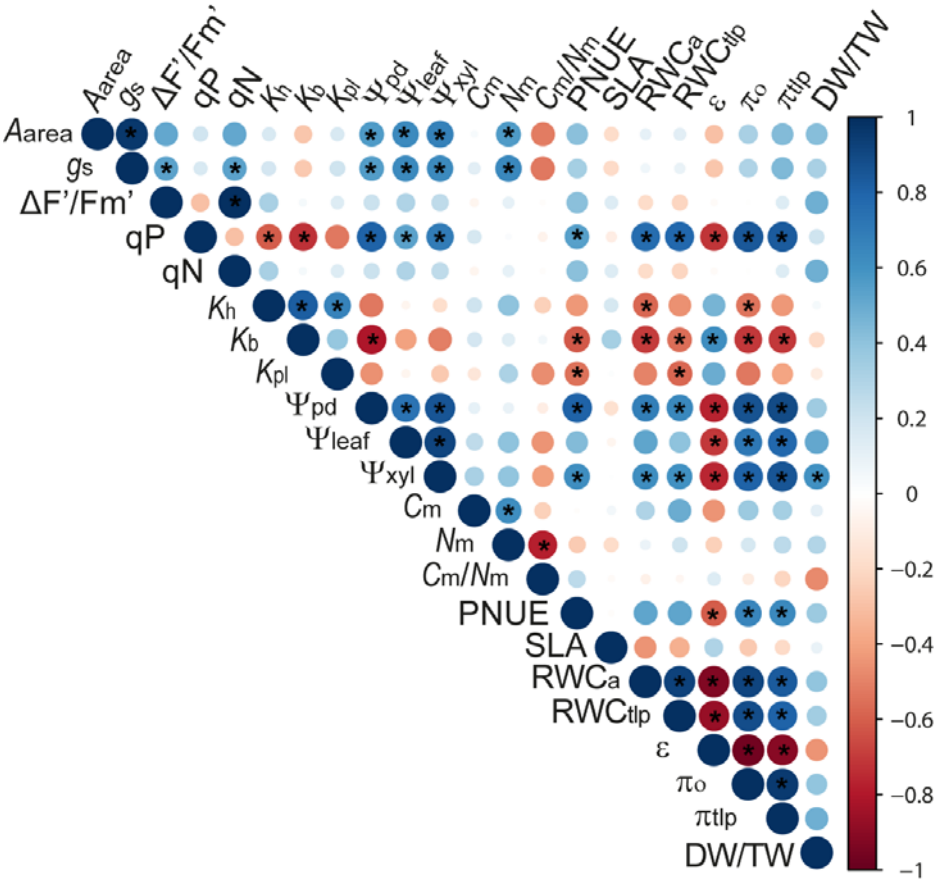


Figure S4. Correlogram for *Quercus ilex*, Spearman's correlations between the net photosynthetic rate on a leaf area basis (A_{area}), stomatal conductance of water vapour (g_s), effective quantum yield (ΔF'/Fm'), photochemical quenching (qP), non-photochemical quenching (qN), leaf-specific apparent hydraulic conductance in the soil-plant-air continuum (K_h), branching system apparent hydraulic conductance (K_b), petiole-leaf lamina apparent hydraulic conductance (K_{pl}), predawn leaf water potential (Ψ_{pd}), afternoon leaf water potential (Ψ_{leaf}), afternoon leaf water potential in non-transpiring leaves (Ψ_{xyl}), elemental leaf carbon content (C_m), elemental leaf nitrogen content (N_m), ratio between C_m and N_m (C_m/N_m), photosynthetic nitrogen-use efficiency (PNUE), specific leaf area (SLA), relative apoplastic water content (RWC_a), relative water content at the turgor lost point (RWC_{tlp}), modulus of elasticity (ε), osmotic potential at full turgor (π_o), osmotic potential at the turgor lost point (π_{tlp}) and ratio of leaf dry weight and leaf turgid weight (DW/TW) measured during late summer and in the afternoon for the gas exchange measurements. *Blue* and *red circles* indicate positive and

negative correlations respectively. Size of the circle and intensity of the colour indicate the magnitude of the correlation, being higher with larger size and more intense colour. Significant correlations at $P < 0.05$ are indicated with asterisk (*).

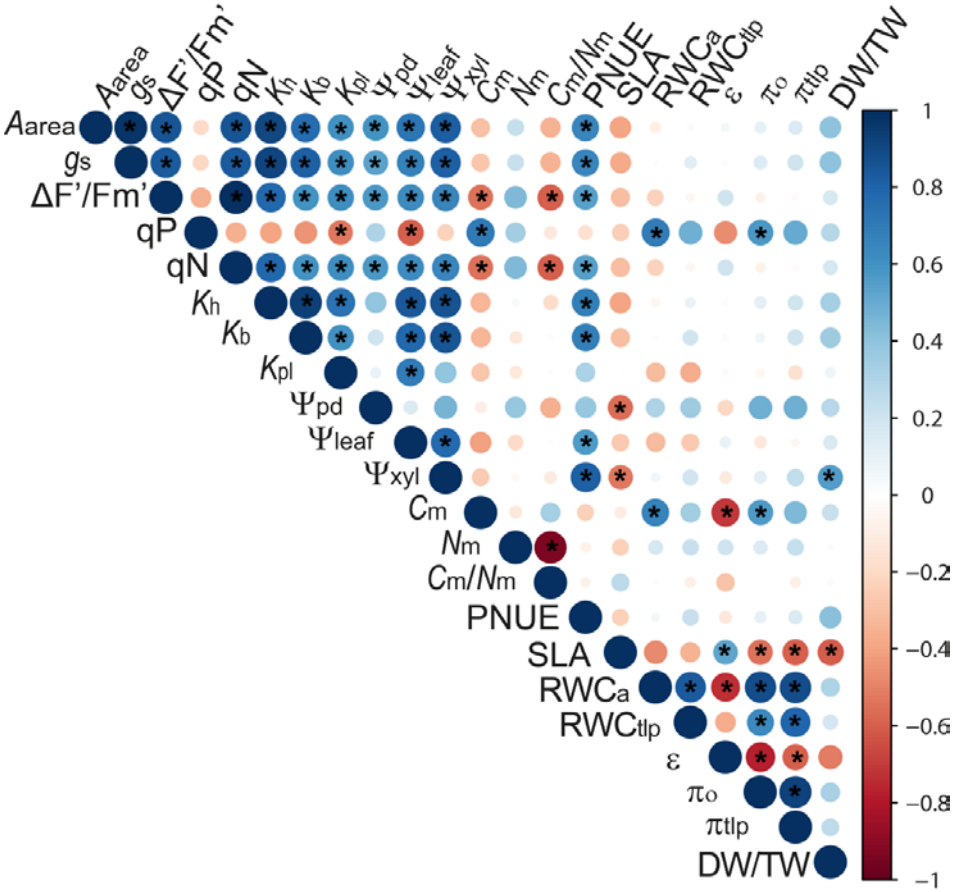


Figure S5. As in Fig. S4 for *Quercus faginea*.

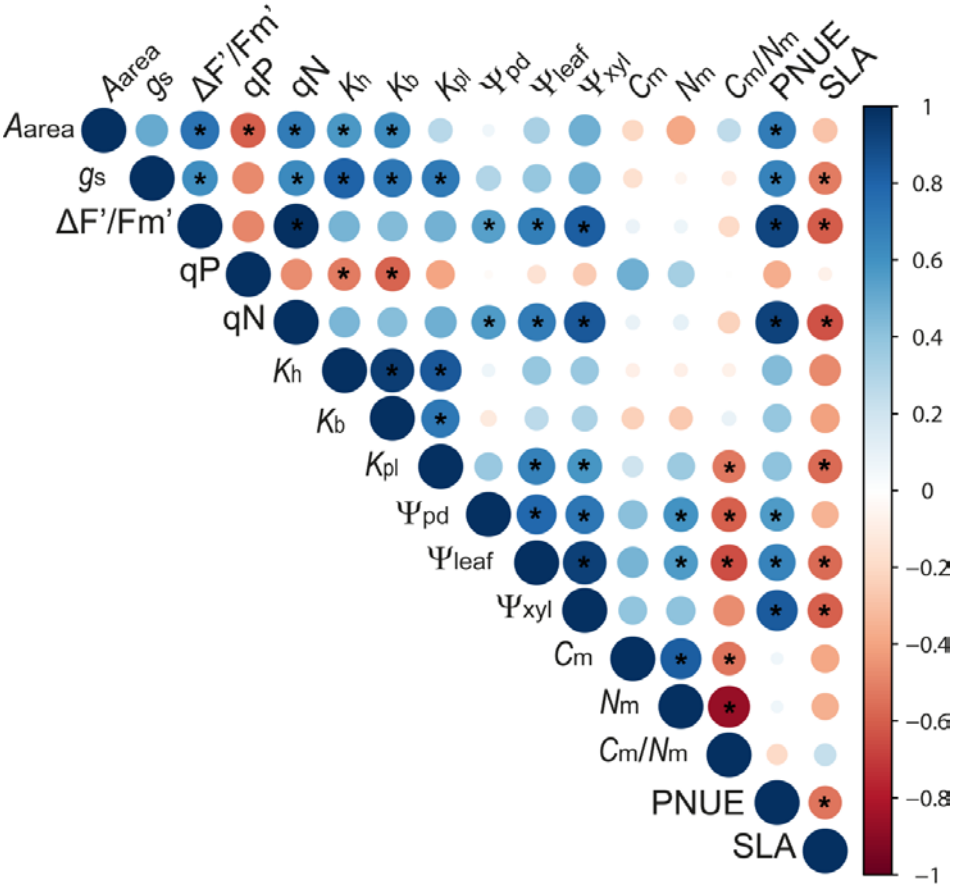


Figure S6. As in Fig. S4 for *Pinus nigra*.

Table S1. Mean ± SE of temperature (*T*, ºC), vapour pressure deficit (VPD, kPa) and relative extractable water (REW) calculated daily and at morning (M) and afternoon (A) during the two sampling periods, early (ESu) and late summer (LSu) for each of the study years (2011-2013).

Year	Period	*T* (ºC)			VPD (kPa)			REW
		Daily	M	A	Daily	M	A	Daily
2011	ESu	22.31 ± 1.27	22.97 ± 1.25	28.08 ± 0.71	1.81 ± 0.09	1.66 ± 0.12	2.89 ± 0.05	0.73 ± 0.01
	LSu	20.44 ± 1.37	20.68 ± 1.89	23.78 ± 5.73	1.56 ± 0.35	1.31 ± 0.50	1.98 ± 1.25	0.11 ± 0.00
2012	ESu	26.64 ± 0.59	28.50 ± 0.48	34.39 ± 0.47	2.85 ± 0.10	2.87 ± 0.04	4.57 ± 0.14	0.55 ± 0.01
	LSu	23.30 ± 0.16	24.42 ± 0.22	30.11 ± 0.28	2.29 ± 0.21	2.25 ± 0.24	3.63 ± 0.33	0.13 ± 0.00
2013	ESu	18.52 ± 0.03	20.46 ± 0.34	26.15 ± 0.35	1.59 ± 0.09	1.71 ± 0.20	2.80 ± 0.20	0.83 ± 0.01
	LSu	21.00 ± 0.31	20.77 ± 0.96	27.14 ± 1.41	1.70 ± 0.01	1.34 ± 0.16	2.68 ± 0.31	0.40 ± 0.01

Table S2. Summary statistics for the linear mixed models (*F*) testing the effect of year, period and its interaction; on the predawn leaf water potential (Ψ_{pd}), afternoon leaf water potential (Ψ_{leaf}), afternoon leaf water potential in non-transpiring leaves (Ψ_{xyl}), total daily sap flow per basal area (Q_{v7}), leaf-specific apparent hydraulic conductance in the soil-plant-air continuum (K_h), branching system apparent hydraulic conductance (K_b), petiole-leaf lamina apparent hydraulic conductance (K_{pl}), specific leaf area (SLA), elemental leaf carbon content (C_m), elemental leaf nitrogen content (N_m), ratio between C_m and N_m (C_m/N_m) and photosynthetic nitrogen-use efficiency (PNUE) for *Quercus ilex*, *Quercus faginea* and *Pinus nigra*. Kruskal-Wallis test was used when data were not normal (X^2). Significant differences are indicated with asterisk (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). n.s. indicated no significant differences.

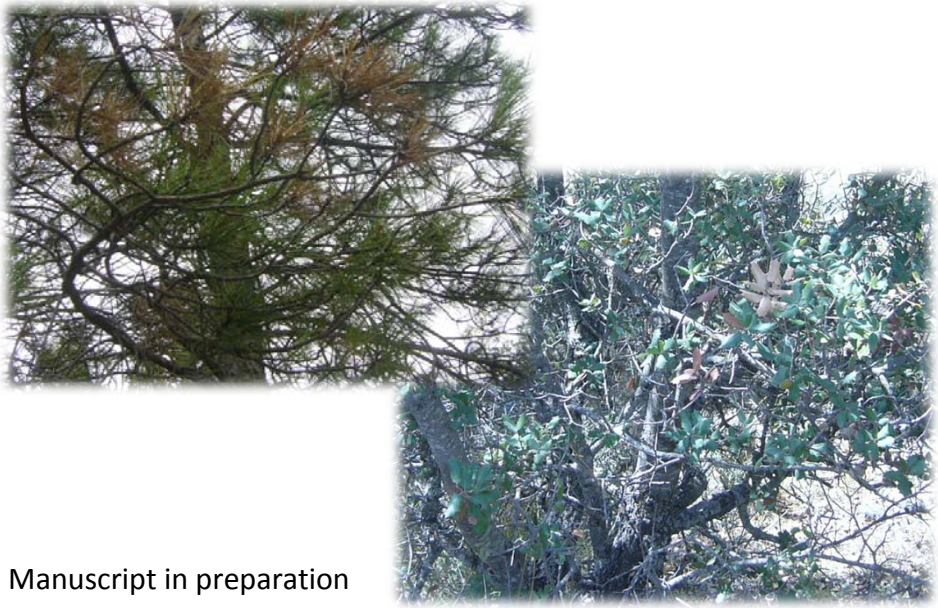
Table S2

		Quercus ilex				*Quercus faginea*				*Pinus nigra*			
		Intercept	Year	Period	Year x Period	Intercept	Year	Period	Year x Period	Intercept	Year	Period	Year x Period
Ψ_{pd}	X^2		7.60*	13.95***	23.92***		9.87**	13.63***	23.79***		n.s.	21.04***	25.11***
Ψ_{leaf}	F	847.91***	11.79***	20.53***	4.15*	1673.47***	6.53**	0.52n.s.	15.95***	1147.35***		34.67***	
Ψ_{xyl}	F	306.73***	59.08***	109.77***		839.69***	32.53***	13.67**		4.57*	7.34**	45.26***	4.23*
Q_{v7}	F	93.85***		34.63***		3472.93***	4.42*	21.86***		14304.71***	48.88***	14265.86***	34.32***
K_h	F	25.21***	4.42*	0.36n.s.	11.79***	920.81***	40.17***	2.71n.s.	6.03**	4.46*	6.91**		
K_b	F	28.95***	2.20n.s.	0.41n.s.	18.48***	0.22n.s.	25.48***			1.44n.s.	8.53**		
K_{pl}	F	52.86***				105.90***	1.36n.s.	0.52n.s.	4.82*	81.31***	13.18***		
SLA	F	29908.16***	21.06***	0.95n.s.	9.22**	4181.22***	11.34***	0.06n.s.	8.77**	9747.91***	32.23***	134.63***	34.35***
C_m	X^2		n.s.	n.s.	n.s.		n.s.	n.s.	n.s.		n.s.	n.s.	n.s.
N_m	F	31.27***	3.69*			178.46***		22.21**		3103.59***		21.02***	
C_m/N_m	X^2		n.s.	n.s.	n.s.		n.s.	3.89*	n.s.		n.s.	12.87***	18.53**
PNUE	F	329.20***	7.40**	19.61***		1342.30***	7.40**			107.59***	3.88*	27.25***	

CAPÍTULO 3

Extreme droughts affecting Mediterranean tree species: the importance of timing and intensity

Alicia Forner, Fernando Valladares, Damien Bonal, André Granier, Charlotte Grossiord, Ismael Aranda



Manuscript in preparation

Images from the front page: dry needles from *P. nigra* and from *Q. ilex* in the Alto Tajo Natural Park.
Author: Alicia Forner.

ABSTRACT

Mediterranean tree species have synchronized their vegetative activity to climatic seasonality, so that extreme droughts occurring during key phenological times can have disproportionate effects on species performance. We investigated the impact of the timing and intensity of extreme droughts on growing season length, growth, transpiration and water-use efficiency of three tree species (*Pinus nigra*, *Quercus ilex* and *Quercus faginea*) coexisting in a continental Mediterranean ecosystem. Annual, seasonal and monthly water stress intensity was calculated for four consecutive years (i.e. 2009-2013) to characterize drought events at these different time scales. Intense droughts were observed not only at annual but also at seasonal and monthly scales particularly during 2011 and 2012. All three species were affected by drought in terms of water-use estimated by sap flow, growth and the growing season length, but with significant species-specific differences. *Q. ilex* showed the highest (63%) and *P. nigra* the least (45%) growth reduction during the driest growing season. *P. nigra* showed higher decrease in tree transpiration in the driest year than the two *Quercus* species. *P. nigra* showed the highest plasticity in water-use efficiency and successfully avoided higher the extensive secondary growth reductions observed in the two *Quercus* species in extremely dry years. Resilience to extreme droughts was different among species, with *Q. faginea* showing poorer recovery of growth after very dry years. *P. nigra* recovered after extreme drought events better than the two oaks despite its higher water use reduction in dry years. Our results revealed that the timing of

extreme drought events, i.e. their onset, duration and temporal scale, can affect tree functioning to a larger extent than drought intensity.

Key words: *Quercus ilex*, *Quercus faginea*, *Pinus nigra*, water-use efficiency, extreme drought, carbon isotope composition

INTRODUCTION

Mediterranean species are adapted to cope with long dry summer seasons, when soil water resources are scarce and temperatures are high. These plants are able to cope with strong water stress by demonstrating a high potential for physiological and morphological plasticity. For instance, plants in dry climates are known to decrease their specific leaf area (Valladares and Sanchez-Gomez 2006), increase their rooting depth (David et al. 2007), take up water in deeper soil horizons (Voltas et al. 2015) exert stronger stomata control (McDowell et al. 2013) and increase water-use efficiency (Medrano et al. 2009). These plants have also synchronized their main vegetative activity with the climatically most favorable periods of the year (i.e. spring and autumn seasons for Mediterranean regions). In fact, Mediterranean tree species are able to adjust their growing season length to climatic conditions and some species can advance leaf emergence when exposed to increasing temperatures (Gordo and Sanz 2009, Morin et al. 2010), despite the risk of early frosts, while other species delay the foliar development (Adams et al. 2015). Nevertheless, those adaptations do not give full insurance to overcome the negative effects derived from a more frequent exposure to extreme droughts as predicted with climate change in the Mediterranean region in the future (Della-Marta et al. 2007, IPCC 2014). Several consecutive extreme droughts can exceed the drought tolerance thresholds and result in episodes of tree decline and mortality events (Breshears et al. 2005, 2008, Allen et al. 2010, McDowell 2011, Klein 2015).

Even though growth reductions have been observed in Mediterranean forests under drought (Sarris et al. 2007, Barbeta et al. 2013), forest responses to extreme droughts are difficult to predict due to the nonlinear relationship between climatic perturbations and tree growth (Lloyd et al. 2013, Vicente-Serrano et al. 2015). Dry conditions have been observed to enhance water-use efficiency (WUE) (Granda et al. 2014, Vicente-Serrano et al. 2015). However, higher WUE does not always imply growth enhancement neither at mesic nor at xeric sites (Lévesque et al. 2014). Moreover, increases in WUE in the long-term do not avoid growth decreases due to drought conditions (Peñuelas et al. 2008). The relationship between growth and WUE seems to be species-specific and particularly sensitive to local environmental conditions (Camarero et al. 2015).

Integrated WUE, by synthesizing the tradeoff between growth and water loss is a key variable to assess plant performance in arid regions, but its estimation is not trivial. WUE inferred by integrating tree growth in terms of basal area increment (BAI) and water loss in terms of tree transpiration estimated from sap flow measurements (WUE_{tree}), offers a realistic integrative view of drought stress effects on the strategies followed by different species in optimizing carbon uptake under water limitations (Sánchez-Costa et al. 2015). WUE_{tree} gives a whole plant perspective and allows the exploration of a wide range of time scales, from days or months to years (McCarthy et al. 2011). However, water-use efficiency estimated from carbon isotope composition in leaves ($\delta^{13}C$), intrinsic water-use efficiency (iWUE), gives

also an integrated information of the tradeoff between net CO_2 assimilation and stomatal conductance during the period the carbon was fixed (i.e. during leaf formation) (Farquhar et al. 1989). Moreover, $\delta^{13}C$ has been used as an indicator of intensity of drought exposure in plants (O'Leary 1995, Saurer et al. 1995), and to establish ecological strategies with regard to water-use from single individuals to population and species (Aranda et al. 2010, de Miguel et al. 2012, Lázaro-Nogal et al. 2015). Therefore, a combination of both WUE estimates is likely to better describe the interplay between growth and water-use during extreme droughts.

Intensity of drought estimated from precipitation, temperature and soil moisture has been widely recognized as a factor determining the extent of drought effects over tree species (McDowell 2011). However, less attention has been paid to the timing of drought, which has been suggested as an important factor as drought intensity for Mediterranean ecosystems. Drought stress can affect species during their key phenological phases by impairing their reproductive success (Aragón et al. 2008), or during resumption of cambial activity in temperate biomes (Camarero et al. 2010, Rozas et al. 2011). Therefore, effects of annual, seasonal or monthly droughts on key phenological phases of the species could affect species functioning (e.g. growth and water-use efficiency). Decreases in net primary production due to drought could be compensated by longer growing periods if species could take advantage of mild conditions produced before or after drought periods and during short but intense rain events. However, this

is still unclear because lengthening the growing period is not necessarily enhancing annual growth, since climatic conditions at autumn, especially solar radiation, are not favorable enough to compensate growth reductions during drought. However, not many studies have addressed how climate change affects the functioning of trees at the end of the growing season (Richardson et al. 2013). Studies have shown that lower spring precipitation in a given year could reduce spring growth of the following year (Morán-López et al. 2014). Therefore, drought stress at short or long scales not only reduces growth of the current year but also affects the following growing season, as has been observed in dendrochronology studies (Badeau et al. 1996, Linares et al. 2010), which farther complicate the estimation of the influence of altered phenologies on actual growth. Moreover, the annual growth depends not only on the precipitation of the current or previous year but also and mainly on the past years' precipitation (Sarris et al. 2007), which could indicate the existence of carry over effects on growth due to drought events.

Therefore, the evaluation of how both intensity and timing of an extreme drought affect different functional processes, from growth to water economy, closely connected with success of Mediterranean tree species under stressful conditions is essential to elucidate ecosystems responses to future scenarios. The main objective of this study was to identify how the timing and intensity of an extreme drought impacts on growth, tree transpiration and water-use efficiency of three coexisting Mediterranean tree species. Specifically, we hypothesized that timing of

extreme droughts can affect more tree functioning that drought intensity, and that those effects are species-specific. We also hypothesized a decoupling between growth and water use according to species, so species with higher increases in water-use efficiency during drought would experience lower decreases in growth than other species with lower increases in water-use efficiency.

MATERIAL AND METHODS

Study site

The study was conducted at the Alto Tajo Natural Park, Guadalajara (central Spain). Soils are calcisols from Cretaceous and Jurassic limestone that rendered shallow and poorly developed soil (Ferrero et al. 2006). The climate is continental Mediterranean with hot and dry summers and cold winters. Mean annual temperature and precipitation are 10.3 ºC and 490.5 mm, respectively (Molina de Aragón, 40º50'40" N, 1º53'07" W, 1951-2013 period; data provided by the Spanish Meteorological Agency, AEMET).

We selected a stand at the Armallones site (40º46'37"N, 2º19'42"W, 1,079 m a.s.l.), where the three main woody species studied have similar importance in terms of basal area: *Quercus faginea* (38.7%), *Pinus nigra* (35.1%) and *Quercus ilex* (25.8%). It has a western orientation with a 48% slope and a total basal area of 13.7 m^2 ha^{-1}. We randomly selected healthy and fully sun exposed representative trees with similar DBH (diameter at breast height, i.e. at 1.3 m height) of Portuguese oak, *Q. faginea* (*n* = 5), black pine, *P. nigra* subsp. *salzmanii* (*n* = 4) and Holm oak, *Q. ilex* subsp. *ballota* (*n* = 3). *Q. faginea*, *P. nigra* and *Q. ilex* mean diameters of selected trees were in the range of 25.5 ± 3.0 cm, 23.5 ± 2.5 cm and 21.4 ± 1.5 cm respectively. The low density of the stand allowed selecting scattered trees with well sun exposed crowns, and with a low interference with neighbor trees. Mean leaf area index (LAI) of the study site is 1.9 ± 0.3 $m^2.m^{-2}$.

Microclimatic conditions were recorded continuously since 2010 to 2013 at the study site using a HOBO® (Onset Computer Co., Bourne, MA, USA) weather station. It was formed by a rain gauge (RGA-MoXX), temperature and relative air humidity (12-Bit Temperature/RH Smart Sensor), solar radiation (S-Lib-MOO3) and wind speed (Wind Speed Smart Sensor, S-WA-MOO3) sensors. Readings of each sensor were recorded every 30 min with a data logger (HOBO® H21-001; Onset Computer Co.).

Drought intensity and timing

Drought intensity and duration was assessed by calculating two drought indexes: a water stress index, I_s and P/PET. Calculation of both drought indexes allowed comparisons with other studies and a more accurate description of drought intensity both regional and local scale. Regional climatic data for the long-term series (1951-2013) was obtained from the closest weather station (at ca. 36 km from the study site), Molina de Aragón (40º50’40”N, 1º53’07”W, 1,063 m a.s.l.), from AEMET (Spanish Meteorological Agency).

I_s was calculated by using the water balance model “Biljou” (Granier et al. 1999). The relative extractable water (REW) was calculated at a daily time scale as the ratio of available soil water to maximum extractable water. REW ranges from 1.0 (field capacity) to 0.0 (permanent wilting point). In addition, REW_c was defined as the REW below a threshold of 0.4. This threshold is considered the point below

which water stress reaches levels in which water is limiting tree transpiration, and causing stomatal closure for most forest tree species (Granier et al. 1999). The water stress index I_S (unitless) used as surrogate of drought intensity was calculated for a given period as the sum of the daily differences between REW and REW_c and divided by 0.4, with higher values corresponding to higher water stress. I_S was calculated annually since 1961 due to the lack of some temperature and wind speed data between 1951 and 1961. The beginning date (BEGWS) and duration (NDWS) of water stress, i.e. when REW < 0.4, was calculated to study the importance of timing, i.e. how the duration and the beginning of the drought matters to tree functioning. Moreover, both BEGWS and NDWS complement the analysis of the temporal evolution of water stress over the last half century.

P/PET was obtained by dividing the sum of precipitation (*P*, mm) by the sum of potential evapotranspiration (PET, mm) (following Thornthwaite and Mather, 1957) for annual, seasonal and monthly timing scales. P/PET was calculated annually, seasonally and monthly for the period 1951-2013. Moreover, this index was also calculated annually using the microclimatic data recorded in the study site for the period 2010-2013, allowing the comparison of regional and local drought intensities. Cross-correlation between the annual series calculated with AEMET data and microclimatic data were calculated to assess the representativeness of regional data for the local climate.

Growth and growing season length

Plant growth was continuously monitored from 2010 to 2013 by measuring stem circumference changes at breast height of the selected trees using automatic band dendrometers (DRL26, EMS Brno, Czech Republic). A rotary position sensor with a stainless steel tape, which encircles the tree trunk, measured length variations every minute (accuracy 1µm). The sensor was fixed to the trunk by the strength of the tape without using invasive fixing parts. Dead bark was brushed off before the dendrometers were installed. Data were stored in a data logger every 10 min as a mean of the ten previous measurements recorded every minute. All data were thoroughly revised for possible abrupt or unusual jumps due to freezing events or resin, which can impede tap movement. Unusual jumps (>0.1 mm between two records) were erased from the data. Basal area increment (BAI, cm^2) was calculated to obtain the stem increment and calculate relative growth rates according to: $BAI = \pi(r^2_t - r^2_{t-1})$ and $RGR = (\ln r^2_t - \ln r^2_{t-1})/t$; where *r* is the tree radius, and *t* is the time interval considered (Hoffmann and Poorter 2002, Jump et al. 2006). The whole growing season BAI (BAI_{gs}) was calculated as the difference between BAI at the beginning (spring) and end of the growing season (autumn). The whole growing period was divided in two parts, spring and autumn, in order to quantify the relevance of growth in both seasons. We considered beginning of the growing when the slope of the BAI evolution over time was higher than 5% and increases continuously the following days. The end of the growing was considered to be over when the slope of the BAI evolution

over time was lower than 5%. BAI and RGR estimated in spring (BAI_{sp} and RGR_{sp}) and autumn (BAI_{su} and RGR_{au}) were calculated using r_t and r_{t-1}, with r representing the mean of tree radius 7 days before and after the beginning and the end of the considered growing period. The beginning and length of the growth during spring and autumn were determined for each tree considering changes in the slope of the BAI evolution over time for the four study years (2010-2013). The effect of the trunk refilling during spring and autumn rainfall on the growth calculation was removed subtracting the diameter decrease produced during the previous summer and winter. We assumed that during spring and autumn, trees achieved the maximum replenishment of internal water capacitances, so diameter decrease during drought was only due to water loose (Betsch et al. 2011). Therefore, diameter increases after drought events were due to water recovery and to reactivation of secondary growth.

Tree transpiration

Tree transpiration (*E*, mm) was calculated based on sap flux density measurements that were continuously monitored from 2010 to 2013 in selected trees, using sap flow density sensors (EMS 51, Environmental Measuring Systems, Brno, Czech Republic). Sensors were installed at breast height on the north side of each trunk to avoid direct sun radiation. The stem heat balance method (THB) was applied to sap flow measurement (Čermák et al. 2004). Sensors were covered with

reflective insulation to avoid temperature gradients. Sap flow (dm^3) was recorded every ten minutes using the average of 1-min measurements. A baseline was calculated using meteorological conditions and the software Mini 32 (EMS 51, Environmental Measuring Systems, Brno, Czech Republic) was used to subtract heat losses from the sensors. Unusual values due to freezing events or energy supply problems were removed from the study.

Missing sap flow data were estimated using means of the three previous and three following days when the gap was lower than 4 hours (20% of daily data) located at night or randomly along the day. That minimized the possible underestimation of the tree transpiration at the hours of maximum sap movement. This operation was only done in months with lower than 7 days gap (23% of monthly data). Sap flow in trees when data gaps exceed 7 days per month was estimated using correlations with other individuals of the same species. Those data were only estimated in that way if seasonal (spring, autumn or growing season) data gap were lower than 32% of the whole considered period. Estimations of sap flow data using correlations between individuals of the same species were only calculated when $R^2 \geq 0.65$. Tree transpiration in the whole growing season (E_{gs}), spring (E_{sp}) and autumn (E_{au}) were calculated by dividing the sum of sap flow (dm^3) for each season by the crown projected area (m^2), so tree transpiration was expressed in mm. The crown projected area was the sum of the triangle areas calculated after measuring the length of the crown in the

orientations North-South, East-West, Northwest-Southeast and Southwest-Northeast.

Water-use efficiency

Tree-level water-use efficiency (WUE_{tree}, cm^2 $BAI.m^{-3}$ H_2O) was calculated for each individual by dividing BAI by the sum of sap flow for the same period; the whole growing season (WUE_{gs}), spring (WUE_{sp}) and autumn (WUE_{au}).

Five individuals per species were selected to collect two healthy, full-expanded and sunny leaves per individual both in spring and summer in the years 2011-2013. Four of the five selected individuals of *P. nigra* and *Q. faginea* were equipped with the dendrometers and sap flow sensors. In the case of *Q. ilex*, just two had it. Leaf samples were oven-dried at 65ºC for 48 h and finely ground (MM300, Retsch). Between 1 and 2 mg of the powdered leaf material from each tree was weighted out (Radwag XA 52/2X Semi-Micro Balance, Nevada Weighing, USA) and placed into tin capsules (Sn 98 capsules, Lüdiswiss) for carbon isotope composition analysis ($\delta^{13}C$, ‰). Analyses were carried out at the Technical Platform of Functional Ecology (OC081) at the INRA Forest Ecology and Ecophysiology Unit, using an EA-GC/IRMS (Delta S, Finnigan MAT, Bremen, Germany; ± 0.2%). $\delta^{13}C$ is a proxy of the intrinsic water-use efficiency (iWUE) integrated over time of plant growth (Dawson et al. 2002, McCarroll and Loader 2004) and calculated through the

equations of Farquhar (Farquhar et al. 1982, 1989) (Supplementary data).

Statistical analysis

Percentiles (5%, 50% and 95%) for the water stress index I_s and beginning date (BEGWS) and duration (NDWS) of water stress were calculated annually for the time series 1961-2013 to identify extreme events and unusual drought periods along the last 5 years (2009-2013) and at regional scale. Same percentiles were calculated annually, seasonally and monthly for the water stress index P/PET for the time series 1951-2013 to identify unusual drought events at different temporal scales. A linear regression model was used to assess the temporal evolution of the P/PET using the microclimatic data. Linear models were used to assess the effect of year and season in P/PET over the last five years (2009-2013). Further, we tested the correlation between P/PET calculated using the AEMET climatic data set and P/PET calculated using the climatic data obtained from the study site using Spearman's rank correlation coefficients.

Linear mixed-effects models (LMMs) were used to assess the effect of year, species, season and their interactions (fixed factors) on BAI, RGR, *E*, WUE_{tree} and $\delta^{13}C$ (Zuur et al. 2007). The study of each season (spring and autumn, or summer in the case of $\delta^{13}C$) was assessed with LMMs and considering as fixed factors year and species. Individual trees were considered as random effects. Models were fitted based on a

restricted maximum likelihood method using the nlme package from R statistical software (Pinheiro et al. 2007). Tukey post hoc analysis was applied to significant results using the package lsmeans (Russell and Maxime 2015). Differences in those variables between species for each year were analyzed using a nonparametric Kruskal-Wallis test. The same test was used to identify independently the effect of year, species and their interaction on the beginning and length of the growth plant in both spring (Beg_{sp}, Len_{sp}, respectively) and autumn (Beg_{au}, Len_{au}, respectively).

Further, relationships between growth and tree transpiration with intrinsic water-use efficiency were assessed for each species using Spearman's rank correlation coefficients to analyze if increases in water-use efficiency could reduce effects of drought on growth and transpiration. Spearman's correlations were also calculated between NDWS and BEWS and BAI_{sp}, E_{sp} and $\delta^{13}C_{su}$ for all species in order to test the effect of timing on growth, transpiration and water-use efficiency. These analyses were carried out using the R statistical software (version R3.2.0; R Development Core Team 2015, Vienna).

RESULTS

Extreme drought events at different time scales

Three of the five last years (2009, 2011 and 2012) showed higher values of water stress index (I_s) than average for the time series 1961-2013 (Fig. 1).

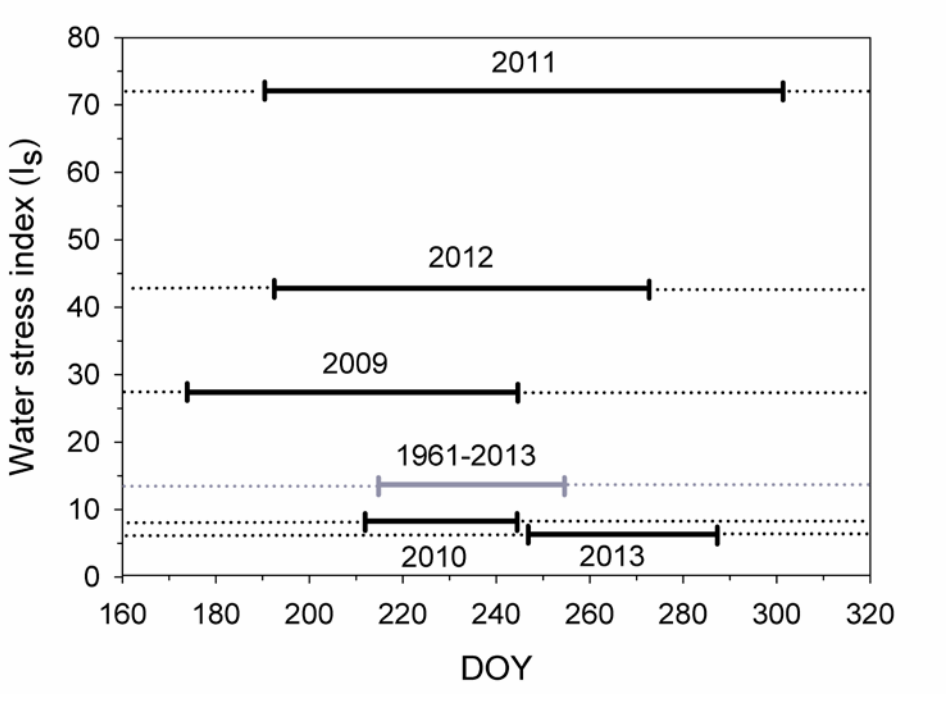


Figure 1. Annual water stress index (I_s) for the years 2009-2013 and the mean for the period 1961-2013 (grey line) (y axis). Higher values of the index indicate more water stress. The beginning and length of the line indicates the day of the year (DOY) when the drought began and ended in the corresponding year (x axis).

The years 2010 and 2013 had lower but similar values to that mean. The year 2011 reached levels of water stress between nine and eleven times higher than 2013 and 2010, respectively. 2011 was also the most stressful year over the last 52 years at our study site, showing not only extremely high I_s but also unusually high duration (NDWS) (higher values than the 95% percentile of the time series 1961-2013). Despite 2011 being around 1.7 times more stressful than 2012, water stress of the latter was also very high (value equal to the percentile 95%), showing 7 and 5 times more water stress than 2013 and 2010, respectively (Fig. 1). Moreover, beginning of water stress (BEGWS) was latter than the median of the long-term time series for 2010 and 2013, while the opposite was true for 2011 and 2012 where water stress occurred earlier. Despite the water stress during 2009 was shorter than the driest years (2011 and 2012), it was longer than the median and started the earliest considering the last five years. Therefore, 2012 and 2011 could be defined as significantly dry years while 2010 and 2013 were significantly wet years for our study period (2010-2013). Moreover, our study period was preceded by very dry years as for example 2009, which climatic conditions could have had effects on the following years (carry over effects).

P/PET showed a significant decrease since 1951 (P = 0.011) indicating increased water stress at the study site in the last decades. Moreover, there were no significant differences in P/PET between the last five years. The high and significant correlation (R^2 = 0.910, P < 0.001) between P/PET calculated from both climatic data sets (AEMET and

microclimatic station at our site) reinforces the validity of the drought intensity results obtained from the long time series for making inferences at our study site. Moreover, this consistency allowed the generalization at a regional scale of the results obtained at our study site regarding the effects of drought in the studied species.

There were some extremely dry years previous to our study period (2010-2013) as for example the year 2009 (P/PET values lower than the 5% percentile of the time series 1951-2013) (Supplementary Fig. S1a), mostly due to the unusually high temperatures recorded (mean temperatures for summer and autumn were 20.8 ºC and 12.7 ºC, respectively). Drought intensity was extremely high and at the threshold of unusual values (lower than the 5% percentile) in summer 2011 and 2012 (Supplementary Fig. S1c) due to very low precipitation (total summer precipitation was 16.1 mm and 41.6 mm for the year 2011 and 2012 respectively). Water stress was also extreme in autumn 2009 (Supplementary Fig. S1d) due to a combination of low precipitation (total precipitation of 60.4 mm) and high temperatures. Regarding extreme events at a monthly scale, May and November 2009 were at the threshold of extremely dry months (P/PET values lower than the 5% percentile), while June 2009 showed an extremely high drought (Supplementary Fig. S2e, k, f). Both, September 2011 and December 2012 showed unusually low levels of water availability (Supplementary Fig. S2i, l), due to extremely low precipitation (total precipitation of 2.7 mm and 3.8 mm in September 2011 and December 2012, respectively).

Trends in growth, growing season length and tree transpiration

Growth

Plant growth, measured as basal area increment (BAI), differed significantly between years (F = 3.41, P = 0.0224), seasons (F = 69.81, P < 0.0001) and species (F = 9.03, P = 0.0071). *P. nigra* showed significantly higher BAI than *Q. ilex* and *Q. faginea*, which did not differ. Growth integrated over the whole growing season (BAI_{gs}) and in spring (BAI_{sp}) followed the same patterns, with the highest growth in *P. nigra* and in 2011 for all species. Moreover, both BAI_{gs} and BAI_{sp} changed significantly between years and species also in a similar manner (Table1, Fig. 2a, b). BAI_{sp} was higher than in autumn (BAI_{au}) for the three species with the exception of *Q. ilex* in 2010 and 2012, which showed similar growth in autumn than in spring. BAI_{sp} was explained mainly by year and species, while no effect of both factors was observed in BAI_{au} (Table 1, Fig. 2b, c). *P. nigra* showed higher BAI_{sp} than *Quercus* species in all years (2010: χ^2 = 6.72, P = 0.0347); 2011: χ^2 = 6.63, P = 0.0362; 2012: χ^2 = 67.28, P = 0.0262), except in 2013 (χ^2 = 5.68, P = 0.0585) (Fig. 2b). The year 2011 resulted in significantly higher growths in spring than the other three years for all the species. After the extremely dry year (2011), BAI_{sp} decreased in 2012 by 85%, 68% and 53% for *Q. ilex*, *Q. faginea* and *P. nigra*, respectively.

Number of days of water stress (NDWS) was correlated significantly with BAI_{sp} only in *Q. ilex* and in *Q. faginea* (rho = 0.80, Supplementary data Table S1). Significant correlations were not found

between BAI_{sp} and beginning of water stress (BEGWS) in any of the species.

Relative growth rate (RGR) was also higher in *P. nigra* than in *Quercus* species, with slightly higher values in *Q. faginea* than in *Q. ilex* (Fig. 3a-d). Spring relative growth rate (RGR_{sp}) was significantly higher for *P. nigra*, which showed highest values in 2010 (Fig. 3a). *Q. faginea* showed higher RGR_{sp} in 2011 while *Q. ilex* did it in 2013 (Fig. 3b, d). Therefore, RGR_{sp} varied significantly among species and not among years, but with significant species per year interactions (Table 1). The highest RGR_{au} was found in *P. nigra* in 2011 (Fig. 3b).

Table 1. Summary statistics for the linear mixed models testing the effect of year, species and its interaction on the basal area increment and the relative growth rate in spring (BAI_{sp} and RGR_{sp}, respectively) and autumn (RGR_{au} and BAI_{au}, respectively) and the growing season for the former (BAI_{gs}). Kruskal-Wallis test results are shown for the beginning and length of spring (Beg_{sp}, and Len_{sp}, respectively) and autumn (Beg_{au} and Len_{au}, respectively). Bold numbers represent statistically significant results ($P < 0.05$).

		Intercept	Year	Species	Year x Species
BAI_{gs}	*F*	58.85	10.57	5.81	
	P	**<0.0001**	**0.0001**	**0.0240**	
BAI_{sp}	*F*	64.14	16.56	12.23	
	P	**<0.0001**	**<0.0001**	**0.0027**	
BAI_{au}	*F*	25.02			
	P	**<0.0001**			
RGR_{sp}	*F*	8491.35	2.06	25.13	3.22
	P	**<0.0001**	0.1329	**0.0002**	**0.0192**
RGR_{au}	*F*	13063.27	22.37	16.57	
	P	**<0.0001**	**<0.0001**	**0.001**	
Beg_{sp}	X^2		9.18	22.57	35.00
	P		**0.0269**	**<0.0001**	**0.0002**
Beg_{au}	X^2		35.25	2.96	41.19
	P		**<0.0001**	0.2280	**<0.0001**
Len_{sp}	X^2		10.14	18.01	31.08
	P		**0.0063**	**0.0004**	**0.0011**
Len_{au}	X^2		0.46	27.40	31.44
	P		0.7950	**<0.0001**	**0.0009**

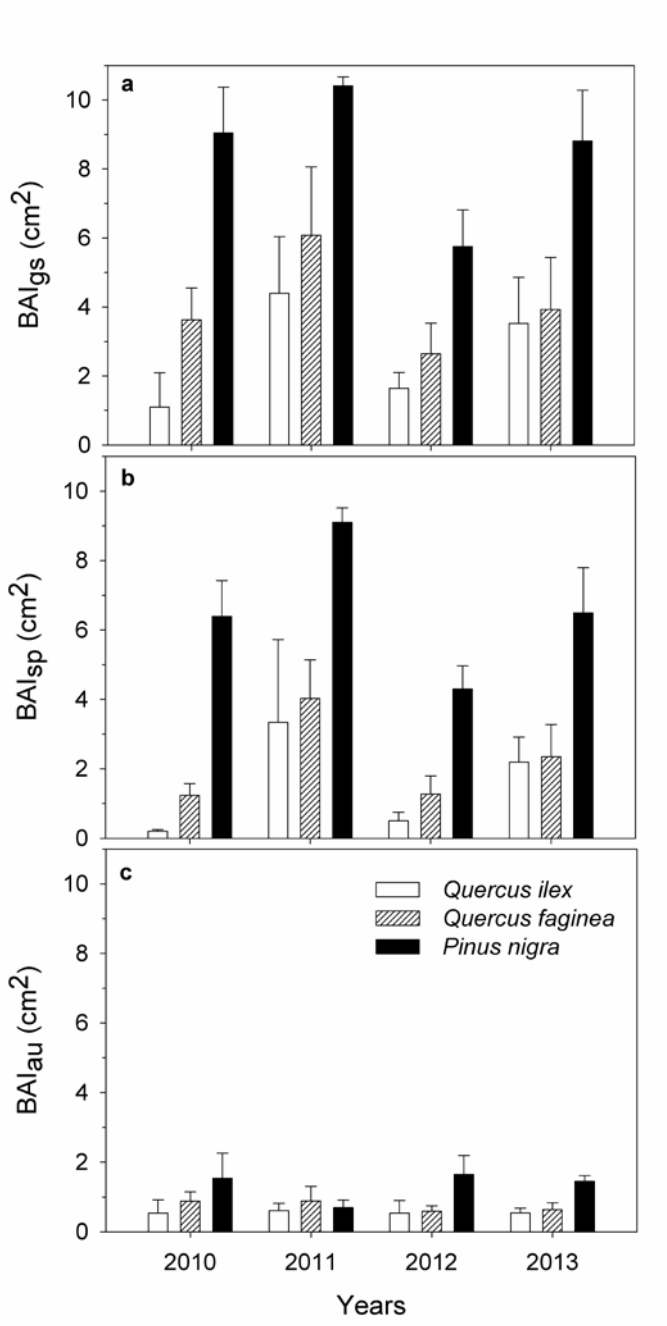


Figure 2. Mean of basal area increment (cm^2) in the growing season (BAI_{gs}, a), spring (BAI_{sp}, b) and autumn (BAI_{au}, c) for each species and study period (2010-2013).

Growing season length

Beginning and length of spring growth (Beg_{sp} and Len_{sp}, respectively) varied among years and species (Table 1, Fig. 3a-d). In the dry years (2011 and 2012) both *P. nigra* and *Q. ilex* advanced Beg_{sp} due to warmer spring temperatures, while *Q. faginea* did not change significantly the beginning of growth (Fig. 3b, c). In general, Len_{sp} was longer in 2011 and 2013 (Fig. 3b, d). The beginning of autumn growth (Beg_{au}) depended on the years (Table 1), being later in 2011 (Fig. 3b). The length of the autumn growth (Len_{au}) varied significantly among species but not among years (Table 1).

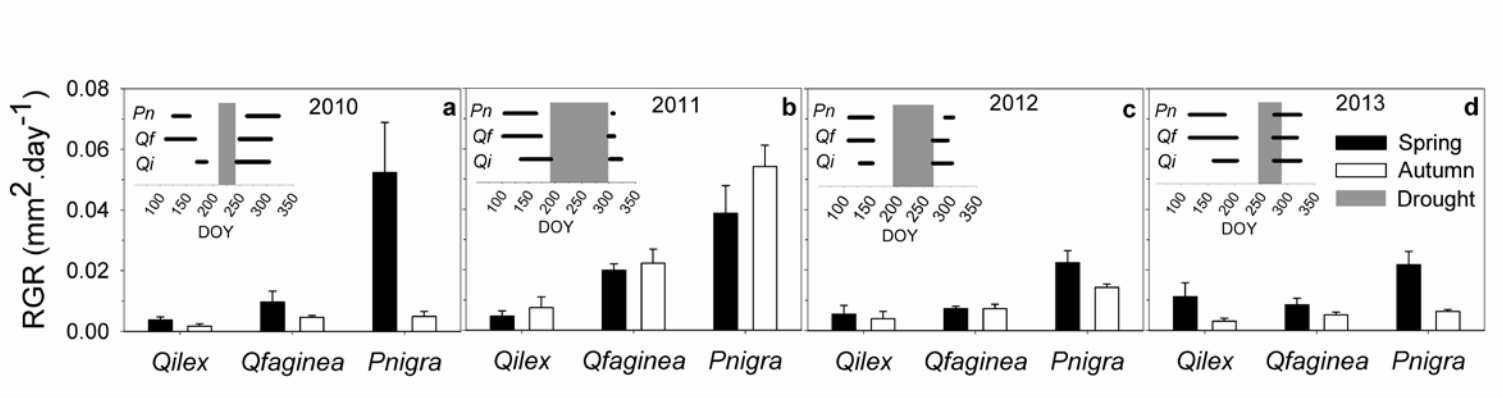


Figure 3. Mean of relative growth rate ($mm^2.day^{-1}$) in spring (RGR_{sp}) and autumn (RGR_{au}) for each species and study period (2010-2013, a-c). Small figures inside represent the beginning and length of growth (Julian days) in spring (Beg_{sp}, Len_{sp}) and autumn (Beg_{au}, Len_{au}) for each species (*Pn*: *Pinus nigra*; *Qf*: *Q. faginea*; *Qi*: *Q. ilex*) and year. Bars in grey represent the beginning (BEGWS) and length (NDWS) of the drought for each year considering the water stress index (I_s).

Tree transpiration

Tree transpiration integrated value through the whole growing season (E_{gs}) and in spring (E_{sp}) showed higher values in 2011 and lower in 2012 (Fig. 4a, b). The effect of the year had a borderline significance (F = 3.44, P =0.0660) for E_{gs}. As for radial growth tree transpiration in spring (E_{sp}), was higher than in autumn (E_{au}) and with similar patterns to those recorded in the growing season (E_{gs}) (Fig. 4a-c). Year and season effects were significant (F = 6.34, P = 0.0172; F = 14.45, P = 0.0006, respectively) considering 2012 and 2013 (without 2011 data). E_{sp} and E_{au} varied significantly among years but not among species (E_{sp}: F = 6.06, P = 0.0098; E_{au}: F = 5.73, P = 0.0436, without 2011 data). In 2012 E_{sp} was significantly lower than in 2011 and 2013 (Fig. 4b). Significant correlations were not found between E_{sp} and BEGWS or NDWS in any of the species (Supplementary data Table S1).

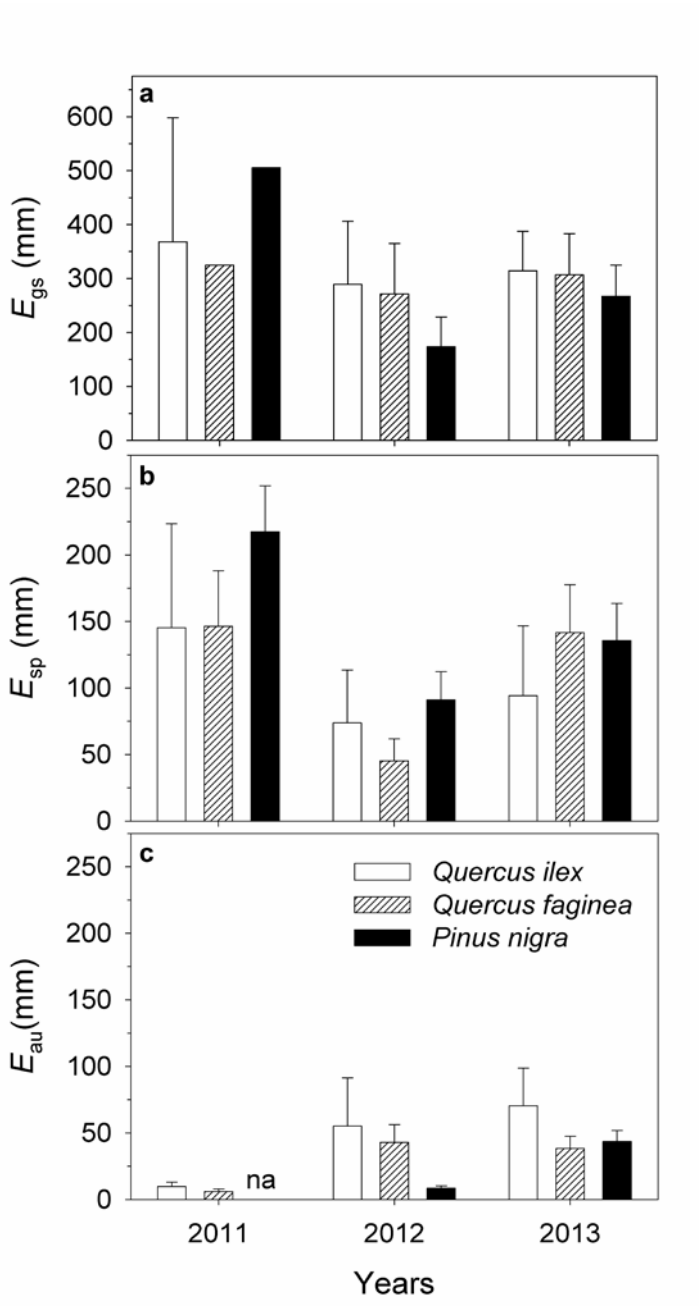


Figure 4. Mean of tree transpiration (mm) in the growing season (E_{gs}, a), spring (E_{sp}, b) and autumn (E_{au}, c) for each species and study period (2011-2013). Data from only one tree were indicated with an asterisk (*). Data for *P. nigra* in 2011 was not available (na).

Regulation of water-use efficiency across years and species

Integrated water-use efficiency (WUE_{tree}) varied among species (F = 10.40, P = 0.0046) but not among years (F = 0.34, P = 0.5633), although the interaction of year with season (F = 5.48, P = 0.0288), species (F = 5.62, P = 0.0107) and both (F = 5.99, P = 0.0084) were significant considering all data (except those of 2011 due to the lack of autumn data for *P. nigra* in that year). Water-use efficiency in spring (WUE_{sp}), autumn (WUE_{au}) and growing season (WUE_{gs}) depended on species (Table 2). However, WUE_{au} was affected by year, as it was the interaction of year with species, while WUE_{sp} was not (Table 2). WUE_{tree} was higher in *P. nigra* in the two seasons and for the whole growing period (WUE_{sp}, WUE_{au} and WUE_{gs}), followed by *Q. faginea* (Table 2). Significant differences among species were found in 2013 for WUE_{au} and WUE_{gs} and in 2012 for the WUE_{au}. Those differences were marginally significant in 2012 for WUE_{sp} (F = 4.85, P = 0.0883) and WUE_{gs} (F = 5.56, P = 0.0622).

Intrinsic water-use efficiency (iWUE) inferred from $\delta^{13}C$ differed among species (F = 28.90, P < 0.0001). $\delta^{13}C$ differed among years (F = 15.46, P < 0.0001). Carbon isotope composition in spring ($\delta^{13}C_{sp}$) and summer ($\delta^{13}C_{su}$) showed significant differences among years and species (Table 2). *P. nigra* showed the highest $\delta^{13}C$ and WUE_{tree} values regardless of the temporal period of integration. However, *Q. ilex* showed lower efficiencies than *Q. faginea* if we considered WUE_{tree} (WUE_{gs}, WUE_{sp} or WUE_{au}) but higher if $\delta^{13}C$ ($\delta^{13}C_{sp}$ or $\delta^{13}C_{su}$) was considered (Table 2). Species were more efficient in the use of water in

2012, followed by 2013 and with lower $\delta^{13}C$ values in the extremely dry year 2011. Differences among species were only significant for $\delta^{13}C_{su}$ in 2012 and 2013 (Table 2). $\delta^{13}C$ from summer showed the highest differentiation among species across years. We observed from $\delta^{13}C_{su}$ all species showed the highest intrinsic water-use efficiency when water stress index (I_s) was higher than the mean 1961-2013 (as observed in 2012), but not at extremely high dry years as observed in 2011, when species showed slightly lower intrinsic water-use efficiency (lower $\delta^{13}C_{su}$, Supplementary Fig. S3). *P. nigra* showed higher $\delta^{13}C_{su}$ than both *Quercus* species regardless of the I_s, showing also higher variability between years. Significant correlations between $\delta^{13}C_{su}$ and BEGWS or NDWS were not found in any of the species (Supplementary data Table S1).

No significant correlations were found between leaf $\delta^{13}C$ and BAI, either between $\delta^{13}C$ and tree transpiration (E) in the two *Quercus* species. However, $\delta^{13}C_{su}$ in *P. nigra* was significantly correlated with BAI_{sp} (r = -0.608, P = 0.0400), BAI_{gs} (r = -0.636, P = 0.0404) and E_{sp} (r = -0.721, P = 0.0242) and marginally with E_{au} (r = -0.714, P = 0.0881). Marginally significant correlation was also found in *P. nigra* between $\delta^{13}C_{sp}$ and BAI_{au} (r = 0.582, P = 0.0655). *P. nigra* showed the highest sensibility to drought in terms of inter-annual changes in intrinsic water-use efficiency due to it was able to increase the $\delta^{13}C_{su}$ 9% in 2012 (change in $\delta^{13}C$ of 2.3 ‰), after the high drought observed in 2011, with reductions of the BAI_{gs} of 40%. However, *Quercus* species showed higher reductions in BAI_{gs} (43 % and 50 % in *Q. ilex* and *Q. faginea*, respectively)

and lower increases in $\delta^{13}C_{su}$ (change in $\delta^{13}C$ of 1.5‰, 5% in both species).

Table 2. Mean of integrated water use efficiency (WUE_{tree}, cm^2 BAI.m^{-3} H_2O) in the growing season (WUE_{gs}), spring (WUE_{sp}) and autumn (WUE_{au}) and carbon isotope composition in spring ($\delta^{13}C_{sp}$) and summer ($\delta^{13}C_{su}$). Moreover, summary statistics for the linear mixed models testing the effect of year, species and its interaction on those variables. Bold numbers represent statistically significant results ($P < 0.05$). Different letters indicate results of post hoc analysis done for each year and variable with significant differences between species. na: not available data.

	Year	*Q. ilex*	*Q. faginea*	*P. nigra*	Intercept		Year		Species		Year x Species	
					F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
WUE_{gs} ($cm^2.m^{-3}$)	2011	0.55 ± 0.20	2.01*	3.23*	64.33	**<0.0001**			8.42	**0.0087**		
	2012	0.40 ± 0.09	0.59 ± 0.07	2.32 ± 0.50								
	2013	**0.69 ± 0.24**[a]	**1.14 ± 0.34**[ab]	**2.31 ± 0.36**[b]								
WUE_{sp} ($cm^2.m^{-3}$)	2011	0.43 ± 0.08	2.33 ± 0.23	3.90 ± 1.89								
	2012	0.35 ± 0.11	2.43 ± 0.84	3.40 ± 0.53	225.78	**<0.0001**	0.10	0.9034	6.89	**0.0153**	2.23	0.1390
	2013	2.27 ± 1.06	1.48 ± 0.45	3.31 ± 0.54								
WUE_{au} ($cm^2.m^{-3}$)	2011	4.10 ± 1.25	8.14 ± 2.27	na								
	2012	**0.65 ± 0.16**[a]	**1.37 ± 0.51**[ab]	**9.28 ± 2.11**[b]	10.09	**0.0112**	12.31	**0.0127**	16.67	**0.0009**	16.66	**0.0036**
	2013	**0.56 ± 0.15**[a]	**1.37 ± 0.27**[ab]	**2.27 ± 0.08**[b]								
$\delta^{13}C_{sp}$ (‰)	2011	-28.05 ± 0.50	-27.84 ± 0.57	-26.87 ± 0.26								
	2012	-26.34 ± 0.41	-27.09 ± 0.61	-25.34 ± 0.79	9447.16	**<0.0001**	6.79	**0.0031**	5.79	**0.0066**		
	2013	-27.10 ± 0.30	-27.18 ± 0.48	-26.44 ± 0.46								
$\delta^{13}C_{su}$ (‰)	2011	-27.88 ± 0.57	-27.97 ± 0.45	-26.44 ± 0.40								
	2012	**-26.63 ± 0.21**[ab]	**-27.34 ± 0.69**[a]	**-24.05 ± 0.21**[b]	28051.74	**<0.0001**	8.66	**0.0008**	27.45	**<0.0001**		
	2013	**-27.26 ± 0.23**[a]	**-27.52 ± 0.49**[a]	**-25.29 ± 0.26**[b]								

DISCUSSION

Extreme events and the importance of timing

In agreement with global trends observed in Mediterranean ecosystems since the 70s (Bladé and Castro Díez 2010) our study site experienced an increase of drought conditions since 1961 (P/PET decreases and REW increases). Two of the studied years, 2011 and 2012, showed between two and five times more intense and lasting water stress than the mean 1961-2013 (Fig. 1). In fact, 2011 showed an extreme drought at annual scale, not only in terms of intensity, but also in duration, which have also been observed in other previous studies (Forner et al. 2014, Dorman et al. 2015). Extreme drought events seem to have increased since 1880 in Mediterranean area, especially since the last two decades (Della-Marta et al. 2007, Sánchez-Salguero et al. 2015). The first effects on forest ecosystems began to be observable in recent years (Peñuelas et al. 2000, 2001, Carnicer et al. 2011, Granda et al. 2013). However, not only annual patterns in the intensity and duration of droughts are important, seasonality in scarcity of precipitation and evaporative demand are relevant as drivers of tree growth and function (Dorman et al. 2015) and how species can manage with water limitations at short and long time scales. Lower growth, growth length and transpiration in spring 2012 were due to very low water availability (low P/PET) in spring 2012 and especially in March and May of that year. Moreover, it was probably also affected by the carry over effects originated by the almost extremely drought occurred in summer 2011 and by the very dry autumn and winter of 2011, especially September of 2011. Moreover,

drought effects on tree functioning were aggravated by the early, long-lasting and intense summer drought occurred in 2011, which delayed and decreased resumption of autumn growth and transpiration of trees. In 2013, the lower stressful conditions (high P/PET) occurred in spring and especially in March, that as well as the previous rainy winter allowed trees to recover spring growth and transpiration and expanded spring growth length. Moreover, the less intense, shorter and latter summer drought in 2013 allowed earlier and longer autumn growth and transpiration of trees.

Furthermore, we observed that intense and long-lasting droughts from summer, as that happening in 2011, brought about higher changes in intrinsic water-use efficiency between spring and summer than those that occurred in years with lower intensity and duration of drought (e.g. 2012 and 2013). In fact, we observed that in 2012 and 2013 both *Quercus* species did not increase intrinsic water-use efficiency between spring and summer, and *P. nigra* did, probably due to its higher plasticity to drought.

Differential species responses in growth and tree transpiration

Pinus nigra was able to maintain high growth rates during drought as has been already observed in previous studies (Martín-Benito et al. 2010, Granda et al. 2013, Forner et al. 2014) and in other pine species as *P. halepensis* coexisting with Mediterranean oaks (Sánchez-Costa et al. 2015). Spring growth was the main determinant of the annual growth

pattern of black pine due to its higher value than the other period of growth, i.e. autumn (Fig. 2). In fact, early summer rainfall and temperatures affect spring growth, and therefore the overall year growth as observed in some Mediterranean species (Levanič et al. 2015). However, we observed an interesting species-specific pattern related to autumn growth in *Q. ilex*, which were higher (2010) or similar (2012) to the ones recorded in spring. This late season growth in *Q. ilex* leads to the formation of double rings, the autumn one being as wide as the spring one when 50% of growing season precipitation occurs during the second growth period of the seasons and after the summer drought (Campelo et al. 2007). This autumn growth has been observed in other species as *P. halepensis* and *J. thurifera* in xeric sites (Camarero et al. 2010), although it is less likely in other deciduous *Quercus* species phylogenetically close to *Q. faginea* such as *Q. pubescens* (Zweifel et al. 2007), or eurosiberian pines phylogenetically close also to *P. nigra* such as *P. sylvestris* (Camarero et al. 2010). In this respect, the pattern observed for *Q. ilex* seems to point out to a higher capacity to compensate the low growth during dry springs with a higher potential over the other two species to restate growth after improvement of the dry summer conditions in autumn. This would be in concordance with the lower sensitivity of *Q.* ilex leaves to water potential decreases observed by Peguero-Pina et al. (2015) during the dry summers of 2011 and 2012. *Q. faginea* could not be restoring growth in autumn due to premature leaf withering occurring during extreme dry summers as a mechanism for protecting stem from xylem cavitation, as observed in other *Quercus* species (Peguero-Pina et al. 2015).

The strongest reductions in growth (BAI_{gs}) due to the extreme drought of 2011 were found in *Q. ilex* (63%), followed by *Q. faginea* (56%) and *P. nigra* (45%). Strong reductions in growth as a consequence of important droughts were also found in *Q. ilex* and *Q. faginea* in other studies (Sánchez-Costa et al. 2015). Our results were in agreement with Forner et al. (2014) who found higher growth declines in *Q. faginea* than in *P. nigra* due to the extreme drought occurred in 2011, and with Montserrat-Martí et al. (2009) who showed that *Q. faginea* was more affected by drought than *Q. ilex*. Growth recovery during 2013 was higher for *Q. ilex* and *P. nigra* (68% and 66% respectively) than for *Q. faginea* (37%), which is in agreement with Granda et al. (2013), who found completely recovery for *P. nigra* and *Q. ilex* two years after extreme droughts, but not for *Q. faginea*. Our lower percentages of recovery could be due to the extreme drought in summer 2011 followed by a very dry winter and an extreme drought in December 2012, a combination that affected negatively *Q. faginea* growth (Corcuera et al. 2004a, 2004b, Granda et al. 2013). Dry winters impact productivity in evergreen species as *Q. ilex* (Allard et al. 2008) or *Q. suber* (Costa-e-Silva et al. 2015). Our results suggest that dry winters could impact the recovery of growth even in winter-deciduous oaks, maybe by impacting negatively cambial activity and formation of a new functional ring porous xylem.

P. nigra and *Q. ilex* were able to adapt the beginning of spring growth to climatic conditions and advanced spring growth in warmer years, as has been observed in *Quercus* species under warming

conditions (Morin et al. 2010). Lower flexibility of *Q. faginea* to adjust the growing season length may be explained by the requirement of a certain heat hours to break dormancy, and/or a control of leaf flushing by photoperiod length (Sanz-Pérez et al. 2009).

As observed in growth, maximum water-use in the growing season was also determined by the spring sap flow for all species (Fig. 4). As expected and in agreement with Forner et al., (2014), stronger reductions in tree transpiration due to previous dry year were found in *P. nigra* (66%; *Q. ilex*: 21%; *Q. faginea*: 17%). *P. nigra*, as other *Pinus* species in the Mediterranean region, have an isohydric performance (e.g. studies in *P. halepensis* by Maseyk et al. 2008), closing stomata early during drought in order to reduce water loses and to maintain water potential above its hydraulic failure threshold, which reduces carbon uptake (Tardieu and Simonneau 1998, Poyatos et al. 2008, Aranda et al. 2012), and secondary growth in dry years (Martín-Benito et al. 2010). Results from recovery in water use contrast with those related to growth as *Q. faginea* recovered 66% of the tree transpiration decrease observed in the extremely dry year while *P. nigra* and *Q. ilex* only recovered 28% and 32% of tree transpiration respectively.

Species-specific response of water-use efficiency to extreme droughts

P. nigra showed higher water-use efficiencies compared with *Quercus* species and higher plasticity to drought events, i.e. it showed higher plasticity in WUE_{tree} between dry and wet years, increasing it under drier

conditions. This increase probably due to drought-induced stomatal closure, restricted water losses more than CO_2 uptake, and pointed to a higher inter-annual climatic plasticity in the coupling of carbon uptake *vs.* water losses for pines. Moreover, when we calculated the mean of the $\delta^{13}C_{su}$ differences between dry and wet years for the three species, results were similar to the ones obtained by Grossiord et al. (2014b) for a Mediterranean ecosystem which included *P. nigra* and *Q. faginea* and where intrinsic water-use efficiency was measured in tree rings. Species mean variations in $\delta^{13}C_{su}$ between 2012 and 2013 were 1.24‰ in *P. nigra*, 0.63‰ in *Q. ilex* and 0.18‰ in *Q. faginea*, suggesting that the latter was the least sensitive to drought in terms of changes in water-use efficiency, which is also suggested by sap flow results obtained by Grossiord et al. (2014a). Moreover, the highest plasticity in water-use efficiency of *P. nigra* was also corroborated by higher increments in intrinsic water-use efficiency between spring and summer, keeping even similar increments in wet years (2013), when *Quercus* species decreased their intrinsic water-use efficiency. *P. nigra* not only showed higher plasticity to seasonal drought in terms of intrinsic water-use efficiency but also to annual drought, due to it had the highest increment in intrinsic water-use efficiency in 2012 (caused by the extreme drought of 2011), avoiding as such as high growth reductions as *Quercus* species. Therefore, our results showed that an increase in water-use efficiency due to drier conditions do not enhance growth, as observed in long-term studies (Levesque et al. 2014), different forest biome types (Peñuelas et al. 2011) and short-term Mediterranean studies (Olano et al. 2014, Vicente-Serrano et al. 2015).

CONCLUSIONS

Timing of the extreme droughts is relevant not only because they take place at different scales and in different years but also because it has important implications for plants growth and their growing season length, tree transpiration and water-use efficiency. Different responses among species to extreme drought events may be due to their timing more than to the intensity of the events, although a combination of both may exacerbate the differences. Therefore, our results revealed that the timing of extreme drought events, i.e. their onset, duration and temporal scale, can affect tree functioning to a larger extent than drought intensity. Resilience to extreme droughts is different among species and seems to be higher in *P. nigra* and lower in *Q. faginea*. Our results show the higher plasticity of *P. nigra* not only at annual but also at seasonal scale in terms of intrinsic water-use efficiency, which mitigates growth decreases caused by extreme droughts. Moreover, it could be concluded that water use and growth of tree species, despite being highly interrelated, could be decoupled and not influenced in the same way by timing and intensity of the drought. The particular tuning of growth and water use according to current and past climatic conditions differed also among species. This reinforces the need to ascertain, in the fate of Mediterranean forest tree species under climate change, not only growth performance but also the water-use strategy at different scales from days to inter-annual variation.

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SUPPLEMENTARY DATA

Leaf carbon isotope discrimination of the bulk leaf organic matter (Δ^{13}, ‰) was calculated from leaf carbon isotopic ratio after Farquhar et al. (1989) according to (1), and assuming a $\delta^{13}C$ of air ($\delta^{13}C_{air}$) equal to -8‰. Carbon isotopic discrimination is related to Ci/Ca after Farquhar et al. (1982) according to (2), and where "a" and "b" are specific coefficients of fractionation during CO_2 uptake.

(1) $$\Delta^{13}C = \frac{(\delta^{13}C_{air} - \delta^{13}C_{sample})}{(1 - \delta^{13}C_{sample}/1000)}$$

(2) $$\Delta^{13}C = a + (b - a)\left(\frac{Ci}{Ca}\right)$$

(3) $$IWUE = \frac{A_{net}}{g_{wv}} = \frac{g_{CO2}(Ca - Ci)}{g_{CO2}/1.6}$$

The $\delta^{13}C$ was used as surrogate of bulk iWUE by combining equation 1, 2 and 3 (Kruse et al., 2012).

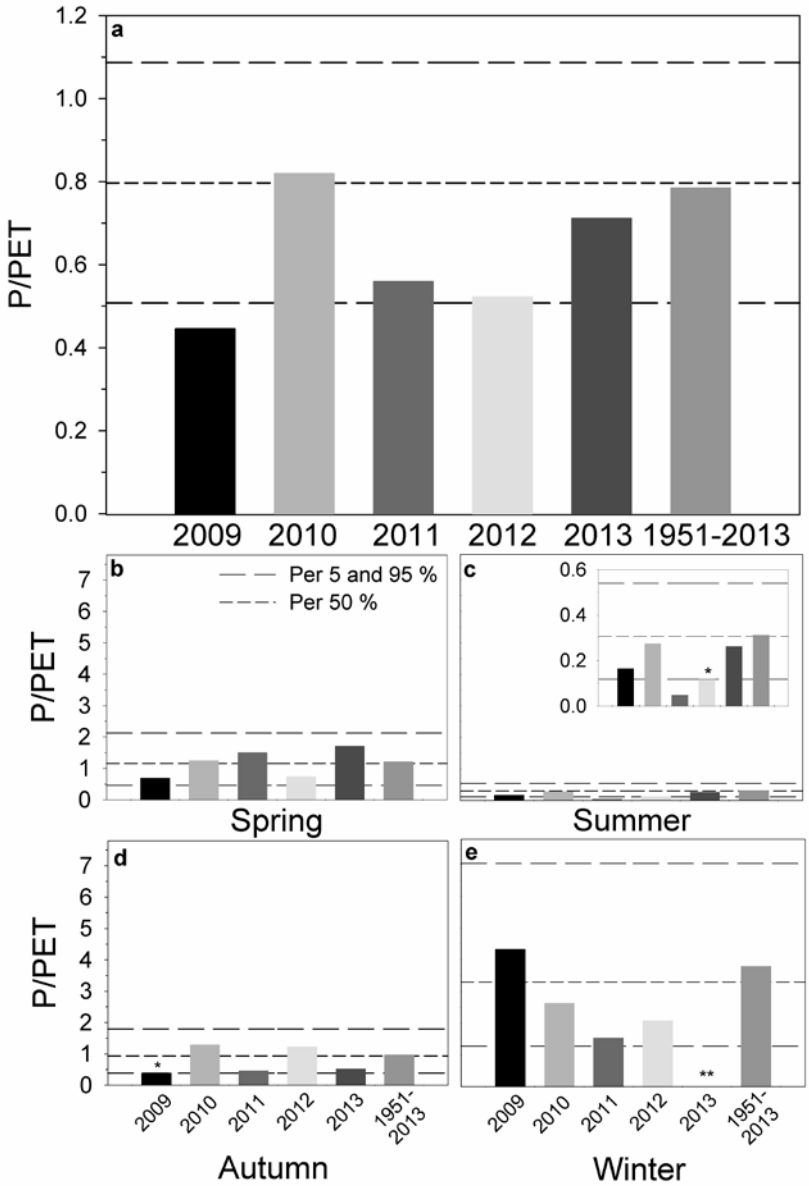
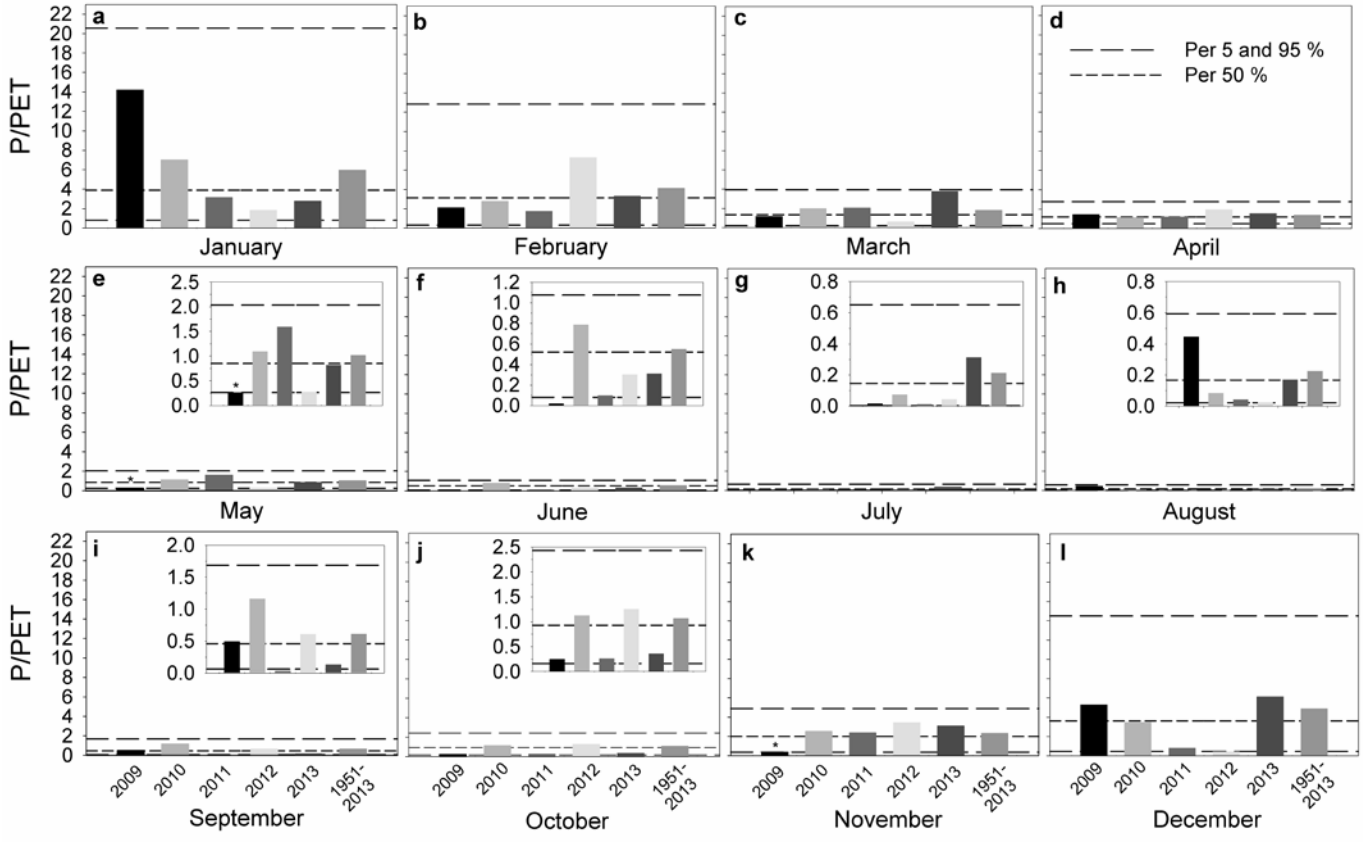


Figure S1. Annual (a) and seasonal (b-e) drought index as precipitation divided by potential evapotranspiration (P/PET) for the years 2009-2013 and the mean for the period 1951-2013. Lower values of the index correspond to more drought stress. Percentiles of 5%, 50% and 95% are shown for annual and seasonal P/PET values using the time serie 1951-2013. Values for the

studied years (2009-2013) equal to the percentiles are indicated with an asterisk (*).When the scale doesn't allow a clear display of the results an extention of it has been done inside the same. Data for winter 2013 was not available (**).

Figure S2


a
b
c
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Per 5 and 95 %
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P/PET
January
February
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l
September
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2009
2010
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Figure S2. Monthly drought index, as precipitation divided by potential evapotranspiration (P/PET) for the years 2009-2013 and the mean for the period 1951-2013. Lower values of the index correspond to more drought stress. Percentiles of 5%, 50% and 95% are shown for mensual P/PET values using the time serie 1951-2013. Values for the studied years (2009-2013) equal to the percentiles are indicated with an asterisk (*).When the scale doesn't allow a clear display of the results an extention of it has been done inside the same.

Table S1. Spearman's correlation coefficient between beginning and number of days of water stress (BEGWS and NDWS, respectively) and basal area increment (cm^2) in spring (BAI_{sp}), transpiration (mm) in spring (E_{sp}) and carbon isotope composition (‰) in summer ($\delta^{13}C_{su}$). Significant effects ($P < 0.05$) are shown in bold.

	BAI_{sp}			E_{sp}			$\delta^{13}C_{su}$		
	Q. ilex	*Q. faginea*	*P. nigra*	*Q. ilex*	*Q. faginea*	*P. nigra*	*Q. ilex*	*Q. faginea*	*P. nigra*
BEGWS	-0.40	-0.40	-0.20	-0.50	-0.50	-0.50	0.50	0.50	0.50
NDWS	**0.80**	**0.80**	0.40	0.50	0.50	0.50	-0.50	-0.50	-0.50

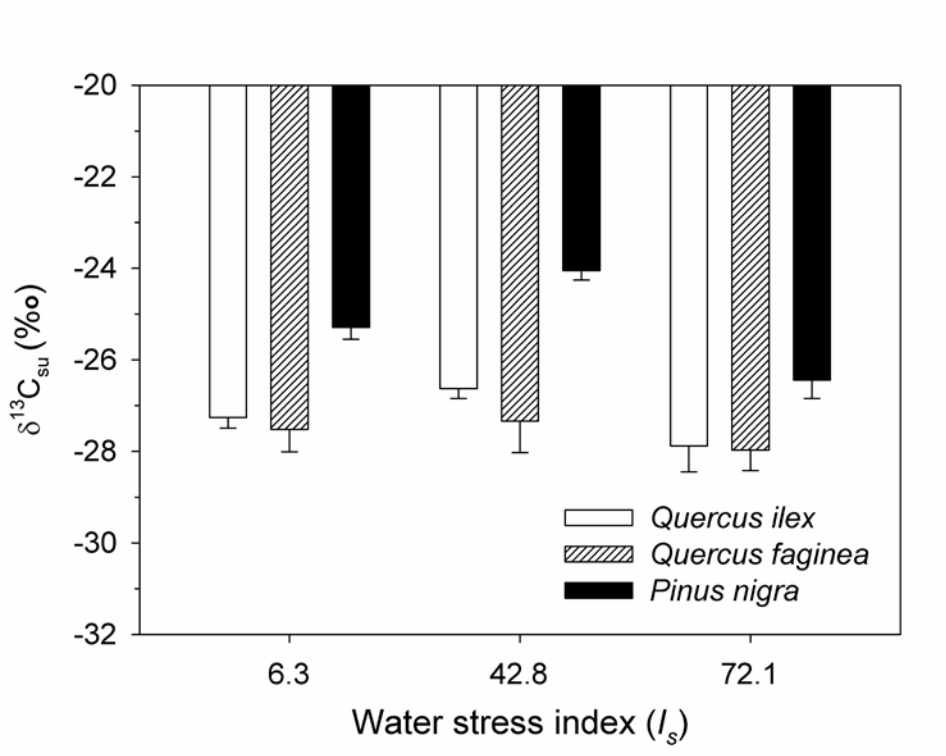

Figure S3. Mean of carbon isotope composition in summer ($\delta^{13}C_{su}$, ‰) for each species and water stress index (I_s, unitless) calculated for the study period (2011-2013). The values of I_s, 6.3, 42.8 and 72.1 correspond to the years 2013, 2012 and 2011 respectively.

CAPÍTULO 4

Differential impact of the most extreme drought event over the last half century on growth and sap flow in two coexisting Mediterranean trees

Alicia Forner, Ismael Aranda, André Granier, Fernando Valladares

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Images from the front page: sapflow sensor and dendrometer installed in study species in the Alto Tajo Natural Park.
Author: Alicia Forner.

ABSTRACT

Extreme climatic events such as intense droughts are becoming more frequent in Mediterranean regions, but our understanding of their impact on tree performance is still fragmentary. We analyzed growth and sap flow responses for a 3-year period including the most stressful drought over the last half century in the evergreen *Pinus nigra* and the deciduous *Quercus faginea*, two dominant tree species in the continental plateau of the Iberian Peninsula. Our aim was to quantify the differential impacts of this event on the performance of both species and their modulation by local microclimate. Growth was registered with digital dendrometers and water use was assessed by continuously recording sap flow in 8-9 coexisting adult individuals of each species in two sites. *Q. faginea* spring growth rate decreased by 60% during the dry year at the dry site, while the decrease in *P. nigra* was around 36%. *P. nigra* exhibited larger sap flow reductions during the dry season and also larger decreases during the extreme year, but in contrast to *Q. faginea*, it was able to recover growth and sap flow values after the extreme drought. Minor microclimatic differences between sites had significant effects on growth and water use, with slightly more mesic conditions significantly attenuating the impact of drought on both species. Findings suggest that the study species were near to their tolerance thresholds so that even moderate increases in the intensity and frequency of unusual droughts have important consequences for individual tree performance, and eventually species coexistence and ecosystem processes.

Key words: extreme drought, *Pinus nigra*, *Quercus faginea*, climate change, Mediterranean forests

INTRODUCTION

Water scarcity is one of the most important limiting factors for tree species in Mediterranean ecosystems and is expected to become even more relevant due to the increased temperature and aridity projected for Mediterranean regions (IPCC 2007). Mediterranean tree species are well adapted to dry periods and have developed different strategies to cope with multiple limiting factors. This explains the complex coexistence observed in Mediterranean ecosystems and promotes high biodiversity levels (Blondel and Aronson 1995). Different traits, such as alternative root systems, water use efficiencies, growth rates, phenologies or stomatal regulation of water use are associated with different functional strategies to cope with drought (Kneitel and Chase 2004), allowing species coexistence at small spatial scales (Chesson and Huntly 1997). One important strategy for trees to keep relatively high water status during summer drought is developing a deep root system that allows the use of deep water resources that are not available for most plants (David et al. 2007).

Complementary water use strategies also play an important role for coexistence of species coping with drought. Isohydric and anisohydric performances are two strategies that represent extremes in the range of water use by plants (McDowell et al. 2013). Both strategies have been observed in Mediterranean tree species (Chirino et al. 2011; Quero et al. 2011). For example, *P. sylvestris* (considered an isohydric species) quickly closes stomata when a water potential threshold is exceeded to avoid hydraulic failure by cavitation, while *Q. pubescens*

(considered an anisohydric species) keeps relatively high transpiration rates during extended dry periods with low hydraulic failure (Poyatos et al. 2008). In general, pine species are characteristic of harsh environments and maintain an isohydric performance under water stress conditions (Himmelsbach et al. 2012; Klein et al. 2013; Brito et al. 2014). This contrasts with the functional strategy of many oaks (*Quercus* spp.), which are more anisohydric species as observed, for example in temperate beech-oak forests (Aranda et al. 2005) and in typical Mediterranean stands (Chirino et al. 2011; Himmelsbach et al. 2012). Although it is difficult to forecast which strategy could be more successful under severe droughts, several studies have shown that isohydric species were more affected by extreme drought events (Allen and Breshears 1998; Leuzinger et al. 2005; Poyatos et al. 2008; Linares et al. 2010; Morán-López et al. 2014). These differences can break tree species coexistence and cause shifts in species distribution, as observed with the replacement of *Fagus sylvatica* and *Calluna vulgaris* by *Quercus ilex* by Peñuelas and Boada (2003). In turn, extreme droughts may result in modifications of carbon sequestration rates and net carbon balance (Lindner et al. 2010) due to the increment of defoliation events (Carnicer et al. 2011), and decreases of Mediterranean forest net production as simulated by Ciais et al. (2005) and Nadal-Sala et al. (2013) under climate change scenarios and extreme drought events, respectively.

It is unlikely, however, that any of these traits can fully offset the impact of the increased frequency of extremely dry periods forecast for

Mediterranean ecosystems (Della-Marta et al. 2007; IPCC 2012). Lack of individual recovery after the incidence of an extreme drought could lead to unstable coexistence on long time scales (Chesson 2000). In fact, a significant increment of defoliation in Mediterranean forests due to extreme drought events has been observed by Carnicer et al. (2011) over the last two decades, with defoliation trends being consistent with significant increments in tree mortality rates. Despite all these reports of severe impacts of extreme droughts and heat events on forests (revised for instance in Allen et al. 2010), plant communities are very resilient and several mechanisms have been discussed to explain the lack of vegetation changes despite the occurrence of extreme climatic events (Lloret et al. 2012a). This apparent lack of impact on vegetation could be more frequent than typically assumed due to publication bias towards positive results, in this case towards significant vegetation dieback following extreme events (Lloret et al. 2012a).

Several studies have addressed the impact of extreme droughts in Mediterranean species (e.g. Peñuelas et al. 2000; Peñuelas et al. 2001; Poyatos et al. 2008; Camarero et al. 2012; del Cacho and Lloret 2012) although long-term studies including periods before, during, and after an extreme drought event are still scarce (Lloret 2012b; Morán-López et al. 2014). Monitoring the response of species to summer drought over several years allows for a better understanding of not only how plants cope with extreme events, but also how these events could amplify water stress effects on species (Peñuelas et al. 2001; Bréda et al. 2006). Studies with high temporal resolution focused on the actual

water use and the alternative functional strategies are paramount for understanding tree species coexistence across ranges of water availability and how it can be affected by a changing climate. At the tree level, measurement of sap flow is widely used to evaluate water consumption spanning over different time intervals from daily to annual scales (Granier et al. 2000; Aranda et al. 2005). In addition, this methodology has been used at different biological scales ranging from individuals (Kunert et al. 2010; Zapater et al. 2012), to the entire forest canopy (Granier and Breda 1996; Granier et al. 2000; Aranda et al. 2012).

Sap flow measurements allow researchers to make inferences about ecological strategies according to species-specific behaviors (Martínez-Vilalta and Piñol 2003; Chirino et al. 2011; Zapater et al. 2012), microclimatic impacts in the functional responses of trees (David et al. 2007), and influences in forest water use of structure and age of trees (Lagergren and Lindroth 2004; Forrester et al. 2010; Gyenge et al. 2011; Drake et al. 2012). Sap flow is also related with tree growth, as the latter results from net carbon balance which is always in compromise with water consumption. Moreover, carbon uptake is functionally coupled with the control of water loss performed by stomatal regulation of transpiration, establishing a species-specific trade-off between water use and growth that is modulated at different time scales. Therefore, water use and growth are considered to be highly integrated in the overall functional response of plants under water limiting conditions. Both response variables (water use and growth) have been treated

thoroughly in previous studies, but few studies have focused on the interaction of both factors as an important way to understand tree response to climate change, with the exception of those inferring water use efficiency from the carbon isotopic signal of plant tissues (e.g. Maseyk et al. 2011; Granda et al. 2014; Voltas et al. 2013). Despite the recognition of the importance of the microhabitat for plant performance, little attention has been paid to the role of microclimate on the regulation of tree response to drought (Zweifel et al. 2007) and how it could condition water use and growth depending on the species (Zweifel et al. 2009). All this supports the need for ecophysiological studies of extreme drought events, integrating both water use and growth and exploring how the responses are modulated by local microclimatic conditions.

We carried out a field study of two coexisting tree species, one evergreen (*P. nigra*) and one deciduous (*Q. faginea*), with two contrasting plant water strategies (isohydric and anisohydric respectively) experiencing the most intense water stress event over the last half century. We determined accumulated basal area increment and sap flow over the period, spring growth rate, and beginning and duration of sap flow and growth as key and interrelated response variables at the whole plant level that depict main tradeoffs determining plant fitness in dry ecosystems. The main objective of this study was to quantify the effects of such extreme drought in growth, phenology and water use of both species. We also tested the importance of local microclimatic conditions for modulating the impacts of extreme drought

events by comparing two slightly different sites. Specifically, we hypothesized that: (i) unusually dry periods have a strong impact on both species becoming disproportionally adverse at the dry microsite, (ii) extreme droughts have a different effect on the functional properties of the study species in relation to the contrasting water strategies of each species, resulting in distinct impacts on growth and water use, and eventual whole plant vulnerabilities to increased frequencies of extreme droughts. We further discuss the implications of these two hypotheses for the coexistence of *P. nigra* and *Q. faginea*, which is maintained in part by differential inter-plays between water use and growth.

MATERIAL AND METHODS

Study sites

The study was carried out in an area located in Alto Tajo Natural Park, Guadalajara (Central Spain). The climate is continental Mediterranean with hot and dry summers and cold winters. Mean annual precipitation and temperature is 490.8 mm and 10.2 ºC, respectively, according to climatic data of the closest meteorological station Molina de Aragón (1951-2012, data provided by the Spanish Meteorological Agency, AEMET). Soils are calcisols (World Soil Classification, FAO) from Cretaceous and Jurassic limestone that rendered shallow and poorly developed soil (Ferrero et al. 2006).

Two sites separated approximately by 8 km were chosen. The Armallones site (AR) (40º46’37”N, 2º19’42”W, 1079 m a.s.l.) has a west orientation with a 48% slope and a basal area of 13.7 $m^2.ha^{-1}$, where *Quercus faginea* (38.7%), *Pinus nigra* (35.1%), *Quercus ilex* (25.8%) and *Juniperus oxycedrus* (0.2%) coexist as the main woody species. The Huertapelayo site (HP) (40º47’33”N, 2º17’14”W, 907 m a.s.l.) has a north orientation, 68% slope and a basal area of 11.4 $m^2.ha^{-1}$. The main woody species are *Pinus nigra* (62.4%), *Quercus faginea* (35.1%), *Juniperus phoenicea* (1.6%) and *Juniperus oxycedrus* (0.9%).

We randomly selected healthy and fully sun-exposed representative trees with similar DBH (diameter at breast height 1.3 m of height) of black pine, *Pinus nigra* subsp. *salzmanii* [n = 8; 4 in each site] and Portuguese oak, *Quercus faginea* (n = 5 [AR] and 4 [HP]). *P. nigra* mean

diameters were in the range of 73.7 ± 7.9 cm and 80.0 ± 9.3 cm for AR and HP, respectively, and 56.0 ± 4.4 cm and 68.0 ± 7.2 cm for *Q. faginea*.

Meteorological data and drought intensity

Climatic data were obtained from the closest weather station (at ca. 36 km from the study area), Molina de Aragón (40º50'40"N, 1º53'07"W, 1,063 m a.s.l.) from AEMET (Spanish Meteorological Agency). Seasonal means of rainfall and temperature were calculated for two time series during 1951-2012 and 1970-2012, as a faster rate of change has been observed since the 70's in Spain (Bladé and Castro Díez 2010) and at global scale (Alexander et al. 2006; Bladé and Castro Díez 2010).

Microclimatic conditions were also measured continuously during the study period at the study sites (2009-2012). At each site, we installed a soil moisture sensor (CR10X-ECH_2O; Decagon Devices, Inc., Pullman WA, USA) at 70 cm depth to measure relative soil volumetric water content (θ). Readings of each sensor were recorded every 2 hours. Air temperature was recorded every hour using a temperature sensor with an accuracy of ± 0.3 ºC installed in a dendrometer increment sensor (DRL 26, Environmental Measuring Systems, Brno, Czech Republic) at both sites (n = 3 [AR] and 4 [HP]). Also, we installed a HOBO® (Onset Computer Co., Bourne, MA, USA) weather station at the Armallones site with a rain gauge (RGA-MoXX), temperature and relative air humidity (12-Bit Temperature/RH Smart Sensor), solar radiation (S-Lib-MOO3) and wind speed (Wind Speed Smart Sensor, S-WA-MOO3)

sensors. Readings of each sensor were recorded every 30 minutes with a data logger (HOBO® H21-001; Onset Computer Co.). Air vapor pressure deficit (VPD) and potential evapotranspiration (PET) were calculated following Campbell (Campbell and Norman 1998) and the Penman equation (Penman 1948), respectively.

Drought intensity for the study period was characterized by estimating the daily relative extractable water in the soil (REW). REW values were only calculated for the Armallones site because the required meteorological data for its calculations were not available for the Huertapelayo site. REW was calculated daily as the ratio of available soil water to maximum extractable water. It ranges from 1.0 (field capacity) to 0.0 (permanent wilting point). Water stress is assumed to occur when REW drops below the threshold 0.4 (REW_c), under which soil water becomes limiting for transpiration, and stomatal closure happens for most temperate forest tree species. Radial growth has been observed to cease in forest trees at REWc (Granier et al. 1999). Daily REW was simulated by running the BILJOU© water balance model (Granier et al. 1999) with measurements of daily rainfall, air temperature and relative humidity, global radiation and wind speed from the Armallones meteorological station. BILJOU© also uses stand characteristics: leaf area index (LAI), dates of bud burst and leaf fall for deciduous species and soil properties. LAI was estimated with a LAI 2000 Plant Canopy Analyzer (PCA, LI-COR, Lincoln, NE, USA) in 2012 (data provided by Pollastrini, M). LAI was kept constant over the period of 1961-2012 to check if there was a climatic drift independently of the LAI

effect on drought intensity variation. Meteorological data for autumn and winter of 2011 was not available because of storage memory failure. Data gap filling for that period was performed using the Agri4cast database (Institute for Environment and Sustainability, IES, European Commission; http://mars.jrc.ec.europa.eu/mars/About-us/AGRI4CAST). As missing data occurring during the winter, when evapotranspiration is low and soil is at its field capacity, errors in REW calculation were considered negligible. Maximum extractable water in calcisol soils is low due to a high stone content, so we fixed it at 80 mm (according to Gaultier et al. 2000).

The beginning date (BEGWS) and duration (NDWS) of water stress (REW < 0.4), as well as a water stress index (I_s), was calculated to compare drought intensity between years. I_s for a given period is the sum of the difference between REW and REW_c when REW < REW_c and divided by REW_c. Higher values of I_s correspond to higher drought stress. I_s was calculated for the period 1961-2012 to analyze the temporal evolution of water stress over the last half century.

Growth and sap flow measurements

Plant growth was monitored from September 2009 to October 2012 measuring stem diameter changes at the breast height of the selected trees using automatic dendrometer bands (DRL26, EMS Brno, Czech Republic). A rotary position sensor with a stainless steel tape, which encircles the tree trunk, measured length variations every hour

(accuracy 1 µm). The sensor was fixed to the trunk by the strength of the tape without using invasive fixing parts. Dead bark was brushed off before the dendrometers were installed. Data were stored in the same data logger as sap flow measurements. All data were thoroughly revised looking for possible abrupt or unusual jumps due to freezing events or resin, which can impede tape movement. Those data were corrected erasing the unusual jump (> 0.1 mm between two records). Basal area increment (BAI) was calculated to obtain the stem increment and calculate growth rates according to: $BAI = \pi(r^2_t - r^2_{t-1})$; where *r* is the tree radius and *t* is the hour of the day (Jump et al. 2006). Accumulated basal area increment per month (BAI_c) was calculated by summing the BAI of that month and all previous monthly BAI since the beginning of the study period (September 2009). BAI evolution over study period allows observing differences of growth not only between seasons but also between years, species and sites, giving detailed information of growth patterns. The beginning and duration of the growth period during spring was determined for each tree considering changes in the slope of the BAI evolution over time for both study sites and for the three study years (2010-2012). We considered the end of the spring growth period to be over when the slope of the BAI evolution over time was lower than 5%. As spring is the main growth season for most of Mediterranean species (Durante et al. 2009) and studies showed that growth of both study species is favored by spring rainfall (Granda et al. 2013), daily spring growth rates (SGR, $cm^2.day^{-1}$) were calculated. SGR was obtained by dividing BAI for the whole growth period by its duration in days.

Sap flow, as a surrogate of tree performance in terms of transpiration control, was continuously monitored at the same time as growth in selected trees at each site using the sap flow sensors EMS 51 (Environmental Measuring Systems, Brno, Czech Republic) installed at breast height on the north side of each trunk. These sensors are based on the stem heat balance (THB) method applied to a stem section with internal heating of tissues (Čermák et al. 2004). Sensors were covered with reflective insulation to avoid the influence of natural temperature gradients in the trunk and the effect of climatic conditions, e.g. radiation. Sap flow was measured every minute; 10-minute averages were recorded by an EMS data logger (Environmental Measuring Systems, Brno, Czech Republic). Heat losses from the sensors were subtracted calculating a baseline taking into account meteorological conditions and using Mini 32 software. After revising the data, unusual values due to freezing events or energy supply problems were removed from the study.

Sap flow per tree ($L.h^{-1}$) was calculated by multiplying the measurements in the trunk sector by the tree perimeter (Čermák et al. 2004). We calculated sap flow per unit of basal area (Q_v, $m^3.day^{-1}.m^{-2}$) considering the individual tree basal area and the integration of the sap flow throughout the day. Five representative periods of tree transpiration along the year were selected independently of the study years (2009-2012). The first period (p1) corresponds to the beginning of a significant water use by both species during early spring (REW ~ 1), when deciduous trees as *Quercus faginea* are developing their leaves.

The second period (p2) shows the maximum tree transpiration which was recorded during the late spring. The third period (p3) considers tree performance at the peak of summer stress when minimum values of sap flow were recorded ($REW < REW_c$). The fourth period (p4) depicts sap flow recovery during early autumn rainfall ($REW_c < REW < 1$). Finally, the fifth period (p5) considers the minimum values recorded during late autumn and early winter. We selected seven representative days with similar meteorological conditions during each study period (Supplementary data Table S1). The relative sap flow was calculated (Q_r) to observe the decrease in water consumption by trees along the summer drought and later recovery due to autumn rainfall. Q_r was obtained as the ratio between sap flow (Q_v) and the maximum sap flow ($Q_{max,}$ p2), both per unit of basal area, measured for the considered species, in both sites and every year.

Growth phenology was assessed from growth resumption in spring until maximum seasonal growth was recorded. The beginning and duration of growth give information of the possible positive effects of increasing temperatures, i.e. advance and extension of growth period due to high temperatures if water resource is not limited. The beginning, maximum and duration of water use was calculated to determine the changes of water use during dry years.

Statistical analysis

Percentiles (5%, 50% and 95%) for temperature (T) and rainfall (P) from both time series (1951-2012 and 1970-2012) were obtained to identify unusually seasonal values along the study period (2009-2012).

A non-parametric Mann-Whitney U test was used to check significant differences in seasonal T and Ɵ values between the two study sites. Differences in phenological variables and annual basal area increment (BAI) between years for each species and site were analyzed using a non-parametric Kruskal-Wallis test. These analyses were performed using Statistica 8.0 (StatSoft Inc., Tulsa, OK, USA). A linear regression model was used to assess the temporal evolution of the water stress index over time. Percentiles (5% and 95%) for water stress index from 1961 to 2012 were calculated to identify unusual water stress over the last 50 years. Differences in accumulated basal area increment between species at each site were analyzed by a linear model. Linear mixed-effects models (LMMs) were used to assess the effect of year (fixed factor) in growth rate (Zuur et al. 2007). Individual trees were considered as random effects. The model was fitted based on a restricted maximum likelihood method using the nlme package (Pinheiro et al. 2007). To test differences in sap flow per unit of basal area between periods (2009-2012), linear models were made for each species and site. Further, we assessed the relationship between REW and spring growth rate and relative sap flow per tree using Spearman's rank correlation coefficients. These analyses were carried out using the

R statistical software (version R3.0.1: R Development Core Team 2013, Vienna).

RESULTS

Climate trends and extreme events

Extreme drought events have increased their frequency over the last decade. Extremely high values of water stress (I_s) were found in three years (1994, 2011 and 2012) during the decade 1992-2012 while no extreme values were found between 1961 and 1992. Additionally, there was a significant increase in water stress (I_s) since 1961 (p = 0.024). Four of the eight most stressful years for vegetation (water stress index, I_s) over the last 50 years took place over the last six years (2007-2012)(Fig. 1). The year 2011 exerted the highest water stress on vegetation over the half century historical record as indicated by the I_s series calculated for 1961-2012 period.

In agreement with increases in water stress during last decade, mean annual decreased by 46.1 mm and temperature increased by 0.6 ºC in the last decade (2002-2012), compared to the long-term data series (1951-2012). Summer and spring temperatures of 2009 were unusually high compared to the long-term data (1970-2012) (Supplementary data Table S2). The spring of 2011 was extremely hot and both summer and winter seasons were unusually dry, which allows considering 2011 as an extremely dry year. These results were corroborated by comparing the long-term data series 1951-2012. Summer rainfall during 2012 was also slightly lower than expected from the series 1951-2012 (Supplementary data Table S2). The year 2010 can be considered a normal year compared with 2009, 2011 and 2012, since

total annual rainfall and mean annual temperatures were the closest to long-term averages. Furthermore, 2010 showed lower water stress index (I_s) compared with 2011 and 2012 (Fig. 1).

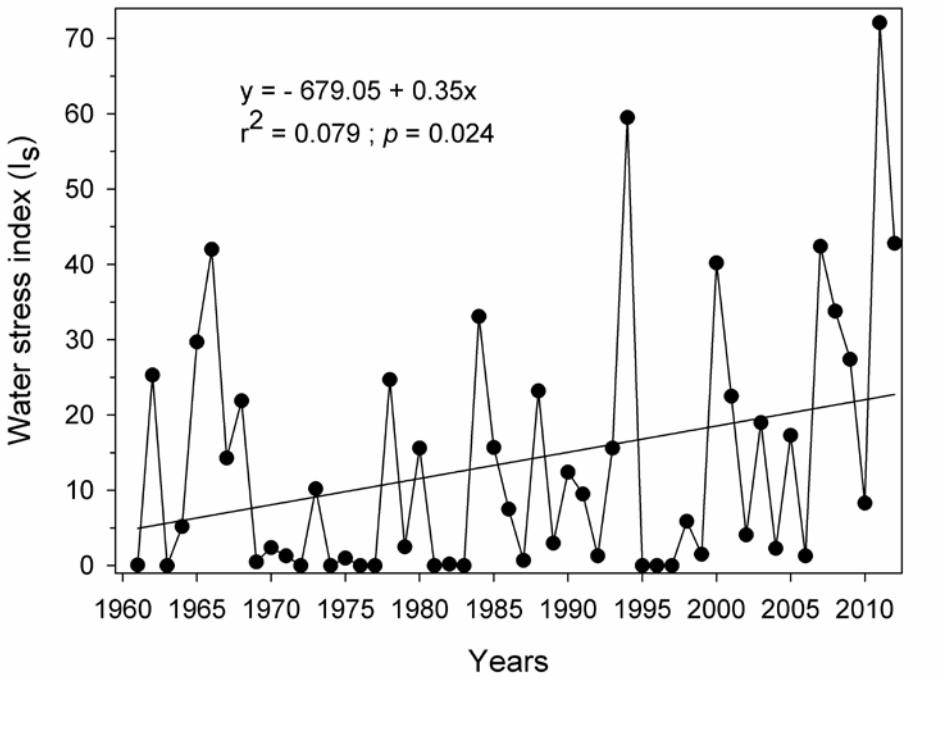

Figure 1. Evolution of annual water stress index (I_s) for the period 1961-2012. Higher values of the index indicate higher water stress. Line represents the fitted linear model.

Microclimatic differences were found between both study sites. The Armallones (AR) site was hotter and drier than the Huertapelayo (HP) site for all study years, with significantly higher air temperatures during the summer of 2009 and 2011 and the winter of 2011 (Table 1).

Table 1. Seasonal mean ± SE temperature (T) and soil volumetric water content (Θ) of the meteorological sensors installed at both study sites (Sp: spring, Su: summer, Au: autumn, Wi: winter). Total rainfall (P) and vapor pressure deficit (VPD) were obtained from the meteorological station located at Armallones site. Potential evapotranspiration (PET) was calculated following the Penman equation. Values between parentheses represent means of total annual rainfall and mean annual temperature for the year indicated above at Armallones site. Water stress index (I_s), beginning of water stress (BEGWS, Julian day) and number of days of water stress (NDWS) were calculated for each year. Significant differences in temperature and water soil content between both study sites are indicated with asterisk (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). $^{+}$ and - indicates means for only two months of the season and data not available, respectively.

Table 1

Site		Armallones								Huertapelayo	
		P (mm)	T (ºC)	VPD (kPa)	PET (mm)	Θ (m^3/m^3)	I_s	BEGWS	NDWS	T (ºC)	Θ (m^3/m^3)
2009 (630.1, 12.6)	Sp	134.6 ± 14.1	11.1 ± 2.7	0.53 ± 0.15	227.6 ± 20.7	-	27.4	174	70	10.7 ± 2.8	-
	Su	32.9 ± 2.6	23.3 ± 1.3*	1.64 ± 0.19	421.4 ± 9.6	-				22.3 ± 1.0	-
	Au	87.0 ± 6.4	13.3 ± 2.7	0.57 ± 0.14	135.9 ± 15.0	0.028 ± 0.003***				12.3 ± 2.8	0.050 ± 0.002
	Wi	375.6 ± 43.9	2.7 ± 0.3	0.11 ± 0.01	37.4 ± 2.4	0.071 ± 0.002***				3.0 ± 0.2	0.142 ± 0.018
2010 (688.8, 11.4)	Sp	247.9 ± 8.6	9.7 ± 2.1	0.36 ± 0.07	200.6 ± 16.1	0.083 ± 0.002***	8.3	212	32	9.8 ± 2.1	0.149 ± 0.003
	Su	92.6 ± 24.4	21.4 ± 2.2	1.21 ± 0.27	385.8 ± 13.5	0.037 ± 0.018***				20.8 ± 1.8	0.094 ± 0.022
	Au	172.4 ± 7.2	10.8 ± 3.7	0.42 ± 0.18	121.8 ± 17.4	0.049 ± 0.015				9.7 ± 3.5	0.054 ± 0.012
	Wi	175.9 ± 16.7	3.5 ± 0.7	0.14 ± 0.04	40.0 ± 3.6	0.053 ± 0.002***				2.5 ± 0.4	0.140 ± 0.002
2011 (-, 12.5)	Sp	214.4 ± 12.9	11.8 ± 2.8	0.39 ± 0.10	212.5 ± 18.7	0.092 ± 0.013***	72.1	191	110	11.5 ± 2.7	0.120 ± 0.016
	Su	36.1 ± 10.9	21.7 ± 1.4*	0.95 ± 0.25	413.7 ± 4.6	0.038 ± 0.023***				21.0 ± 1.0	0.014 ± 0.013^{+}
	Au	-	13.7 ± 3.6	-	145.7 ± 22.0	0.012 ± 0.001***$^{+}$				12.2 ± 3.1	-
	Wi	-	2.8 ± 0.6**	-	41.6 ± 6.0	0.084 ± 0.016^{+}***				1.7 ± 0.4	0.035 ± 0.005^{+}
2012 (561.1, 12.4)	Sp	168.9 ± 31.9	11.0 ± 2.8	0.54 ± 0.17	225.6 ± 19.2	0.088 ± 0.013***	42.8	193	79	10.9 ± 2.7	0.030 ± 0.006
	Su	23.1 ± 2.1	23.6 ± 0.9	1.75 ± 0.16	426.9 ± 6.3	0.019 ± 0.007***				23.0 ± 0.7	0.006 ± 0.003
	Au	234.7 ± 14.0	12.1 ± 3.2	0.41 ± 0.23	119.2 ± 18.7	0.068 ± 0.019				11.8 ± 3.0	0.388 ± 0.233
	Wi	134.4 ± 13.1	3.0 ± 0.3	0.11 ± 0.02	-	0.074 ± 0.002***				2.6 ± 0.2	0.602 ± 0.226

AR was significantly drier than HP comparing annual soil volumetric water content values for almost all seasons. As all those seasons correspond to extremely hot and dry periods (Supplementary data Table S2), the trees at the AR site were expected to experience severe drought stress. Spring air temperatures were significantly different in 2010 and 2011 only for the AR site (p = 0.033, between years). The AR site experienced significantly higher temperatures, between 1.3 and 3.6 ºC, during all study summers ($p < 0.001$) and also during the spring of 2009 and the autumn of 2012 ($p < 0.05$) compared to the long-term time series. Spring potential evapotranspiration (PET) increased around 5.7% per year since 2010. This increase was also found in the summer (6.7% in 2011 and 3.1% in 2012, increase from previous year). Because of this higher PET and rainfall reduction, water stress (I_s) was 88.5% lower in 2010 than in 2011 (Table 1).

Due to low PET and therefore low transpiration, relative extractable water of the soil (REW) was close to 1, soil water was near field capacity from late autumn to early spring for all study years (Fig. 2). REW decreased sharply during the late spring and summer of all study years due to the low water holding capacity. Water stress occurred during each summer of the 4 year periods, but its intensity and duration varied among years. The lowest REW level was reached in the summer of 2011, which confirmed that 2011 was the driest year compared with the other study years. Days under water stress (NDWS) were more than two times higher in the dry year (2011) than in the normal year (2010), being 110 and 32 days, respectively (Table 1). The beginning of the

water stress period (BEGWS) was advanced over the years due to higher temperatures being reached earlier, being up to 19 days earlier in 2012 than 2010, the normal year. Water stress duration was 2 and half months longer in 2011 than 2010, which confirmed a more lasting drought during the extreme dry year.

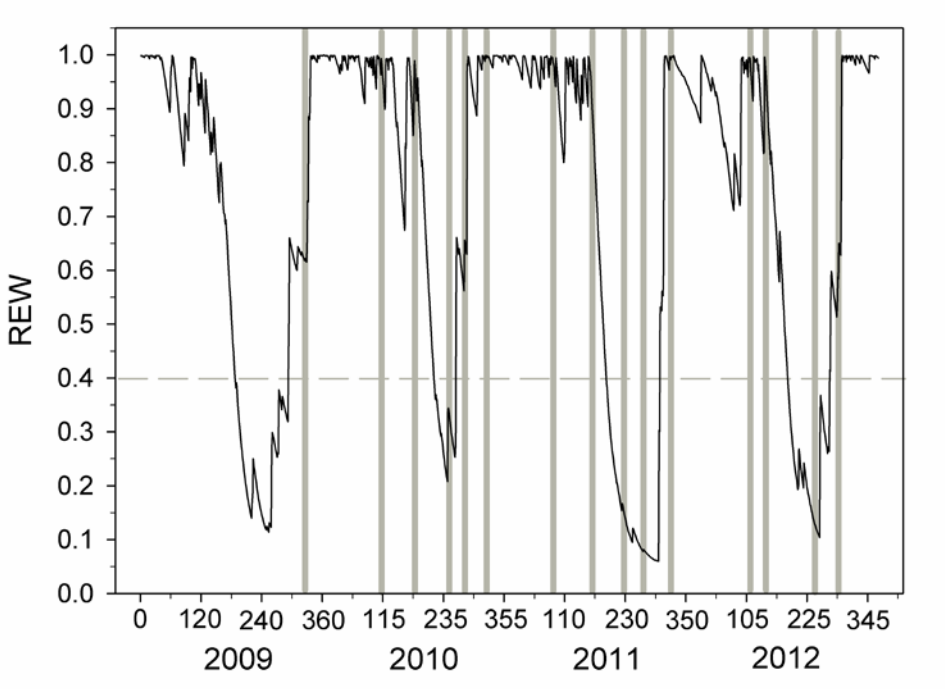

Figure 2. Daily values of modeled relative extractable water (REW) from the soil of Armallones site since 2009 to 2012 (Julian days). Dashed line indicates the critical REW value (REW_c = 0.4) at which tree transpiration begins to decrease. Bars in grey represent periods of sap flow measurements.

Spring growth patterns

Annual growth, measured as annual basal are increment (BAI), was higher in *P. nigra* than in *Q.* faginea at both sites and in all study years. No significant differences in growth were found between sites and years, although higher annual BAI was observed at the mesic site (HP) for both species (Table 2). The exceptionally extreme year (2011) had a higher effect on growth at the dry site in both species. Differences between sites in reductions of annual BAI, derived from the extreme dry year, were higher for *P. nigra* (AR: -38.0%; HP: -9.4%) than for *Q. faginea* (AR: -48.4%; HP: -43.7%). Growth evolution, as the accumulated basal area increment per month (BAI_c), was higher in *P. nigra* than in *Q. faginea* over the study period (2009-2012) and at both sites (Fig.3). Both species showed higher growth in spring, being faster in *P. nigra* at the beginning of this season. No significant growth differences were found between species at each site ($p > 0.05$).

During the spring, the main growth season for the study species, both species showed similar patterns in growth rates (SGR) at each site (Fig. 4), although higher rates were found for *P. nigra* during all years. After the exceptionally extreme year (2011), both species decreased their SGR at both sites, decreasing more at the dry site. At this site, reduction in growth was significantly higher for *Q. faginea* (-59.8%) than for *P. nigra* (-35.9%), while at the mesic site, higher reduction was found for *P. nigra* (-24.2%; -16-7% (*Q. faginea*)).

Table 2. Day of year (Julian day, mean ± SE) for the beginning of spring growth and its duration (days ± SE). Mean ± SE of annual basal area increment (BAI, $cm^2.yr^{-1}$). Data are shown for both study species (*Pinus nigra* and *Quercus faginea*), sites (AR: Armallones; HP: Huertapelayo) and years (2010-2012). Significant *p*-values are shown in bold.

Site	Sp.	Spring growth											
		Beginning				Duration				BAI			
		2010	2011	2012	*p*-value	2010	2011	2012	*p*-value	2010	2011	2012	*p*-value
AR	*P. nigra*	105 ± 5	99 ± 1	109 ± 3	0.082	51 ± 6	65 ± 6	46 ± 2	**0.047**	10.14 ± 1.18	11.22 ± 0.76	6.96 ± 1.16	0.077
	Q. faginea	107 ± 5	87 ± 7	105 ± 1	**0.029**	60 ± 1	88 ± 9	51 ± 8	0.075	3.55 ± 1.15	7.35 ± 2.45	3.79 ± 0.76	0.184
HP	*P. nigra*	101 ± 0	97 ± 1	98 ± 5	0.167	79 ± 2	70 ± 9	74 ± 9	0.387	12.49 ± 2.37	15.34 ± 3.14	13.90 ± 3.47	0.735
	Q. faginea	90 ± 6	73 ± 2	81 ± 2	**0.027**	92 ± 7	108 ± 10	71 ± 3	**0.034**	7.27 ± 2.37	9.07 ± 1.61	5.11 ± 0.97	0.292

The beginning of spring growth was earlier in the mesic site than in the dry site for both species and in all study years, but only significant results were found for 2012 (Table 2). Moreover, spring growth was advanced significally in *Q. faginea* starting between 17 (mesic site) and 20 (dry site) days before, during the exceptionally extreme year 2011 compared to 2010. In addition, growth during 2011 lasted between 9 and 28 days more in both species at both study sites. Growth duration was significantly different between years for *P. nigra* at the dry site but not at the mesic site, while *Q. faginea* had significantly different growth duration between years at the mesic site.

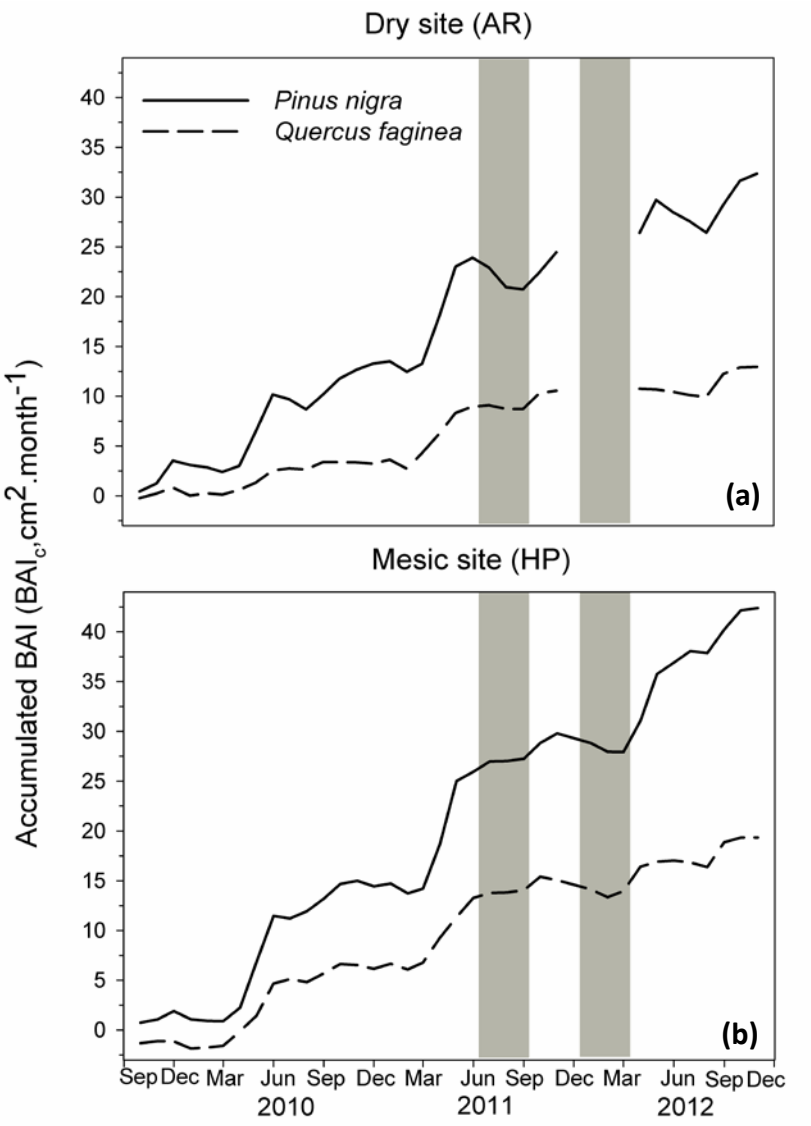

Figure 3. Mean monthly accumulated basal area increment (BAI_c) for the study period 2009-2012 for each species and site [Armallones (a) and Huertapelayo (b)]. Bars in grey represent the two unusually dry seasons over the exceptionally dry year 2011, summer and winter, respectively. No available data since December 2011 to March 2012 at the dry site (AR).

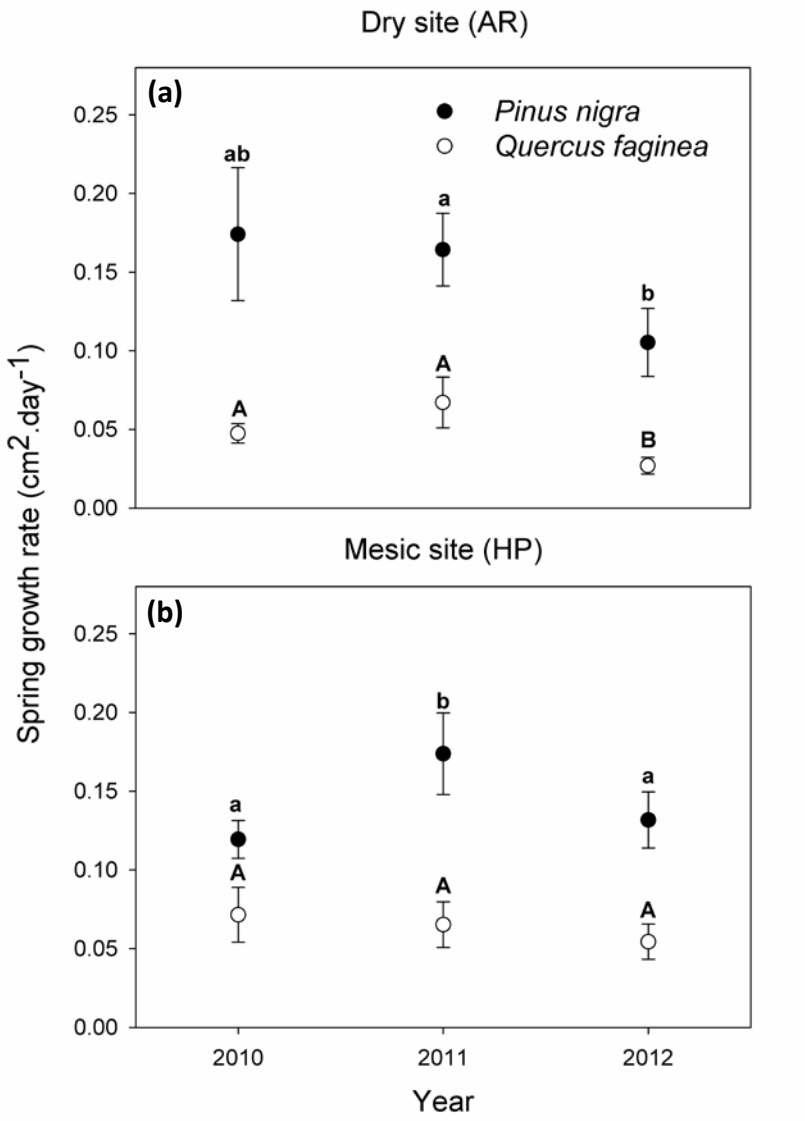

Figure 4. Mean of daily spring growth rate ($cm^2.day^{-1}$) for each species, site [Armallones (a) and Huertapelayo (b)] and study year for the period (2010-2012). Bars are standard mean errors unless eclipsed by symbols. For each species and site, different letters indicate significant differences between years at p = 0.05 (Small letters for *Pinus nigra* and capital ones for *Quercus faginea*).

Sap flow patterns

Higher seasonality in water use, measured as sap flow, was found at the dry site (AR), especially in *P. nigra*. Moreover, differences between species were accentuated during the exceptionally extreme 2011 year (Fig. 5). *Q. faginea* had highest sap flow rates during the late spring, summer and early autumn at the dry site, while at the mesic site similar sap flow rates were found for both species. In all study summers (period p3) the lowest sap flow rates were recorded for *P. nigra* at the dry site, reaching values close to zero in 2011 since limited water resources were available due to the extreme drought. Both species showed higher reductions in water use due to the summer drought (period 2 compared with period 3) at the dry site and particularly after the exceptionally extreme year (*P. nigra*: -25.3%; *Q. faginea*: -22.7%) (Fig. 5). During 2011 and 2012, *P.* nigra was not able to recover water use after the summer drought (period 3 and 4) indicating that autumn rainfall was not abundant enough to reestablish its sap flow. Looking at the seasonal evolution of the relative sap flow rates, *Q. faginea* showed similar patterns at both sites, while *P. nigra* appeared to be more sensitive to water shortage conditions at the dry site (Fig. 6). During the summer drought, both species decreased water use overall both sites, but the species were more affected at the dry site, decreasing their sap flow by 25-45% (years range) in *Q. faginea* and 69-90% in *P. nigra*. Higher reductions in water use were found in the dry year for *P. nigra* but not for *Q. faginea*. At the mesic site, *Q. faginea* was not able to recover its sap flow (period p4) during the exceptionally extreme year but it did

over the following year, while the opposite patters were true for *P. nigra*. Relative sap flow (Q_r) was marginally correlated with REW for *P. nigra* at the dry site (r = 0.829, p = 0.058) but not for *Q. faginea* (r = 0.257, p = 0.658).

Figure 5

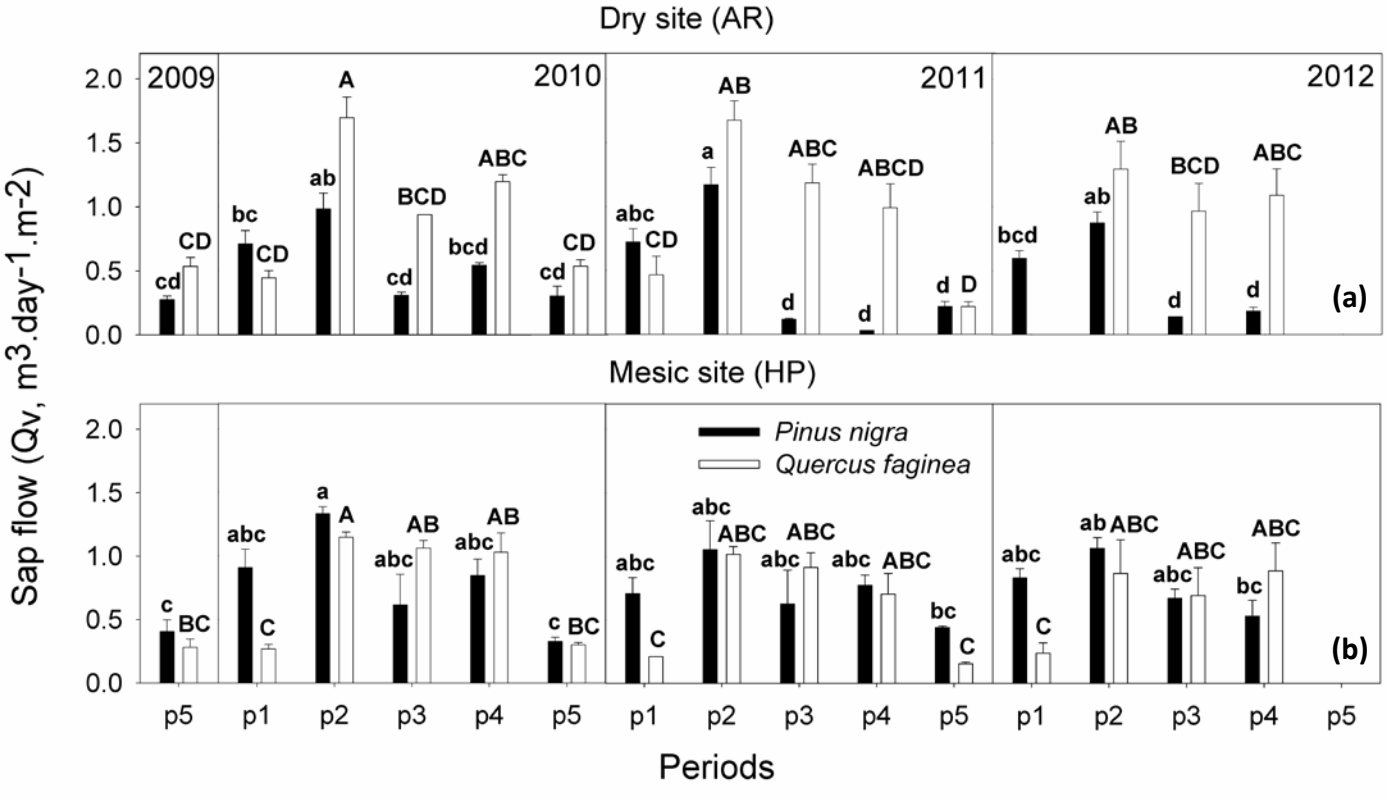
Dry site (AR)
Mesic site (HP)
2009
2010
2011
2012
(a)
(b)
Pinus nigra
Quercus faginea
Sap flow (Qv, m3.day-1.m-2)
Periods
p5
p1
p2
p3
p4
p5
p1
p2
p3
p4
p5
p1
p2
p3
p4
p5

Figure 5. Mean ± SE of daily mean sap flow per basal area (Q_v, $m^3.day^{-1}.m^{-2}$) for each species, site [Armallones (a) and Huertapelayo (b)] and measurement period (p1: beginning of spring water use; p2: maximum evapotranspiration during late spring; p3: minimum values during the peak of summer stress; p4: sap flow recovery during early autumn rainfall; p5: late autumn and early winter minimum values) since 2009 to 2012. For each species and site, different letters indicate significant differences between periods at p = 0.05 (Small letters for *Pinus nigra* and capital ones for *Quercus faginea*). No available data for p5 in 2012 at both sites.

As expected due to their dependence on water resources and temperatures, the beginning of sap flow followed the same pattern as spring growth, being earlier in the mesic site for both species during study years. Due to water scarcity, water use started later in 2011, the exceptionally extreme year, than in 2010 for both species at the dry site (Table 3). Sap flow duration, indicating the spring water use period before summer drought, was opposite to the one observed for growth in pine species at the dry site, being longer for growth and shorter for sap flow in 2011 compared to 2010. However, sap flow as well as growth started later and did not last as long in 2012 as the previous year. Maximum sap flow occurred later in the exceptionally extreme 2011 year for both species and at both sites, although differences were significant only for *P. nigra* at the dry site.

Figure 6

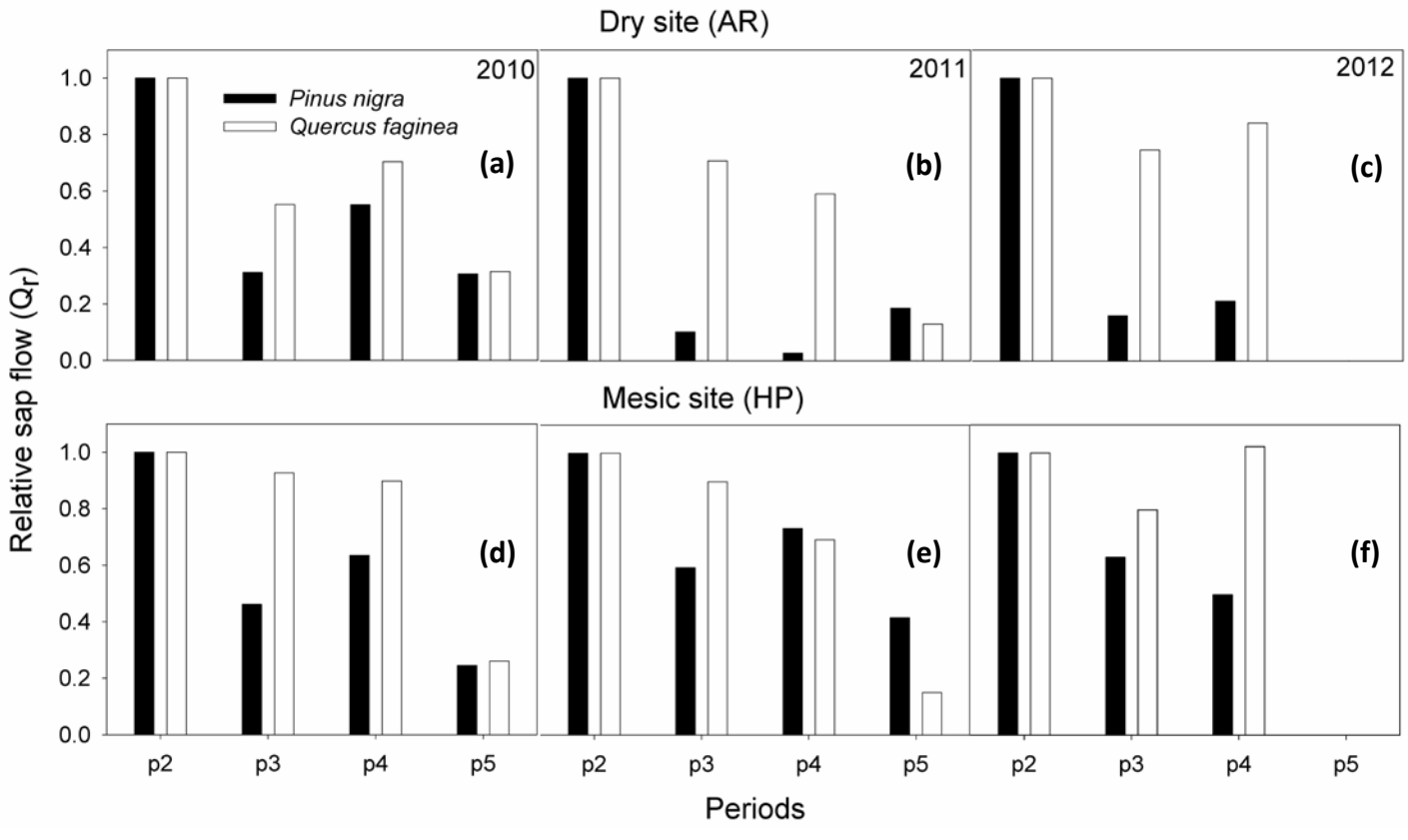
Dry site (AR)
Mesic site (HP)
2010
2011
2012
Pinus nigra
Quercus faginea
(a)
(b)
(c)
(d)
(e)
(f)
Relative sap flow (Q_r)
1.0
0.8
0.6
0.4
0.2
0.0
p2
p3
p4
p5
Periods

Figure 6. Relative sap flow (Q_r) obtained from dividing mean daily sap flow per basal area by the mean maximum sap flow measured that year (period 2, p2) and calculated for each species, year and site [Armallones (a, b and c) and Huertapelayo (d, e and f)]. Periods are considered as following: p2: maximum evapotranspiration during late spring; p3: minimum values during the peak of summer stress; p4: sap flow recovery during early autumn rainfall; p5: late autumn and early winter minimum values.

Table 3. Day of year (Julian day, mean ± SE) for the beginning and maximum of spring sap flow and its duration (days ± SE). Data are shown for both study species (*Pinus nigra* and *Quercus faginea*), sites (AR: Armallones; HP: Huertapelayo) and years (2010-2012). Significant *p*-values are shown in bold.

		Sap flow											
Site	Sp.	Beginning				Maximum				Duration			
		2010	2011	2012	*p*-value	2010	2011	2012	*p*-value	2010	2011	2012	*p*-value
AR	*P. nigra*	62 ± 11	84 ± 6	101 ± 3	**0.092**	159 ± 8	161 ± 0	133 ± 1	**0.023**	126 ± 12	96 ± 6	49 ± 2	**0.007**
	Q. faginea	134 ± 4	149 ± 6	133 ± 1	**0.041**	157 ± 6	161 ± 14	156 ± 10	0.838	55 ± 4	126 ± 77	54 ± 10	0.864
HP	*P. nigra*	86 ± 16	83 ± 7	95 ± 11	0.616	126 ± 8	125 ± 13	128 ± 5	0.938	93 ± 24	109 ± 10	60 ± 10	0.167
	Q. faginea	115 ± 1	118 ± 29	117 ± 8	0.645	146 ± 3	155 ± 12	136 ± 3	0.154	73 ± 4	76 ± 26	64 ± 32	0.692

DISCUSSION

Our study site experienced an increased aridity due to a 0.6ºC increase in mean annual temperature and a 46.1 mm decrease of annual rainfall over the last decade. This is in agreement with warming trends observed in Mediterranean ecosystems since the 70's, both in the Iberian Peninsula (Bladé and Castro Díez 2010) and at the global scale (IPCC 2013). Higher occurrence of extreme drought events over the Mediterranean region (Della-Marta et al. 2007) is particularly relevant for forest ecosystems, which are already showing symptoms of decline by water stress worldwide (Allen and Breshears 1998; Allen et al. 2010; Linares and Camarero 2012). The year 2011 was an extremely dry and warm year at our study site, with a severe and long-lasting drought resulting in very low relative extractable water from late spring to early autumn that resulted in the most stressful year over the last half century. This extreme event occurred in 2011 was preceded by another extreme summer drought with unusual high temperatures during summer just two years before, in 2009, supporting an increased frequency of extreme droughts in the area.

Reductions in the growth of Mediterranean trees have been associated with decreases in annual rainfall since the 70's (Sarris et al. 2007). This pattern was confirmed for *P. nigra* and *Q. faginea* at our study site, not only by our detailed 3-year study but also by dendrochronological studies (Granda et al. 2013). The evergreen species *P. nigra* showed not only a higher basal area increment per year but also higher spring growth rates than the deciduous *Q. faginea*, which is in

agreement with a previous dendrochronological study at the site (Granda et al. 2013). This is in agreement with the higher intrinsic water use efficiency of *P. nigra* (Granda et al. 2014). The effects in growth due to the unusual drought were noticeable the year after the exceptionally dry year. This one-year delay in growth response to drought is based on the reported dependence of growth on previous climatic conditions (Sarris et al. 2007; Morán-López et al. 2014). Reductions in annual growth the year after the extreme drought in relative terms were higher for *Q. faginea* than for *P. nigra*. Opposite results were found by Granda et al. (2013), where *P. nigra* experienced the sharpest growth decline due to the extreme drought events compared with the coexisting *Q. faginea*. These different results may be explained by the different approaches followed in each study to determine tree growth; while our study focused on processes and mechanisms involved in the responses to one unique extreme event, Granda et al. (2013) followed a dendrochronological approach over a time series with many dry and mesic years. However, and in agreement with Granda et al. (2013), *P. nigra* was able to recover after the extreme event while *Q. faginea* was not, suggesting a higher resilience of the former.

As expected, the impact of the extreme drought on growth was more adverse at the dry site for both species, observing a 60% reduction of spring growth rate for *Q. faginea* and 36% for *P. nigra* at the dry site, while decreases at the mesic site were 17% and 24% respectively. Daily stem fluctuations are a combination of both water storage changes and growth processes (Steppe et al. 2005; Zweifel et al. 2005). In the

Mediterranean region, water storage during spring increases significantly due to the abundant rainfall, resulting in increases in stem size due to processes. However, growth processes are still relevant at the daily scale during spring (Drew and Downes 2009) since growth is greater when water is not limiting, which usually occurs during spring for Mediterranean trees (Durante et al. 2009). Some studies have observed high dependency between growth and water availability (e.g. Ibáñez et al. 1998; Ogaya and Peñuelas 2007; Zapater et al. 2012), which is correlated with water use by plants. Water use was assessed through sap flow measurements since it is considered an overall estimator of the water transpired by trees (Köstner et al. 1996; Burgess et al. 2006; Poyatos et al. 2007), and therefore, as an indicator of tree performance in terms of transpiration control. The study species followed the typical Mediterranean transpiration patterns, which peak in late spring and strongly decrease during the summer drought (Tognetti et al. 1998). *P. nigra* was able to better use soil moisture in late winter and early spring, while *Q. faginea* was leafless and with no sap movement for most of this time. As expected from its deep root system and its anisohydric strategy, *Q. faginea* showed higher and more sustained sap flow rates than *P. nigra*. Our ongoing field research on both species is showing different predawn and midday water potentials (unpublished data) that support the higher water availability for *Q. faginea*, particularly during the summer.

Despite the fact that both species were sensitive to water stress and decreased their water use during dry periods, stronger reductions in

transpiration were found in *P. nigra*, an isohydric species that closes stomata during drought. By contrast, *Q. faginea*, a water spender in comparison with *P. nigra*, was able to sustain an active sap flow rate longer over the summer even at the dry site. The pattern observed in *Q. faginea* has been considered characteristic of higher drought tolerance (McDowell 2011). As expected and in agreement with growth results, the impact of the extreme drought on transpiration was higher at the dry site and in *P. nigra* when compared to *Q. faginea* (90% versus 45%). These results were noticeable due to the rather minor differences in annual temperature and soil volumetric water content at the end of the summer between the two sites (from 0.3 to 0.9 ºC and from 0.05 to 0.06 $m^3.m^{-3}$, respectively). Our results, thus, highlight the important role of microclimatic conditions of species responses to drought.

Differences found in growth and water use between the study species in response to the impact of the extreme drought can be explained by differential water strategies. Despite the fact that the distinction among water strategies remains unclear in some cases, with many intermediate behaviors (Franks et al. 2007), we still claim that *Q. faginea* could be adscribed to an anisohydric behavior while *P. nigra* follows an isohydric strategy (Tardieu and Simonneau 1998). *Q. faginea* maintained rather high transpiration rates during summer droughts, while *P. nigra*, as other *Pinus* species do in the Mediterranean region, closed stomata early on to maintain the water potential well above its hydraulic failure threshold but compromised carbon uptake (Martínez-Ferri et al. 2000; Martínez-Vilalta et al. 2002; Anderegg et al. 2014).

Under an extreme and prolonged drought, the drought-avoidance strategy that followed *P. nigra* could result in carbon starvation (McDowell 2011; Sala et al. 2012). In contrast, *Q. faginea* may be in risk of hydraulic failure and death due to cavitation processes during these events. In fact, *Q. faginea* could have been close to hydraulic failure in 2011 since its transpiration rates did not recover during early autumn rainfall while the opposite was true during wetter years. This could originate differences in competitive ability of *Q. faginea* between mesic and dry years. In fact, Zweifel et al. (2007) found that the related species *Q. pubescens* was a less efficient competitor during extremely dry years, showing massive leaf loss and almost a cessation of physiological activity. Although we did not study die-back processes or find higher tree mortality for these two species at the study site, we did observe leaf death in both species during and after the extreme summer drought of 2011. This could be the prelude of a severe defoliation event observed in some species in Europe (Carnicer et al. 2011), where these events were related to tree mortalities. Moreover, mortality is expected to increase since the expected warmer temperatures can greatly accelerate tree decay and death (Adams et al. 2009) as already observed in many forest types around the world (Allen et al. 2010).

Considering all our results, we suggest that under more frequent and extreme drought events *Q. faginea* could experience hydraulic failure, combined with eventual reductions in autumn rainfall, this could compromise species recovery and significantly reduce its growth. Although, *P. nigra* is also prone to starvation during intense drought

events, we suggest that its higher water use efficiency (Granda et al. 2014) may confer competitive advantages over *Q. faginea*. Intrinsic water use efficiency increased during unfavorable years in both species while growth decreased (Granda et al. 2014). Therefore, despite the fact that growth and intrinsic water use efficiency were higher for *P. nigra*, growth reductions during the extremely dry year were higher for *Q. faginea* (Granda et al. 2013; 2014). Thus, we suggest that *P. nigra* may perform better than *Q. faginea* at our study site under a scenario with more frequent and intense extreme droughts. However, due to the complexity of species responses to drought events and as many factors are involved in the overall impact of extreme climatic events over the life cycle of a tree, more studies should address how species-specific responses could lead to future shifts in community composition. Nonetheless, the different responses observed between *P. nigra* and *Q. faginea* may lead to changes in dominance and in community composition, and dynamics presumably favour *P. nigra*. Mild autumn and winter seasons are likely to increase transpiration at the community level if evergreen species, like *P. nigra*, dominate canopy composition in detriment of deciduous species, like *Q. faginea*. This is supported by Brantley et al. (2013), who found that an increase of deciduous species decreased evapotranspiration during winter time. As a consequence of this eventual replacement of the deciduous by the evergreen species, more water resources will be used by the community over the year, which in combination with increases in aridity and evaporative demand under increasing temperatures (Nadal-Sala et al. 2013) could have important and negative consequences at the basin level (Otero et al.

2010) therefore affecting the water cycle at large spatial and temporal scales (Brantley et al. 2013).

Decreases in species growth and changes in forest stand composition due to climate change can deeply affect carbon cycle, decreasing carbon uptake and affecting the role of forests as CO_2 sinks (Grace 2004). In fact, models have simulated a decrease in net primary production in Iberian Peninsula forests after the mid 21st century due to increases in aridity in combination with higher respiration rates and evaporative demand (Nadal-Sala et al. 2013). As a result, forests could even change from their actual role as carbon sinks to carbon sources (Nadal-Sala et al. 2013).

In conclusion, despite the fact that the species experienced the most stressful drought over the last half century and that species performance was significantly affected by this extreme event, in terms of growth and water use, the trees studied did not exceed their tolerance thresholds at our study site as suggested by the lack of mortality after the extreme event. Therefore, the high resilience observed for these Mediterranean species could be counteracting the impact derived from an extreme drought event. Studies considering different time scales, involving mechanistic understanding of species responses to extreme events and monitoring other variables like decline or mortality are needed to make sound predictions of species shifts and dynamics, and its implications for future ecosystem functioning.

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SUPPLEMENTARY DATA

Table S1. Days of the year selected for sap flow and growth analysis for each period and year for both species and at both study sites. - indicates data not available.

Periods	p1	p2	p3	p4	p5
2009	-	-	-	-	November 18th-24th
2010	April 21st-27th	June-July 29th-5th	September 3rd-9th	September-October 25th-1st	November 13th-19th
2011	March-April 30th-5th	June 12th-18th	August 15th-21th	September-October 25th-1st	November 14th-20th
2012	April 21st-27th	May 22th-28th	August-September 29th-4th	October 2nd-8th	-

Table S2. Seasonal mean ± SE total rainfall (P) and temperature (T) of the closest long-term meteorological station from AEMET (Sp: spring, Su: summer, Au: autumn, Wi: winter). Percentiles of 5%, 50% and 95% are shown for each variable using two time series, 1951-2012 and 1970-2012. Values between parentheses represent means of total annual rainfall and annual temperature for the time series or year indicated above. Annual means for 2012 do not include winter data as it is not available. Values for the studied years (2009-2012) beyond the percentile boundaries ($P_{5\%}$ and $P_{95\%}$) are indicated in bold for the time series 1970-2012 and with an asterisk for 1951-2012.

Table S2

		P (mm)				T (ºC)			
		Total	$P_{5\%}$	$P_{50\%}$	$P_{95\%}$	Mean	$P_{5\%}$	$P_{50\%}$	$P_{95\%}$
1951-2012 (491.7, 10.2)	Sp	156.4 ± 7.8	64.4	150.1	253.2	8.8 ± 0.1	7.0	8.8	10.5
	Su	101.9 ± 5.7	41.6	101.6	171.2	18.5 ± 0.1	17.0	18.4	20.2
	Au	134.2 ± 7.6	57.9	126.9	244.5	10.7 ± 0.1	9.2	10.7	12.4
	Wi	99.2 ± 6.4	35.9	89.3	196.1	3.0 ± 0.1	1.3	2.8	4.6
1970-2012 (481.5, 10.3)	Sp	156.5 ± 9.7	64.4	159.8	245.8	8.8 ± 0.2	6.8	9.1	10.8
	Su	102.1 ± 7.2	28.9	101.5	171.2	18.7 ± 0.2	17.0	18.6	20.6
	Au	128.0 ± 8.1	59.5	125.1	208.8	10.7 ± 0.2	9.2	10.8	12.4
	Wi	94.9 ± 8.0	29.4	88.8	196.1	3.1 ± 0.2	1.3	2.8	4.6
2009 (313.0, 11.5)	Sp	92.8 ± 6.6				9.7 ± 2.5			
	Su	60.2 ± 18.1				**20.8 ± 1.0***			
	Au	60.4 ± 9.3				**12.7 ± 2.6***			
	Wi	99.6 ± 1.9				2.9 ± 0.6			
2010 (504.0, 10.6)	Sp	167.9 ± 7.7				9.2 ± 1.9			
	Su	96.3 ± 22.2				19.7 ± 1.6			
	Au	167.3 ± 16.9				10.1 ± 3.4			
	Wi	72.5 ± 3.3				3.5 ± 0.5			
2011 (351.9, 11.2)	Sp	241.7 ± 24.1				**11.2 ± 2.6***			
	Su	**16.1 ± 2.4***				19.2 ± 0.9			
	Au	68.9 ± 15.8				12.3 ± 2.9			
	Wi	**25.2 ± 1.9***				2.2 ± 0.9			
2012 (278.0, 13.4)	Sp	103.2 ± 16.4				9.5 ± 2.5			
	Su	41.6 ± 9.8*				20.0 ± 0.6			
	Au	133.2 ± 1.6				10.8 ± 2.9			

CAPÍTULO 5

The effects of extreme drought on holm oak forests are attenuated by forest fragmentation

Alicia Forner, Teresa Morán-López, Dulce Flores-Rentería, Ismael Aranda, Fernando Valladares

Manuscript in preparation

Images from the front page: small forest fragment in Quintanar de la Orden.
Author: Diego García.

ABSTRACT

Fragmentation and summer drought are two of the main threats to Mediterranean forests. More frequent extreme droughts over the last decades have challenged the capacity of plants to tolerate severe water stress. However, little is known about how these two key drivers interact to affect plant performance and survival. Trees from fragmented Mediterranean forests are expected to suffer higher water stress during summer drought because they are more exposed to high radiation and temperatures due to edge effects, leading to higher water losses by evapotranspiration. However, intraspecific competition may be lower in forest edges, increasing water availability and thus reducing water stress. To test these alternative hypotheses, we monitored ecophysiological traits during two climatically contrasting years in two holm oak (*Quercus ilex*) forests archipelagos of the Iberian Peninsula. In order to evaluate the effect of summer drought on these fragmented forests, in each year we measured before and during the drought and in forest interiors, edges and small fragments located on both forests. Some ecophysiological traits were monitored in both previous- and current- year leaves in order to test the effect of leaf status on the measurements.

Water stress was lowest in trees from small fragments in both study forests and years. In general, previous-year leaves of trees from forest interiors showed the lowest predawn water potential, stomatal conductance and photochemical efficiency of photosystem II. The patterns were also observed during the intense summer drought, where

water stress was further exacerbated at forest edges and interiors. Previous- and current- year leaves were differentially affected by summer drought, and this response was trait-dependent. Our results showed that fragmentation may attenuate water stress, and that this improvement in the availability of water was even more important under extreme dry periods. The study highlights the importance of local-scale studies addressing the interaction of extreme drought events with other global change drivers, such as forest fragmentation, to elucidate the actual tree tolerance to severe water stress. This issue is fundamental to elucidate the real capacity of trees to tolerate severe water stress events in the future. More generally, our study challenges the usually-assumed expectation of detrimental effects of habitat fragmentation.

Key words: *Quercus ilex*, forest fragmentation, water stress, ecophysiological traits, summer drought

INTRODUCTION

Increased aridity associated to climate change and habitat fragmentation are the main global change drivers affecting forest ecosystems in the Mediterranean region (Matesanz and Valladares 2014; Valladares et al. 2014a). Water scarcity, especially during the summer, is expected to increase due to more frequent and intense droughts (IPCC 2014). Such extreme drought events limit water use and plant growth (Flexas et al. 2014; Forner et al. 2014). As a consequence, several physiological processes may be decoupled, decreasing plant resilience and in turn leading to their decline and later death (Lloret et al. 2004; Bréda et al. 2006). Moreover, habitat fragmentation can constraint plant adaptive potential to other environmental disturbances such as extreme droughts. In fact, habitat fragmentation has increased over the last years as a consequence of agriculture expansion (Valladares et al. 2014b), and is expected to increase over the next years (Millennium-Ecosystem-Assessment 2005).

The effects of habitat fragmentation on biodiversity are widely known (Fahrig 2003; Fischer and Lindenmayer 2007), and are caused by the impact of habitat reduction, increased isolation and changes in the physical environment of plant populations (Valladares et al. 2014a). Several negative effects of forest fragmentation are well-documented, including those on acorn dispersal, seedling recruitment, plant-animal interactions and genetic diversity (e.g. Santos and Tellería 1997; Aparicio et al. 2012; Morán-López et al. 2015). Effects of the fragmentation on the genetic structure of populations are particularly relevant (Ortego et

al. 2010; 2014). However, little is known about the interactive effects between drought and forest fragmentation on the functional response of trees (but see Kunstler et al. 2007; Matesanz et al. 2009; Leimu et al. 2010, García-Valdés et al. 2015), since most studies have addressed the separate effect of these processes on plants, obviating their temporal parallelism and dependence (Parmesan et al. 2013). Surprisingly, studies in fragmented forests have not been focused on the plant physiological status despite being crucial to cope with drought, especially in fragmented forests. A few studies suggest that forest fragmentation may exacerbate the negative impacts of climate change (Matesanz and Valladares 2014), arguing that individual plants may be more exposed to higher temperatures and radiation due to edge effects, which would induce higher water losses by evapotranspiration. Therefore, forest fragmentation may increase forest species vulnerability to drought, leading to lower resilience (Travis 2003; Opdam and Wascher 2004). However, a higher proportion of forest edges in small fragments may reduce intraspecific competition (Morán-López et al. 2016), which, in turn, could attenuate water stress during summer drought (Moreno and Cubera 2008). Indeed, recent studies in fragmented holm oak forests revealed that fragmentation may have positive effects on acorn production, soil nutrient availability and water retention, among others (Morán-López et al. 2016; Valladares et al. 2014b).

These interactive effects between fragmentation and drought may be modified by intrinsic characteristics of the site, such as size and shape of the fragment, position of the fragment in the landscape and

surrounding matrix and climatic conditions of the site, among others (Saunders et al. 1991). Moreover, these effects may be also species-specific and depend on intrinsic characteristics of the species such as the phenology, the water regulation strategy or the status of the leaves and xylem conduits when species are exposed to drought. Finally, environmental conditions such as the climatic conditions of the study year and season may affect the drought characteristics and therefore, its interaction with fragmentation. Therefore, understanding the interplay between forest fragmentation and drought, and their feedback effects on forest performance and survival is both complex and necessary.

Holm oak (*Quercus ilex*) is a widely distributed species in Mediterranean ecosystems and has a prominent economical and ecological role, which makes holm oak forests suitable to address the interactive effects of fragmentation and increasing drought intensity. *Quercus ilex* is considered a drought-tolerant species and as such is able to maintain carbon fixation under high levels of water stress (Quero et al. 2011), tolerating low leaf and xylem water potentials. However, this may lead to a potential higher risk of functionality loss of its hydraulic system (Urli et al. 2013). Indeed, holm oak usually operates at the safety limits for hydraulic conductivity, which may be exceeded during extreme droughts (Carevic et al. 2014). In order to maintain hydraulic conductivity under severe water stress, *Q. ilex* has evolved several adaptations to drought such as a deep root system (Moreno et al. 2005), sclerophyllous leaves (Savé et al. 2009), large-area vessels (Camarero et

al. 2014), high tolerance to tissue desiccation (Tognetti et al. 1998) and high ability to resprouting after disturbances (Galiano et al. 2012). Despite these adaptations, previous studies have shown that under extreme water stress conditions, *Q. ilex* has shown high losses of xylem hydraulic conductivity (Martínez-Vilalta et al. 2003), negative carbon balances (Gracia et al. 2001) and symptoms of hydraulic failure (Urli et al. 2013). These observed conditions probably resulted in high defoliation rates and dieback episodes observed in this species in some regions of the Iberian Peninsula (Peñuelas et al. 2001; Camarero et al. 2004).

Trees from fragmented Mediterranean forests are suffering higher water stress during summer drought because edge effects may increase water losses by evapotranspiration related to higher influence of solar radiation on the tree crown. However, forest edge may also reduce intraspecific competition, providing higher water availability, and reducing water stress. Therefore, we test both alternative hypotheses. Previous studies have found that sites with drier environmental conditions reinforce negatively the drought effects on Mediterranean species (Lázaro-Nogal et al. 2013, Forner et al. 2014). Therefore, we also hypothesized that holm oaks from the drier region may have the highest water stress independently of the fragmentation effect, but that positive or negative effects of fragmentation would be more significant in extremely dry years.

To test these hypotheses, we assessed the interplay between fragmentation and summer drought effects on two contrasting holm

oak forests archipelagos of the Iberian Peninsula, and during two climatically contrasting years. Furthermore, we determined whether the degree of aridity of the study region increases water stress endured by holm oak trees. To do this, we studied species responses to summer drought during two years and in two regions with contrasting climatic conditions, Lerma as the cold and wet region (northern region) and Quintanar as the warm and dry region (southern region). To evaluate the effect of summer drought on these fragmented forests, water stress and ecophysiological traits were measured in both years before and during the summer drought (early and late summer, respectively). Finally, some ecophysiological traits were monitored in both previous- and current- year leaves in order to test the effect of leaf status on the measurements.

MATERIAL AND METHODS

Study regions

The study was carried out at two holm oak (*Quercus ilex* subsp. *ballota*) archipelagos of the Iberian Peninsula and with a fragmented forest resulting from the expansion of agriculture. The forests were fragmented in a variety of patch sizes and had a surrounding matrix of cereal and legume croplands, with scattered grape crops. In the northern region the studied fragments were selected near Lerma (42º5'N, 3º45W; 930 m a.s.l.). The fragments showed similar spatial structure and vegetation and were separated by at least 50 m to a maximum of 11 km. The dominant tree is the holm oak, with isolated Lusitanian oak (*Quercus faginea*) and Spanish juniper (*Juniperus thurifera*) and understory shrubs typical of wetter and cooler supramediterranean localities (e.g. *Cistus laurifolius*, *Genista Scorpius*, *Thymus zygis*; see Santos and Tellería (1998); Díaz et al. (1999) for further details). Annual precipitation is 567 mm and annual mean temperature is 11ºC (mean 1982-2013, from Villafría meteorological station, located 39 km away from the study region; 891 m a.s.l.; AEMET data). In the southern region the studied fragments were selected near Quintanar de la Orden (39º35'N, 3º02'W; 870 m a.s.l.).The fragments showed similar spatial structure and vegetation and were separated by at least 50 m to a maximum of 8 km. The dominant tree is the holm oak with the understory composed by shrubby Kermes oak (*Quercus coccifera*) and shrub species typical from xeric mesomediterranean localities (e.g. *Rhamnus lycioides*, *R. alaternus*, *Cistus ladanifer*,

Asparagus acutifolius, see Santos and Tellería (1998); Díaz et al. (1999) for further details). Annual precipitation and mean temperature are 342 mm and 15.8ºC, respectively (mean 1982-2013, from Toledo meteorological station, located 89 km away from the study region; 515 m a.s.l.; AEMET data).

The two study regions were selected to represent contrasting environmental conditions for holm oak forests. The northern region is wetter and colder while the southern region is hotter and drier. Therefore, summer droughts in the southern region are expected to be more intense than in the northern region. In a study carried at the same sites and forest fragments Morán-López et al. (2016) found that water shortage during summer (average P/PET from June to August) was on average 68% more severe in the southern than in the northern region. Similarly, accumulated water deficit from April to August (Di), estimated as the difference between accumulated precipitation and potential evapotranspiration from April to August (Rigling et al. 2013), was 60 % higher in the south than in the north.

Experimental design

We defined three potential situations with regard fragmentation in each region: forest interior and forest edge in large fragments, and small fragments. In each site we selected three large forest fragments with continuity in the forest vegetation (> 100 ha), and 10-11 forest small fragments (mean of 0.4 ha) surrounded mainly by agricultural fields.

Many of the small fragments were no far away than 8-10 km from the large forest fragments. In large fragments we defined forest interiors when there were at least 60 m from the cultivated border (García et al. 1998). Forest edges were selected along long straight borders to avoid influences of border geometry (Fernández et al. 2002). We randomly selected between 26 and 28 trees per fragmentation level and site (total sample size = 163). Intraspecific competition was higher in forest interior than forest edge, being the lowest in small fragments in both regions (Morán-López et al. 2016).

In order to evaluate the impact of summer drought in the ecophysiological response of in the selected trees, we sampled in two periods per each year in both study regions, early summer (ESu, second fortnight of June) and late summer (LSu, second fortnight of August). The objective of measuring in ESu, i.e. before the drought, was to determine the water stress and ecophysiological status of study individuals before the drought affected them. In this way, we could identify the real effect of drought on holm oaks by comparing measurements done before and during drought (LSu). We carry out the study in two climatically contrasted years, 2012 and 2013, due to plant responses to drought may depend on annual climatic conditions, as these will determine the severity of the summer drought and the plant water stress before drought. In the northern region, mean annual temperatures were 10.7ºC and 10.1ºC, and total annual precipitations were 402.3 mm and 654.5 mm, and in 2012 and 2013, respectively. In the southern region, mean annual temperatures were 14.2ºC and

13.7ºC, and total annual precipitations were 328.4 mm and 377.5 mm, in 2012 and 2013 respectively. The drought index P/PET (unitless), calculated dividing precipitation by potential evapotranspiration, was higher, i.e. wetter conditions, in the northern (2012: 0.67 and 2013: 1.12) than in the southern region (2012: 0.45 and 2013: 0.58) in both years, and in 2013 in both regions (Morán-López et al. 2016). Therefore, 2012 was drier and warmer than 2013 in both regions, showing a more severe drought, especially in the northern region, than 2013. Morán-López et al. (2016) found that in the same regions Di was 8.9 % higher in 2012 than the long term mean (1982-2014) and 18.6 % lower in 2013. Therefore, the summer drought of 2012 could be considered as an extreme one compared to the long term mean.

Data collection

In both study periods and years, water stress was assessed from predawn leaf water potential (Ψ_{pd}, MPa) measured in two twigs per tree. To avoid the effect of daily meteorological conditions was confused with the fragmentation effect, we followed a randomized factorial design where each day approximately the same number of trees per fragmented level were measured. On average, a total of 15 trees per day were measured. Excised twigs were collected at predawn, enclosed in sealable plastic bags with air saturated of humidity and CO_2 and kept refrigerated in dark until measurements were performed (Pérez-Harguindeguy et al. 2013). All leaf water potential measurements

were carried out using a Scholander pressure chamber (Scholander et al. 1965).

We also measured stomatal conductance (g_s, mmol.s^{-1}.m^{-2}) in 2-3 leaves per tree with a leaf porometer SC-I (Decagon, Pullman, USA) at mid-morning, when species have maximal conductance (10:00 to 12:00 h). Predawn and midday maximum photochemical efficiency of photosystem II (F_v/F_{mpd} and F_v/F_{mmd}, respectively; unitless) were also measured in dark adapted leaves with a portable pulse-modulated fluorometer FMS2 (Hansatech, Norfolk, UK) and in 2-3 leaves per tree close to those used to measure g_s. Midday measurements were done from 13:30 to 15:30. These three traits were measured in both current-year leaves (CYL) and previous-year leaves (PYL) to test whether differences on leaf status determined the suffered drought effects. CYL and PYL may have differences in biochemical composition due to their different age or to being exposed to previous droughts that determines their vulnerability to a new one. Leaf dry matter content (LDMC, mg.g^{-1}) was calculated in LSu and in both years from three previous-year leaves per tree dividing oven-dry mass by its water-saturated fresh mass. Leaves were saturated in darkness during 12 h. All leaves were sampled in the south of the tree crowns.

Statistical analysis

Leaf dry mass content (LDMC) was calculated in three leaves per tree and afterwards means per tree were calculated.

Linear mixed-effects models (LMMs) were used to assess the effect of year, period, region, forest fragmentation levels and their interaction (fixed factors) on water stress (Ψ_{pd}) ecophysiological traits (g_s, F_v/F_{mpd}, F_v/F_{mmd}) and LDMC (Zuur et al. 2007). Fragment identity was considered as a random factor in the LMMs. Analyses were performed separately in data collected from previous- and current- year leaves (PYL and CYL, respectively). Models were fitted based on a restricted maximum likelihood method using the nlme package from R statistical software (Pinheiro et al. 2007). Differences between measurements performed in PYL and CYL in all study traits and according to year, period, region and forest fragmentation level were tested using a nonparametric Kruskal-Wallis test due to non-normal distribution.

To test which traits were more correlated with water stress we performed Spearman's rank correlation analysis between water stress (Ψ_{pd}) and ecophysiological traits (g_s, F_v/F_{mpd}, F_v/F_{mmd}) for both PYL and CYL. Correlations between water stress and LDMC were only calculated in PYL. All analyses were carried out using the R statistical software (version R3.3.1; R Development Core Team 2016, Vienna).

RESULTS

General patterns of water stress and ecophysiological traits

Water stress, as measured by leaf water potential, differed between regions, years, periods and fragmentation levels (Table 1). Fragmentation effect interacted with year and period, showing a differential response to drought depending on the fragmentation level (Table 1, Fig. 1). The lowest leaf predawn water potentials (Ψ_{pd}) were observed in the southern region and during the driest conditions, 2012 and late summer (LSu) (Fig. 1). Forest fragmentation attenuated water stress in LSu of 2012 in the northern region and in ESu and LSu of both study years in the southern region. In LSu of 2012, forest edges and interiors of both regions showed Ψ_{pd} below -3 MPa. Decreases in Ψ_{pd} between measurements done in ESu and LSu were higher in 2012 in the southern region and in 2013 in the northern region. In this region, decreases in Ψ_{pd} in LSu compared to ESu were higher in forest edges in both years. In the southern region those decreases were higher in forest edges in 2012 (-107 %) and in forest interiors in 2013 (-73 %).

Table 1. Summary statistics for the linear mixed models (*F*) testing the effect of year (Ye), period (Pe), fragment level (Frag), region (Reg) and their interaction on the predawn leaf water potential (Ψ_{pd}), stomatal conductance (g_s), predawn (Fv/Fm_{pd}) and midday (Fv/Fm_{md}) maximum photochemical efficiency of photosystem II and leaf dry matter content (LDMC) for *Quercus ilex*. Significant differences are indicated with asterisk (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$). n.s. indicated not significant differences.

		Ψ_{pd}	g_s		Fv/Fm_{pd}		Fv/Fm_{md}		LDMC
			PYL	CYL	PYL	CYL	PYL	CYL	
Intercept	*F*	**1502.95*** **	**4353.17*** **	**2582.40*** **	**10868.02*** **	**18811.39*** **	**278952.33*** **	**300356.80*** **	**1891071.70*** **
Ye	*F*	**822.22*** **	**85.15*** **	**18.04*** **	**35.98*** **	0.02$^{n.s.}$	**94.46*** **	**8.44** **	**36.2*** **
Pe	*F*	**1412.41*** **	**104.91*** **	**103.92*** **	**42.62*** **	**67.69*** **	**168.39*** **	**6.58* **	-
Reg	*F*	**19.36*** **	0.20$^{n.s.}$	0.45$^{n.s.}$	**5.81* **	**41.23*** **	1.83$^{n.s.}$	0.50$^{n.s.}$	**8.8** **
Frag	*F*	**17.48*** **	1.88$^{n.s.}$	2.57$^{n.s.}$	**18.56*** **	**20.23*** **	**22.23*** **	**23.79*** **	**3.2* **
Ye x Pe	*F*	**111.25*** **	1.75$^{n.s.}$	**11.84** **	**9.16** **	**30.59*** **	**67.98*** **	**91.53*** **	-
Ye x Reg	*F*	0.64$^{n.s.}$	**44.27*** **	**41.81*** **	**68.41*** **	**143.09*** **	**31.49*** **	**87.16*** **	**31.5*** **
Ye x Frag	*F*	**13.49*** **	**9.89*** **	**24.69*** **	**9.28*** **	**11.39*** **	**12.91*** **	**7.94** **	
Pe x Reg	*F*	0.14$^{n.s.}$	0.05$^{n.s.}$		0.89$^{n.s.}$	**37.88*** **	1.52$^{n.s..}$	**59.50*** **	-
Pe x Frag	*F*	**16.18*** **	**8.91** **	**15.06*** **	**23.13*** **	**12.07*** **	**19.56*** **	**9.40*** **	-
Reg x Frag	*F*	2.57$^{n.s.}$			2.37$^{n.s.}$	**6.45** **	**5.87** **	**4.38* **	
Ye x Pe x Reg	*F*	**31.15*** **	**9.07** **		**8.85** **			0.16$^{n.s.}$	-
Ye x Pe x Frag	*F*	**5.68** **			**4.66** **	**4.03* **	**9.81*** **	**4.49* **	-
Ye x Reg x Frag	*F*	**3.11* **			2.40$^{n.s.}$		3.78*	**4.90** **	
Pe x Reg x Frag	*F*				**4.51* **		**5.63** **	**12.19*** **	-
Ye x Pe x Reg x Frag	*F*				**4.99** **			**4.91** **	-

Stomatal conductance (g_s) differed between years and periods (Table 1). Forest fragmentation only affected g_s when interactions with year and period were considered. In general, the lowest g_s were measured in LSu and in 2012 for all fragmentation levels (Fig. 2). Previous-year leaves (PYL) showed lower g_s than current-year leaves (CYL). In both regions, g_s measured in LSu of 2012 was significantly higher in small forest fragments, for both PYL and CYL. In general, the decrease in g_s between both seasonal periods was higher in 2012 for both regions, except at the forest edges in 2012 in the southern region and at forest small fragments in both regions for PYL. In general, in the northern region higher decreases were observed in forest interiors (2012: -37 %, 40 %; 2013: -28 %, 27%, in PYL and CYL respectively). Forest small fragments showed lower decreases in g_s as a consequence of summer drought in both years and regions.

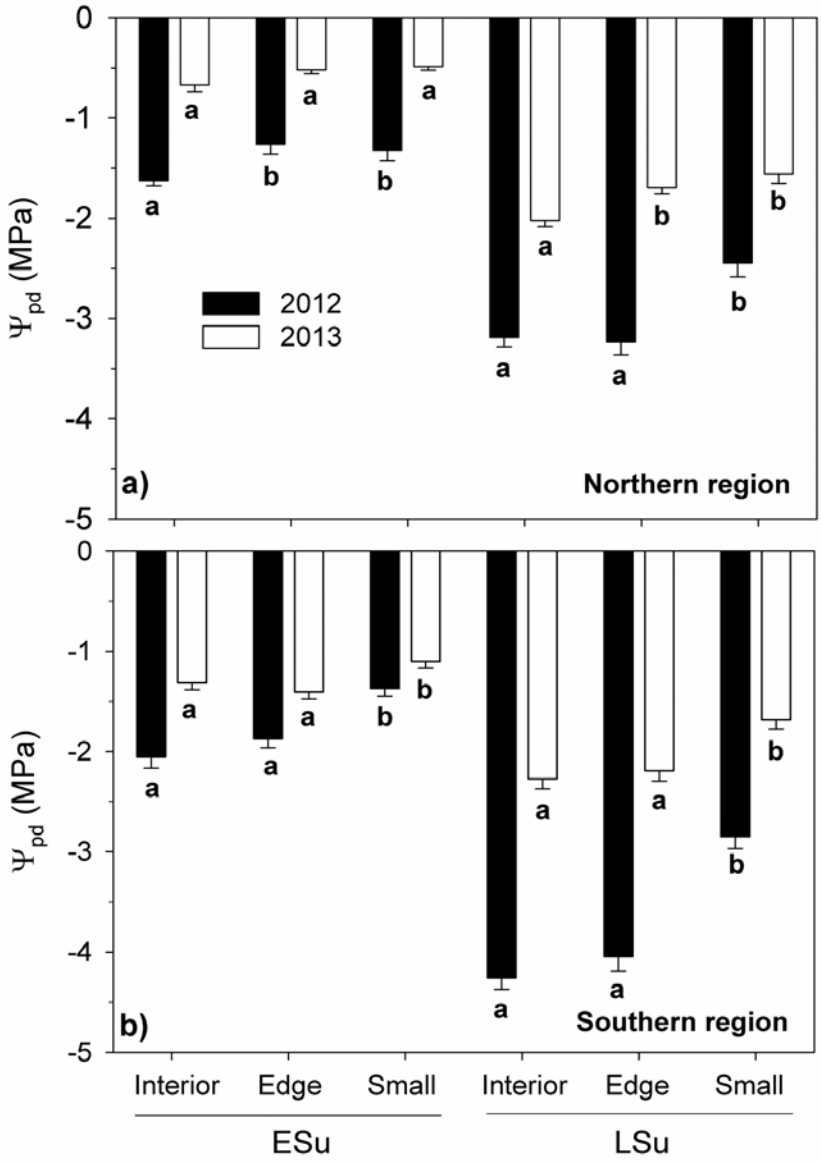

Figure 1. Mean ± SE of leaf water potential (Ψ_{pd}, MPa) measured in each fragmentation level (Interior, Edge and Small) and in early (ESu) and late (LSu) summer of both study years (2012 and 2013) in the northern (a) and southern region (b). For each year and period *different letters* indicate significant differences between fragmentation levels at $P = 0.05$.

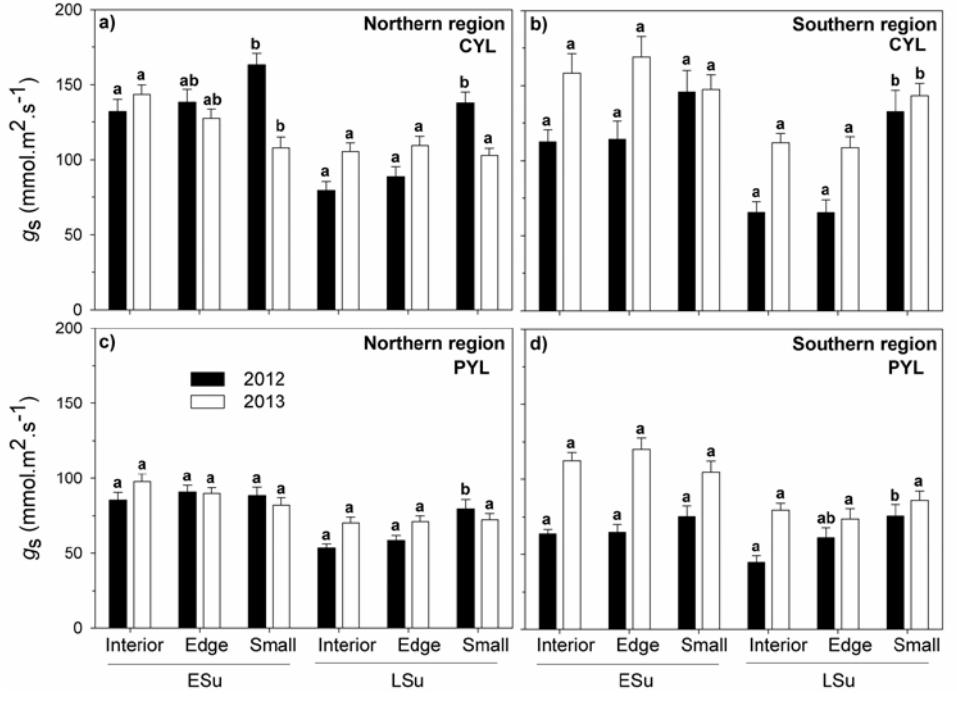

Figure 2. Mean ± SE of stomatal conductance (g_s, mmol.s^{-1}.m^{-2}) measured in each fragmentation level (Interior, Edge and Small) and in early (ESu) and late (LSu) summer of both study years (2012 and 2013) in the northern (a,c) and southern region (b,d). Measurements were done in previous-year leaves (PYL; a,b) and in current-year leaves (CYL; c,d). For each year and period *different letters* indicate significant differences between fragmentation levels at P = 0.05.

Predawn and midday maximum photochemical efficiency of photosystem II (F_v/F_{mpd} and F_v/F_{mmd}, respectively) differed between years, periods and forest fragmentation (Table 1). However, region determined F_v/F_{mpd} but not F_v/F_{mmd} in both PYL and CYL. In general, the effect of forest fragmentation on F_v/F_m depended on its interaction with year, period and region. F_v/F_m was lower at midday than at predawn, and the lowest values were observed in LSu of 2012 in the southern region (Figs. 3,4). F_v/F_{mpd} and F_v/F_{mmd} were significantly higher in forest small fragments in LSu of 2012 in both regions, and in LSu of 2013 in the southern region (Figs. 3b,d;4b,d). In the southern region, fragmentation had an effect on F_v/F_{mpd} in ESu of 2012 in CYL and on F_v/F_{mmd} in both years in PYL (Figs. 3b;4d). CYL of forest interiors showed significantly lower F_v/F_{mmd} than edges and forest small fragments in ESu of 2012 (Fig. 3c). In the northern region, 2013 showed the highest decreases in both F_v/F_{mpd} and F_v/F_{mmd}, excepting measurements in PYL. Conversely, in the southern region 2012 showed the highest decreases in both traits. Both F_v/F_{mpd} and F_v/F_{mmd} decreased more due to summer drought effect in forest interiors in both years and regions for PYL.

Leaf dry matter content (LDMC) differed between years, regions and forest fragmentation (Table 1). Only the interaction between year and region was significant. Differences among interiors, edges and forest small fragments were not clear in the northern region (Fig. 5a). However, in the southern region forest small fragments showed significantly lower LDMC in 2012 and similar values to forest edges in 2013 (Fig. 5b).

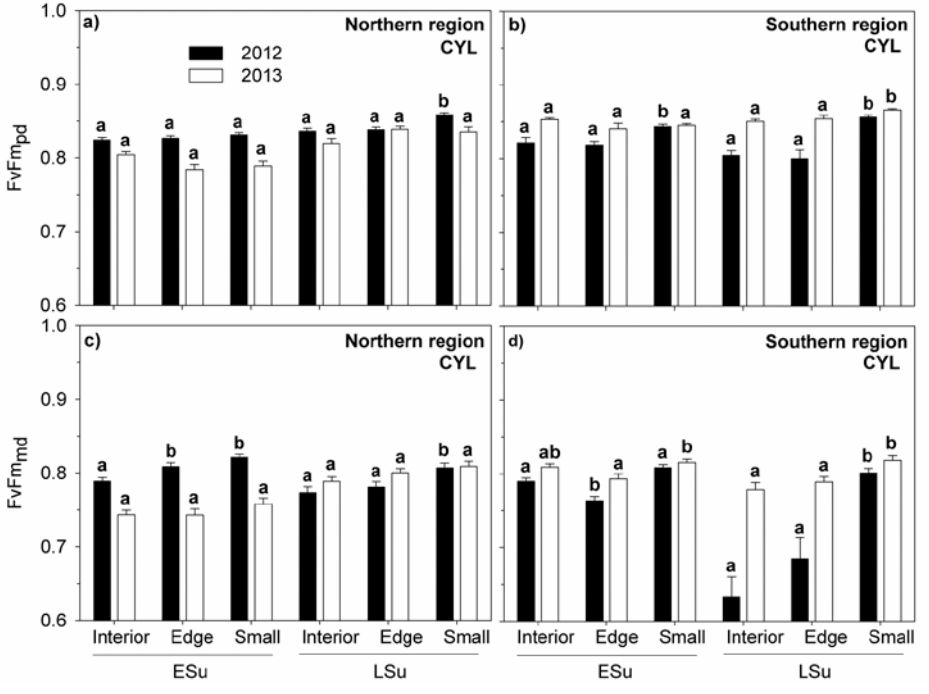

Figure 3. Mean ± SE of predawn (Fv/Fm_{pd}; a,b) and midday (Fv/Fm_{md}; c,d) maximum photochemical efficiency of photosystem II (unitless) measured in current-year leaves (CYL). Measurements were done for each fragmentation level (Interior, Edge and Small) and in early (ESu) and late (LSu) summer of both study years (2012 and 2013) in the northern (a,c) and southern region (b,d). For each year and period *different letters* indicate significant differences between fragmentation levels at $P = 0.05$.

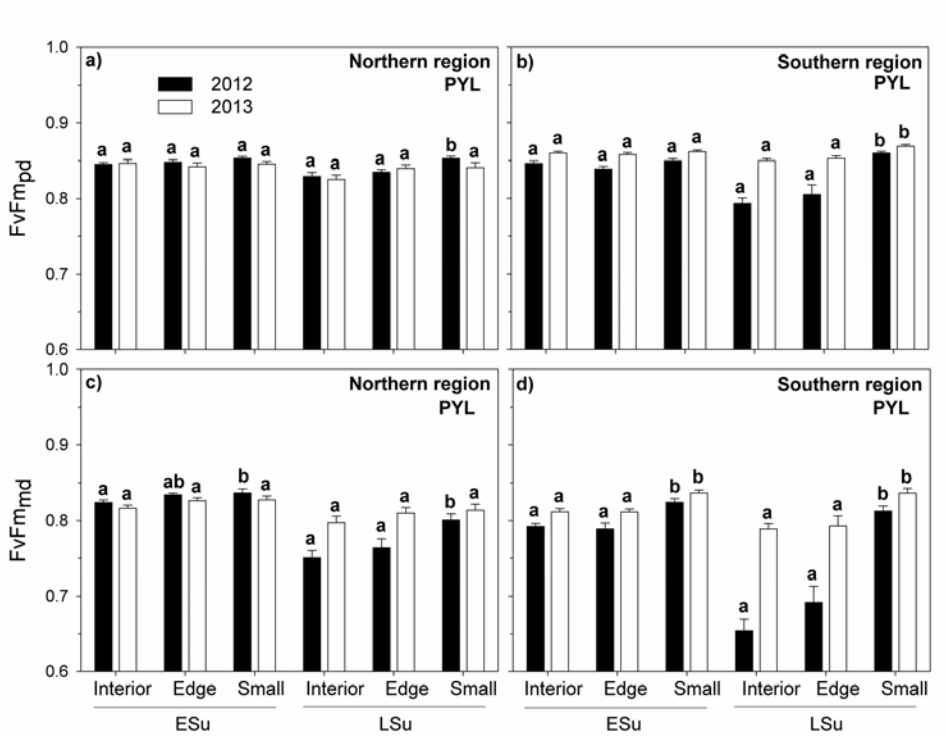

Figure 4. Mean ± SE of predawn (Fv/Fm_{pd}; a,b) and midday (Fv/Fm_{md}; c,d) maximum photochemical efficiency of photosystem II (unitless) measured in previous-year leaves (PYL). Measurements were done for each fragmentation level (Interior, Edge and Small) and in early (ESu) and late (LSu) summer of both study years (2012 and 2013) in the northern (a,c) and southern region (b,d). For each year and period *different letters* indicate significant differences between fragmentation levels at $P = 0.05$.

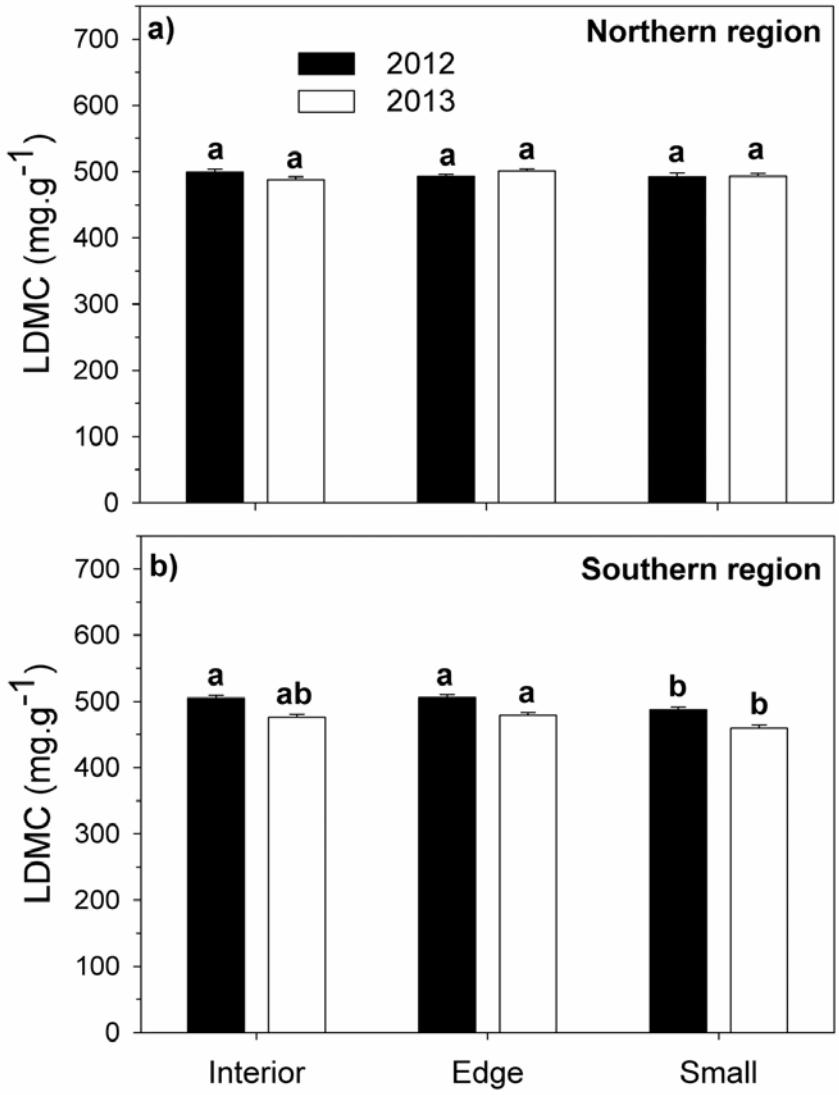

Figure 5. Mean ± SE of leaf dry matter content (LDMC, $mg.g^{-1}$) measured in each fragmentation level (Interior, Edge and Small) in late summer (LSu) of both study years (2012 and 2013) in the northern (a) and southern region (b). For each year and period *different letters* indicate significant differences between fragmentation levels at $P = 0.05$.

Differences between current-year leaves and previous-year leaves

In both regions, g_s differed between current and previous year leaves in interiors, edges and forest small fragments (Table 2). The only exception to this pattern was g_s measured in forest edges from the southern region in ESu of 2013. In the northern region, F_v/F_{mpd} and F_v/F_{mmd} were significantly different between CYL and PYL from all fragmentation levels in ESu of both years. The pattern found in the southern region was of significant differences in leaves from forest small fragments in LSu of 2012 in F_v/F_{mpd} and F_v/F_{mmd}, and in 2013 for the latter variable. Forest interiors and edges showed differences in F_v/F_{mpd} between CYL and PYL in 2012 but not in 2013.

Correlation between water stress and ecophysiological traits

The highest significant correlations (> 0.7) between water stress and ecophysiological traits were found in the driest conditions, LSu of 2012 in the southern region (Table 3). Those high correlations were found in CYL from forest edges for g_s, F_v/F_{mpd} and F_v/F_{mmd} and from forest small fragments for the former variable (Table 3). In PYL those high correlations were found also in forest edges for F_v/F_{mpd} and F_v/F_{mmd} but not for g_s (0.4) (Table 3). Ψ_{pd} in forest interiors was correlated only with F_v/F_{mmd} in CYL in ESu of 2012 and in LSu of 2013 in PYL. LDMC was correlated with Ψ_{pd} only in LSu of 2012 in forest interiors and edges.

Table 2. Summary statistics for Kruskal-Wallis test (χ^2) testing differences in the stomatal conductance (g_s), predawn (Fv/Fm_{pd}) and midday (Fv/Fm_{md}) maximum photochemical efficiency of photosystem II and leaf dry matter content (LDMC) measured in current-year leaves (CYL) and in previous-year leaves (PYL) for *Quercus ilex*. Significant differences are indicated in bold and with asterisk (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$). n.s. indicated not significant differences.

			2012		2013	
			ESu	LSu	ESu	LSu
g_s	NR	I	**18.78*** **	**9.22** **	**20.31*** **	**18.19*** **
		E	**17.89*** **	**10.19* **	**23.05*** **	**17.31*** **
		S	**31.32*** **	**24.23*** **	**7.14** **	**19.69*** **
	SR	I	**21.12*** **	**9.51** **	**4.99* **	**12.30** **
		E	**14.28** **	**7.11** **	$0.12^{n.s.}$	**10.81** **
		S	**16.55*** **	**10.13** **	**10.74** **	**21.05*** **
Fv/Fm_{pd}	Northern region	I	**19.40*** **	$0.58^{n.s.}$	**28.50*** **	$0.19^{n.s.}$
		E	**16.67*** **	$0.59^{n.s.}$	**26.94*** **	$0.08^{n.s.}$
		S	**21.18*** **	$2.09^{n.s.}$	**32.20*** **	$0.28^{n.s.}$
	Southern region	I	**8.82** **	**5.93* **	$1.08^{n.s.}$	$0.11^{n.s.}$
		E	**7.96** **	**6.45* **	$0.15^{n.s.}$	$1.17^{n.s.}$
		S	$2.17^{n.s.}$	**18.29*** **	$1.55^{n.s.}$	$2.66^{n.s.}$
Fv/Fm_{md}	Northern region	I	**22.64*** **	$3.02^{n.s.}$	**34.5*** **	$1.38^{n.s.}$
		E	**15.97*** **	$0.78^{n.s.}$	**32.30*** **	$1.99^{n.s.}$
		S	**11.48** **	$0.10^{n.s.}$	**29.61*** **	$0.70^{n.s.}$
	Southern region	I	$0.00^{n.s.}$	$0.39^{n.s.}$	$0.01^{n.s.}$	$0.29^{n.s.}$
		E	**6.24* **	$3.33^{n.s.}$	$0.25^{n.s.}$	$1.40^{n.s.}$
		S	**5.59* **	**12.99** **	$2.45^{n.s.}$	**6.83** **

Table 3. Spearman's correlations between the predawn leaf water potential (Ψ_{pd}) and the stomatal conductance (g_s), predawn (Fv/Fm_{pd}) and midday (Fv/Fm_{md}) maximum photochemical efficiency of photosystem II and leaf dry matter content (LDMC) measured in *Quercus ilex*. All correlations were performed for previous- (PYL) and current-year leaves (CYL), excepting LDMC which only was measured in PYL. Significant differences are indicated in bold and with asterisk (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$). n.s. indicated not significant differences.

			Previous-year leaves				Current-year leaves			
			2012		2013		2012		2013	
			ESu	LSu	ESu	LSu	ESu	LSu	ESu	LSu
g_s	Northern region	I	$0.053^{n.s.}$	$-0.089^{n.s.}$	$0.291^{n.s.}$	$-0.179^{n.s.}$	$0.073^{n.s.}$	$-0.257^{n.s.}$	$-0.120^{n.s.}$	$-0.033^{n.s.}$
		E	$0.294^{n.s.}$	$0.027^{n.s.}$	$0.020^{n.s.}$	$0.259^{n.s.}$	$-0.144^{n.s.}$	**0.538****	$0.169^{n.s.}$	$0.108^{n.s.}$
		S	**0.454***	**0.619****	$0.245^{n.s.}$	$0.339^{n.s.}$	$0.138^{n.s.}$	**0.631****	$0.156^{n.s.}$	$-0.030^{n.s.}$
	Southern region	I	$0.077^{n.s.}$	$0.152^{n.s.}$	$0.199^{n.s.}$	$0.264^{n.s.}$	$-0.290^{n.s.}$	$0.094^{n.s.}$	$0.284^{n.s.}$	$0.262^{n.s.}$
		E	$0.133^{n.s.}$	**0.407***	$-0.026^{n.s.}$	$0.105^{n.s.}$	$0.381^{n.s.}$	**0.719*****	$-0.264^{n.s.}$	**0.458***
		S	$0.144^{n.s.}$	**0.591****	$0.060^{n.s.}$	$0.175^{n.s.}$	**0.402***	**0.745*****	$0.074^{n.s.}$	$0.313^{n.s.}$
Fv/Fm_{pd}	Northern region	I	$0.200^{n.s.}$	$-0.370^{n.s.}$	$-0.148^{n.s.}$	**-0.382***	$0.313^{n.s.}$	$-0.238^{n.s.}$	**-0.409***	$-0.170^{n.s.}$
		E	$0.092^{n.s.}$	$0.174^{n.s.}$	**-0.406***	$-0.257^{n.s.}$	$-0.047^{n.s.}$	$0.104^{n.s.}$	$-0.293^{n.s.}$	$-0.181^{n.s.}$
		S	$0.028^{n.s.}$	$0.285^{n.s.}$	$-0.218^{n.s.}$	$-0.163^{n.s.}$	$-0.212^{n.s.}$	$0.229^{n.s.}$	$-0.020^{n.s.}$	$-0.166^{n.s.}$
	Southern region	I	$-0.076^{n.s.}$	$0.248^{n.s.}$	$-0.198^{n.s.}$	$0.088^{n.s.}$	$-0.404^{n.s.}$	$0.164^{n.s.}$	$-0.003^{n.s.}$	$0.146^{n.s.}$
		E	$0.108^{n.s.}$	**0.745*****	$-0.089^{n.s.}$	$-0.055^{n.s.}$	$0.073^{n.s.}$	**0.792*****	$-0.118^{n.s.}$	$0.139^{n.s.}$
		S	$-0.163^{n.s.}$	$0.196^{n.s.}$	$-0.024^{n.s.}$	**0.486****	$0.278^{n.s.}$	**0.450***	$-0.184^{n.s.}$	$0.203^{n.s.}$
Fv/Fm_{md}	Northern region	I	$0.363^{n.s.}$	$-0.217^{n.s.}$	$0.197^{n.s.}$	$-0.324^{n.s.}$	**0.595****	$0.030^{n.s.}$	$0.090^{n.s.}$	$-0.147^{n.s.}$
		E	$0.229^{n.s.}$	$0.199^{n.s.}$	$-0.259^{n.s.}$	$-0.113^{n.s.}$	$-0.101^{n.s.}$	$-0.108^{n.s.}$	$-0.231^{n.s.}$	$-0.149^{n.s.}$
		S	$0.000^{n.s.}$	$0.142^{n.s.}$	$-0.185^{n.s.}$	$-0.018^{n.s.}$	**-0.419***	$0.022^{n.s.}$	$0.031^{n.s.}$	$-0.081^{n.s.}$
	Southern region	I	$0.222^{n.s.}$	$0.085^{n.s.}$	$0.103^{n.s.}$	$-0.048^{n.s.}$	$0.118^{n.s.}$	$0.180^{n.s.}$	$0.330^{n.s.}$	$0.148^{n.s.}$
		E	**0.460***	**0.795*****	**0.391***	**0.436***	$0.237^{n.s.}$	**0.773*****	$0.075^{n.s.}$	$0.324^{n.s.}$
		S	$0.169^{n.s.}$	**0.638****	$0.326^{n.s.}$	**0.522****	$0.277^{n.s.}$	$0.271^{n.s.}$	$-0.263^{n.s.}$	**0.444***
LDMC	Northern region	I		$0.200^{n.s.}$		$0.204^{n.s.}$				
		E		$-0.18^{n.s.}$		$0.03^{n.s.}$				
		S		$-0.268^{n.s.}$		$-0.059^{n.s.}$				
	Southern region	I		**-0.501****		$0.098^{n.s.}$				
		E		**-0.487***		$-0.359^{n.s.}$				
		S		$-0.252^{n.s.}$		$0.005^{n.s.}$				

DISCUSSION

Our results show lower water stress on forest fragments compared to continuous forests, both interiors and edges, independently of the study region and year. Differences in water stress, assessed by differences in predawn leaf water potential, among fragmentation levels were enlarged under drier conditions in both regions. Therefore, the highest differences in water stress among small fragments and continuous forests were observed in late summer, i.e. during summer drought, in both years. Moreover, these differences were higher in the driest year, 2012, in both regions, although the highest differences were observed in the driest region, i.e. the southern. Therefore, fragmentation attenuated water stress even under extreme droughts and drier climatic conditions, such as drier years or regions, may intensify this attenuation.

Differences in the fragmentation effect on plant species due to contrasted annual climatic conditions has been observed in the reproduction success of shrubs (Rabasa et al. 2009), perennial herbs (Valdés and García 2011) and trees (Morán-López et al. 2016). However, to our knowledge, there are not previous studies done in Mediterranean trees which address the fragmentation effects on ecophysiological traits in climatically contrasted years. However, some evidences has been observed in holm oak stands when high tree densities, such as in forest interiors, showed higher water stress and lower gas exchange (Moreno and Cubera 2008). This becomes even more relevant under climate change scenarios due to climatic interannual variability may be increased and extremely dry years will be more frequent in the

Mediterranean region (Della-Marta et al. 2007; IPCC 2014). Therefore, studies of fragmentation effects on plant species, especially including interactions with drought, could not be extrapolated to other years or periods.

The attenuated water stress in small fragments could be due to lower intraspecific competition to water resources observed in other studies performed in *Q. ilex* (e.g. Moreno and Cubera 2008 and Morán-López et al. 2016). However, other factors related to differences in habitat quality between forest fragmentation levels could be modulating the drought effects. For example, higher fertility and water holding capacity in soils from small fragments may decrease drought effect on holm oak seedlings (Flores-Rentería et al. 2015).

The lowest predawn leaf water potentials were measured during the severe drought of 2012 in both regions. Those predawn water potentials exceeded the limits of safety for hydraulic conductivity for *Q. ilex*, i.e. -3 MPa, which are reached under severe drought (Carevic et al. 2014). Small fragments showed higher predawn water potentials than the mentioned limit during the severe drought in both regions (northern: -2.5 ± 0.14 MPa; southern: -2.8 ± 0.12 MPa). However, predawn water potentials of interiors and edges were -3.2 ± 0.01 MPa and -3.2 ± 0.13 MPa, respectively in the wetter region. In the drier region, those predawn water potentials were -4.3 ± 0.1 MPa and -4.0 ± 0.2 MPa, respectively. Therefore, forest interiors and edges from both regions may loss around 68% of their hydraulic conductivity under severe drought (Carevic et al. 2014). Indeed, other studies performed in

Q. ilex found that predawn water potentials≤ -4 MPa resulted in 75% loss of hydraulic conductivity (Martínez-Vilalta et al. 2002). Thus, both forest interiors and edges from the driest region may have less than 25 % of their optimal hydraulic conductivity at the end of the summer. Carevic et al. (2014) found that a loss of hydraulic conductivity higher than 68%, i. e. -3 MPa of predawn water potential, *Q. ilex* was not able to recover xylem conductivity after a rain event. They also stablished that 55 % of loss hydraulic conductivity, i.e. -2.5 MPa, was the limit to allow xylem recovery after a rain event. Therefore, this limit was only reached in all fragmentation levels under the driest conditions, late summer of 2012 in the southern region.

Water stress resulting from drier summer and annual conditions was observed thorough decreases in stomatal conductance and predawn and midday maximum photochemical efficiency of photosystem II decreases in all fragmentation levels. As observed in predawn water potential, the effects of drought in those traits were lower in small fragments, and differences with forest interiors and edges were exacerbated in the driest conditions (driest year and region). Stomatal conductance observed in continuous forests during both summer drought (50-80 $mmol.m^2.s^{-1}$) and wetter conditions (125-200 $mmol.m^2.s^{-1}$) agreed with studies performed by Nardini et al. (2000) in *Q. ilex*. Photoinhibitory damage in response to high temperatures and/or water stress was expected when F_v/F_m decreases below the optimum, which is 0.8 according to Maxwell and Jonson (2000) (Valladares and Pearcy 1997). Both F_v/F_m measured at predawn and

midday were not lower than this optimum in small fragments independently of the year and region. Contrary, F_v/F_m measurements performed at midday in forest interiors and edges were lower especially in the driest conditions (<0.7).

These observations make sense considering the highest significant correlations between predawn water potential and ecophysiological traits that were mainly found under higher water stress conditions (late summer of 2012 in the southern region). In fact, lower significant correlations were found under milder water stress, such as observed in the northern region or in the early summer compared to the southern region and late summer, respectively. Moreover, not only water stress determined these correlations, fragmentation also seemed to determine the significance and degree of these correlations, since significant correlations were found mainly in small forest fragments and forest edges. Indeed, highest significant correlations were found in forest edges, which could be explained by the edge effect. Under severe water stress conditions, forest interiors did not show any significant correlations between water stress and ecophysiological traits. The only exception was the correlation with leaf dry mass content, which was only significant in forest interiors and edges in late summer of 2012 in the southern region. Under severe water stress, such differences in forest interiors might result in differences in the selection of phenotypes if those traits are adaptive, i.e. have an effect on the species fitness. In this case, the optimum phenotype would be different in forest interiors,

which is so relevant at such a small scale and under the large selective pressure that could be done by ongoing frequent and intense droughts.

Our study challenges the usually-assumed expectation of detrimental effects of habitat fragmentation. Studies of negative effects of habitat fragmentation cover a wide range of scales and issues: limitation of the expression of plasticity and evolutionary potential of plants under climate change (Matesanz and Valladares 2014), disruption in plant-animal interactions (Leimu et al. 2010), lower seed dispersal effectiveness (Morán-López et al. 2015 oikos) and changes in the structure and metabolism of soil microbial communities (Flores-Rentería et al. 2015), among others. However, few studies also agree with positive effects of forest fragmentation, such as increase of acorn production (Morán-López et al 2016) and of water and nutrient soil availability (Flores-Rentería et al. 2015). Other positive effects of fragmentation are observed thorough studies were lower density stands, such as small fragments. For example, Moreno and Cubera (2008) found predawn and midday water potential, CO_2 assimilation rate and sap flow densities were significantly higher in low density tree stands.

In conclusion, fragmentation may attenuate water stress in holm oak forest, being even more significant this attenuation under severe water stress conditions. This novel result has a relevant importance under the more frequent and intense droughts expected under the

ongoing climate change. Moreover, the study highlights the importance of local-scale studies addressing the interaction of extreme drought events with other global change drivers, such as forest fragmentation, to elucidate the actual tree tolerance to severe water stress. This issue is fundamental to elucidate the real capacity of trees to tolerate severe water stress events in the future. Moreover, the study support the idea that studies focused on the interplay between forest fragmentation and drought may be performed among seasons and years, due to results obtained from one-year studies cannot be extrapolated. More generally, our study challenges the usually-assumed expectation of detrimental effects of habitat fragmentation.

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CAPÍTULO 6

Discusión general

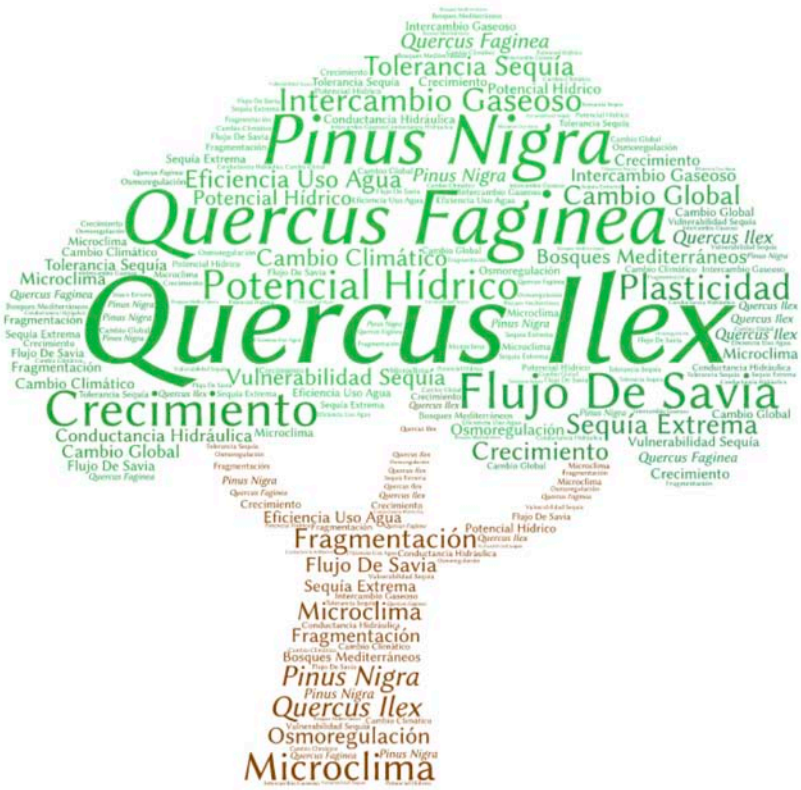

Imagen página anterior: Diseño y realización: JM. Escolano

En esta tesis doctoral se han llevado a cabo varios estudios, a corto y largo plazo, con el fin de analizar la respuesta funcional a la sequía de especies mediterráneas coexistentes y con contrastadas estrategias hídricas. Gracias a la duración temporal y resolución de los estudios, se han identificado patrones en cada una de las especies de estudio, los cuales han sido asociados a un determinado grado de tolerancia a la sequía. Estos resultados permiten discutir sobre cuál podría ser la estrategia hídrica más beneficiosa frente a las cada vez más frecuentes sequías extremas.

La especie evitadora, *Pinus nigra*, presentó mayores descensos en su tasa de intercambio gaseoso y en el consumo de agua que las especies más tolerantes a la sequía, *Quercus faginea* y *Quercus ilex*. Esto fue debido principalmente a cierres estomáticos (Attia et al. 2015), ya que a pesar de la severidad de la sequía, no se observaron daños en la bioquímica de las hojas, ni en las especies evitadoras ni en las tolerantes. Menores tasas de transpiración son características de especies evitadoras cuando se encuentran bajo condiciones de estrés hídrico ya que su gran control estomático les permite cerrar los estomas cuando todavía no se ha alcanzado la máxima intensidad de la sequía (McDowell et al. 2008). Esta clara ventaja a la hora de afrontar las pérdidas de agua tiene un efecto negativo en la fijación de carbono (Martínez-Ferri et al. 2000; Martínez-Vilalta et al. 2002). Es por ello que *P. nigra* presentó mayores disminuciones en la fijación de carbono a nivel de hoja durante la sequía que ambas especies tolerantes. Tanto *Q. ilex* como *Q. faginea*, mantuvieron los estomas abiertos durante las

sequías, soportando mayores descensos en los potenciales hídricos, pero manteniendo la fijación de carbono, lo cual es característico de especies tolerantes (McDowell et al. 2008). Esta ventaja de las especies tolerantes ocasiona un mayor riesgo de pérdida de conductividad hidráulica del xilema, lo cual las acerca al umbral crítico a partir del cual se produce el fallo hidráulico y la cavitación del xilema (Martínez-Vilalta et al. 2002). Entre ambas especies tolerantes, fue *Q. ilex* la que se mostró más tolerante a la sequía, ya que presentó menores descensos en la transpiración foliar y en el crecimiento secundario durante la sequía extrema. Además, en *Q. ilex* se observaron dos mecanismos desarrollados comúnmente para afrontar la sequía, una mayor eficiencia en el sistema hidráulico (Urli et al. 2013), y una mayor capacidad de osmorregulación en las hojas (Meinzer et al. 2014), lo cual le permitió mantener una mayor turgencia en las hojas bajo condiciones de sequía.

Sin embargo, a escala de árbol, *P. nigra* mostró menores descensos en el crecimiento secundario que las especies tolerantes. Esto puede deberse a una mayor eficiencia en el uso del agua, y a un mayor incremento en ésta comparado con las otras especies (Granda et al. 2014). De hecho, son muchos los estudios que han observado incrementos en la eficiencia en el uso del agua ante sequías en las especies mediterráneas (Granda et al. 2014, Vicente-Serrano et al. 2015). Además, la especie evitadora presentó una mayor plasticidad en la eficiencia en el uso del agua lo cual le permitió adaptarse mejor a los cambios en la intensidad de la sequía e incrementar en mayor medida su eficiencia bajo condiciones de estrés hídrico extremo.

Como ya se ha comentado, hay una clara relación entre el crecimiento y la transpiración, la cual se intensifica bajo condiciones de sequía. Sin embargo, en estos estudios se ha observado un cierto desacople entre ambos procesos, ya que se vieron afectados en diferente grado como consecuencia del momento en el que se produjo la sequía y por su intensidad. Además, este desacople dependió de la estrategia de la especie y fue menor en el caso de la especie evitadora. Esto podría ser explicado por su marcado incremento en la eficiencia en el uso del agua, aunque deberían estudiarse otros factores para poder comprender mejor los motivos que lo han generado.

A pesar de la estrategia evitadora de la sequía que mostró *P. nigra*, la especie no pudo evitar por completo los efectos negativos derivados de las sequías extremas, llegando a mostrar potenciales hídricos cercanos a su umbral crítico, a partir del cual se produce la cavitación de los tejidos conductores. En realidad, a pesar de la intensidad de la sequía, ninguna de las especies mostró signos claros de defoliación severa o de mortalidad. Estudios llevados a cabo bajo condiciones de sequía extrema en especies mediterráneas han observado casos de decaimiento y posterior muerte en las especies (Carnicer et al. 2011, Lloret et al. 2012). Esto sugiere que las especies de estudio no sufrieron fallos hidráulicos severos e irreversibles ni procesos de inanición por falta de carbono bajo condiciones de sequía extrema (McDowell 2011). Esto corrobora la elevada resiliencia de los ecosistemas mediterráneos (del Cacho y Lloret 2012) y señala que la aridificación climática aún no ha alcanzado niveles irreversibles para la

funcionalidad de las especies y localidades estudiadas. Sin embargo, no todas las especies presentaron la misma resiliencia a sequías extremas en temas de crecimiento, siendo mayor en *P. nigra* y menor en *Q. faginea*, tal y como ya observó Granda et al. (2013).

Por lo tanto, dado que tanto las especies evitadoras como las tolerantes mostraron mecanismos y adaptaciones para afrontar la sequía y en especial la sequía extrema, y que ninguna de ellas mostró claros síntomas de decaimiento ni de mortalidad, es difícil establecer cuál estrategia sería más eficiente ante la mayor frecuencia, intensidad y duración de las sequías. Cabría esperar que las especies más tolerantes se viesen más afectadas por sequías más intensas, pero puntuales, ya que esto las llevaría a sobrepasar su umbral crítico, generándoles fallos en el sistema hidráulico y cavitación del xilema. Además, se esperaría que las especies evitadoras se viesen más afectadas por sequías más prolongadas o crónicas debido a la falta de fijación de carbono, mostrando síntomas de inanición. Sin embargo, ambas especies pueden morir por inanición o por fallo hidráulico, y de hecho ambos procesos están conectados.

Por una parte, es complicado discernir si las especies van a poder desarrollar mecanismos evitadores eficientes que les permitan no solo evitar el estrés hídrico, sino que también desarrollarse bajo condiciones de agua limitante. En este sentido se ha observado que algunas especies evitadoras sufren mayores cavitaciones y rellenados del xilema a escala diaria, lo que les permite aumentar su capacitancia y usar el agua para transpirar (Sperry et al. 2008; Meinzer et al. 2009), lo cual sería

considerado un mecanismo de tolerancia. Por otra parte, es de esperar que las especies tolerantes presenten mecanismos de recuperación del embolismo muy eficientes (Franks et al. 2007). De todas formas, hay que considerar que las especies no son exclusivamente tolerantes o evitadoras, sino que se mueven en un rango de tolerancia (Franks et al. 2007). Por este motivo, todas las especies deben desarrollar ambas estrategias en mayor o menor medida (Valladares et al. 2004), lo cual en un futuro puede permitir una mejor adaptación a las sequías extremas y a la alta variabilidad climática asociada al cambio climático, combinando tanto mecanismos de tolerancia como de evitación de la sequía extrema.

En esta tesis doctoral también se ha observado que los efectos de la sequía en las especies vegetales no solo dependen de su vulnerabilidad a la sequía y de los mecanismos y adaptaciones desarrollados para tolerar la escasez hídrica. Además, estos dependen de las condiciones en las que se produce la propia sequía, como su intensidad, y el momento en el que se produce, considerando tanto la duración como la escala temporal en la que tiene lugar.

En los estudios llevados a cabo, se observaron sequías de diferente intensidad, llegando incluso a registrar las sequías más extremas de los últimos 50 años. Tal y como se esperaba fueron las sequías más extremas las que generaron mayores efectos en las especies de estudio. Estas sequías extremas se observaron tanto a

escala anual como a escala de estación, lo cual también condicionó los efectos sobre las especies. Hay que tener en cuenta que un año puede presentar una sequía extrema en una de sus estaciones y ser también extremo a escala anual o bien ser un año moderadamente seco o incluso un año húmedo en comparación con la serie histórica del lugar. Esto remarca la importancia de estudiar las sequías a diferentes escalas y también la idea de que frecuentes sequías a varias escalas podrían incrementar su efecto por acumulación y por la falta de recuperación de las especies entre sequías. Además, la duración de la sequía no solo alargó su efecto, sino que también retrasó y disminuyó la recuperación tras el incremento de la disponibilidad. Probablemente fue el efecto combinando entre la intensidad y la duración lo que generó efectos más intensos sobre las especies.

El momento en el que se produjo la sequía tuvo un efecto importante en las especies, especialmente en la especie caducifolia, *Q. faginea*. La escasez de agua observada en primavera adelantó los efectos de la sequía, generando un estrés hídrico propio de condiciones de verano. Ante esta situación tanto *P. nigra* como *Q. ilex*, especies perennes, pudieron adaptarse y activar su metabolismo, adelantando así su fenología, crecimiento y consumo de agua. Sin embargo, *Q. faginea* presentó una menor flexibilidad a la hora de ajustar el inicio del crecimiento, ya que esta especie requiere de un cierto número de horas para romper su latencia y la formación de las hojas depende de la duración del fotoperiodo (Sanz-Pérez et al. 2009). Por lo tanto, el hecho de que la sequía se produjese en un momento del desarrollo de la

especie en el que es más sensible a la escasez hídrica hizo que no pudiese adaptarse a esta.

En concordancia con estos resultados, algunos estudios han encontrado que el momento en el que se produce el estrés condiciona el fallo en la reproducción (Erickson y Markhart 2002). Otros estudios también han observado que las especies son especialmente sensibles a la sequía cuando se encuentran en un determinado momento de su desarrollo, como por ejemplo, el desarrollo reproductivo (Andersen et al. 2002, Aragón et al. 2008).

Por lo tanto, el momento en el que se produce la sequía puede tener mayores efectos que la propia intensidad de la sequía, no solo a nivel de estrés, sino también en otros aspectos de la planta como la fenología o la reproducción.

Otro de los objetivos de la presente tesis fue analizar la modulación de la respuesta a las sequías extremas por parte de las condiciones locales. En nuestro estudio se observó que pequeñas diferencias en la humedad del suelo atenuaron los efectos de la sequía extrema en las especies, independientemente de su estrategia hídrica. Por lo tanto, el microclima atenuó los efectos de la sequía extrema observada a escala regional. Estos resultados concuerdan con las observaciones llevadas a cabo en bosques y matorrales de la Península Ibérica tras la extrema sequía de 1994, en la que un gran número de especies presentó daños severos (Peñuelas et al. 2001). Así pues, la

orientación de la ladera (menor daño en umbrías) (Peñuelas et al. 2001), la litología del suelo (menor daño en suelos profundos y penetrables por las raíces) (Lloret y Siscart 1995), la especie dominante (Peñuelas et al. 1998), la gestión forestal (menor daño en bosques aclarados o menos densos) (Gracia et al. 1999) y la existencia de incendios previos (menor daño en especies rebrotadoras quemadas que en las no quemadas) (Moreno et al. 2004) atenuó los efectos de la sequía extrema.

Esto evidencia la posibilidad de que factores locales puedan atenuar sequías extremas, disminuyendo así los efectos sobre las especies. Es por ello, que es crucial considerar los factores locales a la hora de diseñar experimentos donde se estudie el efecto de la sequía en varias zonas. Asimismo, es necesario tenerlos en cuenta cuando quieran ser evaluados los efectos futuros derivados de un incremento en la frecuencia e intensidad de las sequías.

Finalmente, en esta tesis doctoral se evaluó el papel de la fragmentación de la masa forestal sobre la respuesta ecofisiológica de la encina. Los resultados mostraron que la fragmentación no solo atenuó los efectos de la sequía moderada en los bosques de encinas, sino que también lo hizo bajo condiciones de sequía extrema. De hecho, bajo condiciones climáticas más secas, es decir, años más secos en la región más seca dicha atenuación fue mayor. Estos resultados están en concordancia con estudios llevados a cabo en masas forestales de encina, en las que se observó que masas con densidades de árboles

mayores presentaban mayor estrés hídrico y menor intercambio gaseoso (Moreno and Cubera 2008).

El menor estrés hídrico observado en los árboles de fragmentos pequeños pudo ser debido a una disminución de la competencia intraespecífica (Morán-López et al. 2016a) y a un aumento en la disponibilidad de agua en los suelos (Flores-Rentería et al. 2015). Esto concuerda con el hecho de que los fragmentos forestales continuos presentasen menores potenciales hídricos, los cuales, bajo condiciones más secas, corresponderían con pérdidas de la conductancia hidráulica de entre un 68 % y un 75 % según estudios previos en la encina (Carevic et al. 2014; Martínez-Vilalta et al. 2002). Además, se estima que bajo pérdidas de la conductancia hidráulica superiores a un 68 %, la encina no es capaz de recuperar la conductancia hidráulica (Carevic et al. 2014). Por todo ello, es de esperar que las encinas de los fragmentos continuos tuvieran pérdidas irreversibles, al menos a corto plazo, de la conductancia hidráulica.

Estos resultados positivos de la fragmentación y de su interacción con el clima contrastan con los numerosos estudios en los que se han observado efectos negativos, especialmente sobre la biodiversidad (e.g. Santos y Tellería 1997; Fahrig 2003; Aparicio et al. 2012; Morán-López et al. 2015a). Esto no resulta contradictorio si se tiene en cuenta que la fragmentación puede tener efectos positivos o negativos dependiendo del objeto o proceso estudiado y su escala. Además, los efectos de la fragmentación pueden cambiar a lo largo de las diferentes etapas vitales de las especies. Es por ello, que es necesario

un estudio integrado que aborde diferentes aspectos del bosque, como el suelo, el propio árbol y las interacciones con las plantas o el ambiente, siempre considerando el mayor número de etapas del ciclo vital de las especies.

En este sentido, los efectos positivos de la fragmentación sobre la ecofisiología de la encina, la diversidad microbiana y las propiedades funcionales de los suelos (Flores-Rentería et al. 2015) y la producción de bellotas (Morán-López et al. 2016a) podrían verse contrarrestados por los efectos negativos observados en la dispersión de bellotas (Morán-López et al. 2015b). Esto se debe a que la dispersión de bellotas tiene importantes repercusiones sobre la regeneración, lo cual podría comprometer la diversidad genética de las plántulas, a pesar de la existencia de un flujo de polen extenso a través del paisaje (Morán-López et al. 2015b). En este contexto, dado que el cambio climático afecta tanto a la fenología de la encina como de sus dispersantes, podrían generarse desacoples entre ambos procesos y grupos de organismos, afectando negativamente a dicho mutualismo (Valladares et al. 2014; Morán-López et al. 2016b).

Dada la complejidad del funcionamiento de los ecosistemas fragmentados, y de la interacción entre estos y el cambio climático, especialmente las sequías, es difícil predecir si en su conjunto los efectos sobre dichos ecosistemas van a ser positivos o negativos. Además, el signo de dichos efectos puede variar bajo condiciones de sequías extremas, por lo que son necesarios estudios a largo plazo que tengan en cuenta todos estos factores.

Los resultados observados en esta tesis doctoral muestran claras diferencias entre las especies tanto por las diferentes adaptaciones y mecanismos que han desarrollado con el fin de evitar y/o tolerar la sequía, como por su vulnerabilidad a ésta, ya sea moderada o extrema, y su diferente respuesta a los factores condicionantes de la sequía. Además, las especies han mostrado diferente capacidad de recuperación, así como de eficiencia a la hora de compensar los efectos derivados de la escasez hídrica. A pesar de la gran capacidad de resiliencia observada en las especies mediterráneas (del Cacho y Lloret 2012; LLoret 2012), nuestros resultados sugieren una cierta pérdida de resiliencia, o al menos un retraso en la completa recuperación bajo condiciones de sequía extrema. A largo plazo esta respuesta diferencial de las especies a las sequías extremas puede conllevar cambios en la composición de las comunidades (ej. Peñuelas y Boada 2003), lo que a su vez podría generar cambios en la capacidad de almacenaje de carbono de los bosques y en su balance neto (Lindner et al. 2010). Uno de los motivos implicados en estos cambios puede ser el incremento de los procesos de defoliación en determinadas especies forestales, y su posterior muerte (Carnicer et al. 2011), cuyos casos ya se han observado en especies mediterráneas (Peñuelas et al. 2001).

Ante el incremento en la frecuencia e intensidad de las sequías extremas, es necesario llevar a cabo estudios a largo plazo que permitan conocer el estado de las especies antes, durante y después de una sequía extrema. De esta forma, se tendrá una visión completa del efecto

de la sequía en la especie y de su capacidad de recuperación. Estos estudios también permitirán identificar la acumulación de efectos y su interacción con otros factores del cambio global, como es la fragmentación.

Asimismo, se requieren estudios donde se aborden los efectos de las sequías en las especies desde diferentes enfoques, intentando tener una visión global de todos los aspectos relacionados y de todos los factores implicados. Esto permitirá conocer mejor la respuesta de las especies a la escasez hídrica y cómo éstas van a responder ante su intensificación.

Por último, y con el objetivo de establecer patrones en los efectos de las sequías extremas en los bosques, es necesario llevar a cabo estudios en los que se consideren el mayor número de especies coexistentes posible, abarcando un amplio rango de vulnerabilidades a la sequía.

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APÉNDICES

En este apartado se incluye un resumen de cada uno de los artículos publicados derivados de la participación en otros proyectos de investigación durante el desarrollo de esta tesis doctoral. Los resultados obtenidos complementan los estudios abordados en los anteriores capítulos e incrementan la comprensión de cuestiones como la respuesta diferencial de las especies a la sequía dependiendo de varios factores como su estrategia hídrica, la fragmentación del territorio o los mecanismos adoptados por las especies para sobrellevar las sequías. Por ello, en cada uno de los apéndices se indica la relevancia del estudio en relación a la presente tesis doctoral.

APÉNDICE 1: Species-specific water use by forest tree species: From the tree to the stand

Ismael Aranda, **Alicia Forner**, Bárbara Cuesta, Fernando Valladares

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Los bosques tienen un papel crítico en el ciclo hidrológico, siendo el estudio del uso del agua por parte de los árboles y la masa forestal de suprema importancia en el contexto del cambio global. En las últimas décadas se han descrito efectos muy negativos en la vegetación forestal derivados de sequías cada vez más intensas y frecuentes. Los síntomas de enfermedad y decaimiento de numerosos bosques, especialmente en regiones europeas templadas y mediterráneas, han sido asociados con cambios en los patrones de precipitación. Diferencias intra- e inter-específicas en la morfología y fisiología de las plantas ejercen una gran influencia en el balance hídrico de los ecosistemas forestales, además de afectar a su vulnerabilidad frente a la sequía. La composición y la estructura de la masa forestal influyen en la interceptación de la lluvia, la escorrentía y los flujos de agua de todo el ecosistema. Por lo tanto, los bosques naturales y semi-naturales, así como las plantaciones en expansión y de interés para la industria del biocombustible deben gestionar de forma sostenible el consumo de agua en relación con las expectativas generadas en cuanto a la producción de biomasa. En este artículo se revisa el papel de los motores clave en el uso del agua del bosque, y en particular, el papel de elementos como la composición específica, la respuesta del dosel arbóreo según dicha composición y la sensibilidad de cada especie a la escasez hídrica del suelo. En concreto,

se discute el papel de estos factores en el comportamiento de bosques naturales, pero con referencias también a plantaciones forestales. En un futuro cercano se espera que la escasez hídrica sea uno de los principales problemas de la sociedad, por lo que el uso del agua por parte de los ecosistemas forestales, tanto naturales como plantados, se ha convertido en un tema central en las actuales agendas de investigación.

La participación en la elaboración de esta revisión aportó una visión resumida, completa y conceptual sobre el uso del agua por parte de las especies, algunos de los mecanismos fisiológicos implicados, los factores que los condicionan y sus implicaciones a diferentes escalas. El conocimiento de cómo las especies regulan las pérdidas de agua durante periodos secos es esencial para comprender cómo las sequías extremas afectan a diferentes especies, tanto a corto como a largo plazo. Además, el estudio de la cantidad de agua consumida por diferentes especies, tomando como referencia todo el árbol, se ejemplificó con dos especies típicamente mediterráneas: *Q. ilex* y *P. nigra*. En este ejemplo se observaron claramente las diferentes estrategias hídricas seguidas por estas especies, las cuales han sido objeto de estudio en la presente tesis doctoral (Fig. A1). Durante la sequía, *Q. ilex* presentó un patrón anisohídrico con una ligera disminución del consumo del agua a lo largo del día. Sin embargo, *P. nigra* cerró los estomas muy temprano, mostrando un patrón de

respuesta isohídrico y, por lo tanto, una mayor vulnerabilidad a la sequía en comparación con *Q. ilex*.

Además, otros elementos implicados en el uso del agua por las especies forestales fueron revisados. El conocimiento sobre el uso del agua en relación con la edad, el tamaño o la estructura de la masa forestal, son elementos a tener también en cuenta a la hora de establecer diseños experimentales que eviten una mala interpretación de la respuesta intrínseca de las diferentes especies arbóreas a la sequía. Asimismo, el conocimiento de la relación entre el uso del agua y otros procesos funcionales también específicos de cada especie, como la capacitancia o la transpiración nocturna evaluada a partir del flujo de savia nocturno, permiten estimar con mayor precisión la relación entre el consumo de agua y el crecimiento utilizando sensores de flujo de savia y dendrómetros digitales, respectivamente.

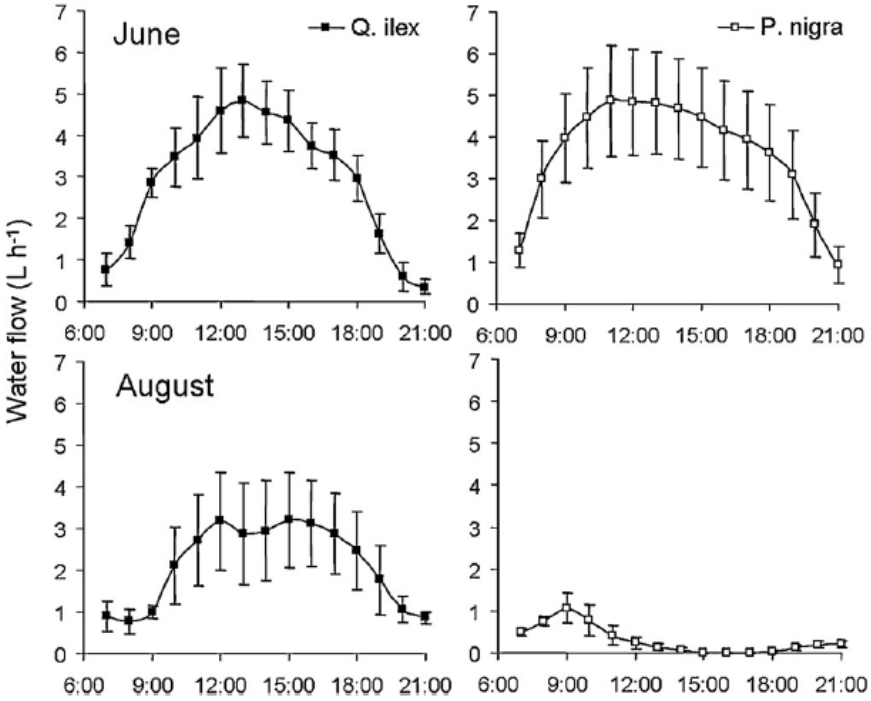

Figura A1. Flujo de savia diario en *Quercus ilex* y *Pinus nigra* durante el final de primavera (gráficas superiores) y mediados de verano (gráficas inferiores). Los valores son medias ± error estándar para cuatro árboles por especie.

APÉNDICE 2: Contrasting water strategies of two Mediterranean shrubs of limited distribution: uncertain future under a drier climate

Ana Lázaro-Nogal, **Alicia Forner**, Anna Traveset, Fernando Valladares

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Las plantas han desarrollado diferentes estrategias para afrontar las sequías, incluyendo características ecofisiológicas alternativas y diferentes niveles de plasticidad. Estas estrategias son críticas para las especies de distribución limitada, las cuales son especialmente vulnerables a las actuales altas tasas de cambios ambientales. El objetivo de este estudio fue evaluar la estrategia hídrica de dos especies con distribución limitada, *Cneorum tricoccon* L. y *Rhamnus ludovici-salvatoris* Chodat y evaluar su variabilidad interpoblacional a lo largo de un gradiente de aridez para estimar su vulnerabilidad a un clima más seco. Se estudiaron diferentes rasgos ecofisiológicos afectados por la sequía: conductancia estomática al vapor de agua, máxima eficiencia fotoquímica del fotosistema II, fraccionamiento isotópico del C^{13} como estimador de la eficiencia en el uso del agua y concentración de clorofila. El estudio se llevó a cabo en dos años climáticamente contrastados, antes y durante la sequía estival. Ambas especies fueron vulnerables a la sequía en el límite de máxima aridez del gradiente, pero mostraron estrategias hídricas contrastadas. *C. tricoccon* siguió una estrategia hídrica consevadora del agua a lo largo del gradiente de aridez, mientras que *R. ludovici-salvatoris* no, mostrando una conductancia estomática mayor, aunque siendo capaz de incrementar la eficiencia en el uso del agua en los sitios más xéricos. Es esperable que

los cambios en la duración e intensidad de los eventos de sequía favorezcan las estrategias de algunas especies en detrimento de las otras. De este modo, *C. tricoccon* es más vulnerable a las sequías crónicas y prolongadas, mientras que sequías cortas pero agudas puede que tengan fuertes efectos en *R. ludovici-salvatoris*. En las comunidades donde estas especies coexisten, estas diferentes estrategias podrían llevar a cambios en la estructura de las comunidades bajo escenarios de cambio climático, con un efecto cascada desconocido en el funcionamiento de los ecosistemas.

En este trabajo se abordaron varios aspectos relacionados con esta tesis, como es el estudio de la vulnerabilidad a la sequía estival de dos especies con diferentes estrategias hídricas. Al igual que se ha observado en la presente tesis doctoral con las tres especies arbóreas estudiadas, la especie más tolerante a la sequía, *R. ludovici-salvatoris*, mantuvo los estomas abiertos durante la sequía, permitiendo la fijación de carbono, pero aumentando el riesgo de que sus tejidos conductores cavitasen (Fig. A2b). Sin embargo, *C. tricoccon*, especie evitadora de la sequía, tuvo conductancias estomáticas muy bajas durante el año más seco, 2006 (Fig. A2a). Además, se estudiaron dos años climáticamente contrastados y varias poblaciones que conformaban un gradiente de aridez, por lo que los efectos de la escasez hídrica en las especies se abordaron usando varias escalas de estudio. Las diferencias en los valores de los rasgos ecofisiológicos observados entre los lugares de estudio remarcaron la importancia de la influencia de las condiciones

microclimáticas del lugar. Los resultados mostraron que la eficiencia en el uso del agua aumentaba con el incremento de aridez, patrón general que también se ha observado en los capítulos anteriores de esta tesis. En este caso el aumento fue mayor en *R. ludovici-salvatoris* que en *C. tricoccon* (Fig. A2c,d). El estudio de otros rasgos ecofisiológicos como la conductancia estomática o la máxima eficiencia fotoquímica del fotosistema II, permitió ahondar en el conocimiento de la respuesta ecofisiológica de las dos especies durante la sequía, y cómo ésta depende en gran medida de la estrategia hídrica de la especie y de las condiciones climáticas de la población de estudio. Por último, este estudio mostró la importancia que puede tener la sequía en especies de distribución limitada, un tema no abordado en esta tesis, pero de gran relevancia en el estudio del potencial adaptativo de los endemismos en un marco climático distinto al actual.

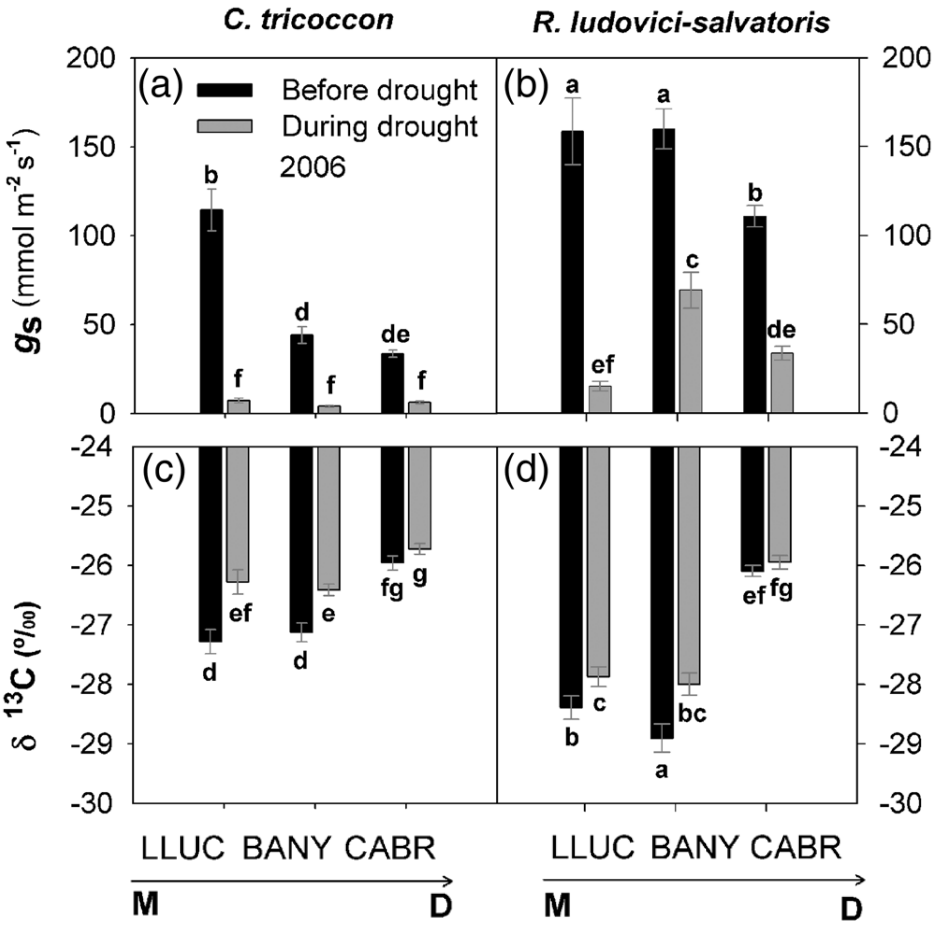

Figura A2. Valores medios de conductancia estomática (g_s) y en el ratio del isótopo de carbono ($\delta^{13}C$) en dos años climáticamente contrastados, en poblaciones de *C. tricoccon* y *R. ludovici-salvatoris*. Figura simplificada.

APÉNDICE 3: Influencia de la fragmentación y el clima en procesos clave para la regeneración del encinar

Fernando Valladares, Dulce Y. Flores-Rentería, **Alicia Forner**, Teresa Morán-López, Mario Díaz

Publicado en Ecosistemas (2014), 23 (2): 37-47

El presente trabajo recoge los principales resultados de tres aspectos relacionados con los efectos del cambio global sobre los fragmentos de encinar rodeados de una matriz agrícola, y su capacidad de regeneración. Se resumen y discuten resultados sobre encinares continuos y fragmentados en dos localidades de clima contrastado. Los resultados sugieren que los procesos de fragmentación podrían atenuar algunos efectos negativos del cambio climático, en concreto la disminución de la productividad de la encina asociada al creciente estrés hídrico. Esta atenuación se debería tanto a la menor competencia por el agua entre los árboles próximos al borde, como a los cambios en las comunidades microbianas y en las propiedades funcionales de los suelos. Dado que el cambio climático opera directamente sobre otros procesos como la fenología de los árboles y de los dispersantes, la fragmentación, como efecto negativo, podría llevar a desacoples entre los ritmos vitales de la encina y sus principales dispersores terrestres –roedores-. El desacople fenológico entre encinas y roedores observado en las poblaciones más áridas tendería a aumentar la vulnerabilidad de los encinares frente al calentamiento global en lo referido al potencial de diseminación de las bellotas. De este modo, la fragmentación tuvo, en su conjunto, un efecto negativo sobre la calidad de dispersión de las

bellotas debido, entre otros, a la falta de zonas de refugio para los dispersantes.

En este trabajo se estudió la interacción de los efectos de la sequía con la fragmentación en las mismas poblaciones de encina que las analizadas en el capítulo correspondiente de esta tesis doctoral (capítulo 5). Este estudio ofreció una visión más amplia que la abordada en dicho capítulo, ya que no solo se estudiaron los efectos sobre el estado hídrico de las encinas, sino que también se abordó el análisis de la dispersión de las bellotas, o los cambios en la composición y riqueza de las comunidades microbianas y en las propiedades funcionales de los suelos. Por lo tanto, es un claro complemento a lo estudiado en esta tesis. Los resultados mostraron efectos positivos de la fragmentación en el estado hídrico de las encinas, en el tamaño de la cosecha y de las bellotas, y en la capacidad de retención del agua del suelo derivado de un incremento en la producción de la materia orgánica, entre otros efectos (Fig. A3). También se observaron efectos negativos en la germinación y la efectividad de la dispersión de las bellotas, entre otros. Este estudio pone de manifiesto la importancia de las condiciones climáticas de la zona de estudio, ya que la magnitud del efecto cambió según fuese la población mésica o xérica, es decir, según las condiciones termo-pluviométricas de la zona (Fig. 3). Por ejemplo, la magnitud del efecto de la sequía en el estado hídrico de las encinas fue mayor (en valor absoluto) en la población xérica. La fragmentación también tuvo

un mayor efecto en la materia orgánica y la disponibilidad de nutrientes del suelo en la población xérica.

Finalmente, este estudio permitió comprender que la evolución futura de los bosques de encinas no solo depende de los efectos individuales de la fragmentación sobre el estado funcional del arbolado, el clima y su interacción. Además, hay todo un conjunto de procesos que se ven también modificados y que pueden tener un impacto directo también sobre la dinámica de los encinares en el futuro.

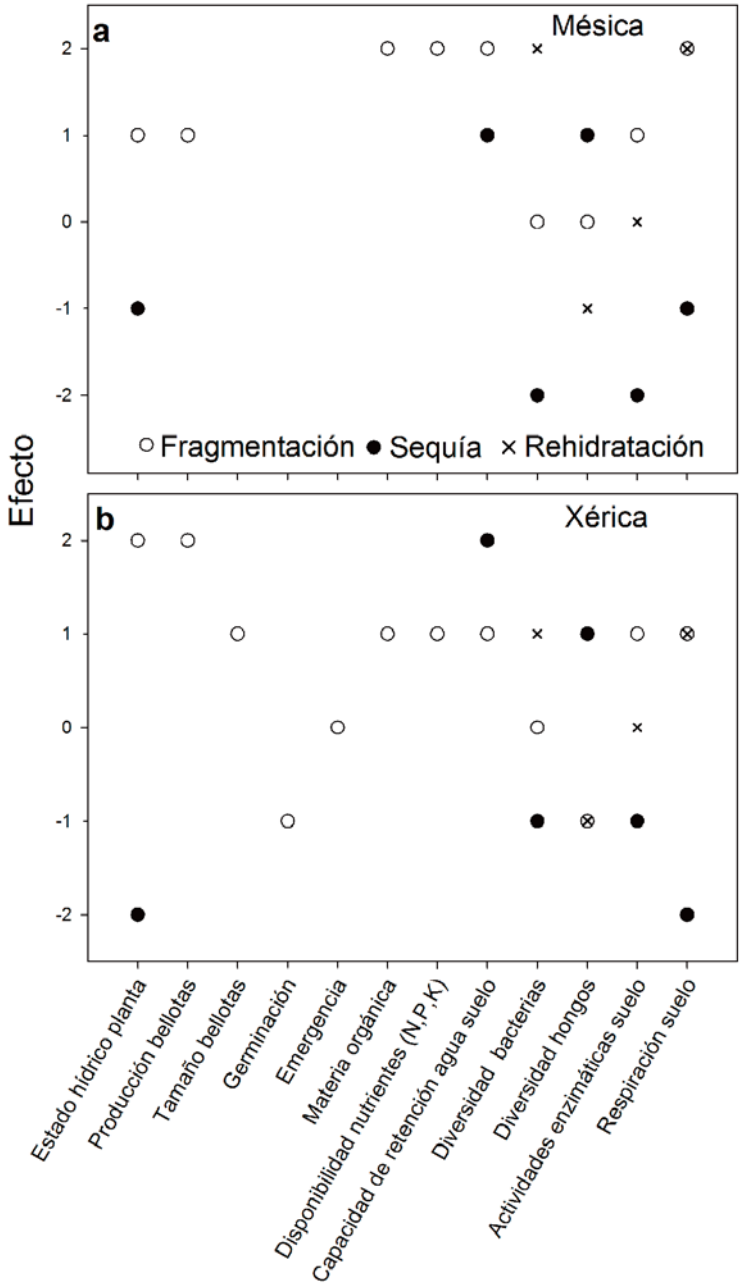

Figure A3. Efectos significativos (positivo, negativo y neutro) de la fragmentación y la sequía sobre varias variables edáficas y ecofisiológicas estudiadas en los encinares de ambas localidades, mésica (a) y xérica (b).

APÉNDICE 4: Influence of species interactions on transpiration of Mediterranean tree species during a summer drought

Charlotte Grossiord, **Alicia Forner**, Arthur Gessler, André Granier, Martina Pollastrini, Fernando Valladares, Damien Bonal

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Investigaciones recientes han demostrado que la interacción entre especies con diferentes rasgos funcionales puede promover los procesos forestales del ecosistema. En el contexto del cambio climático, entender si la interacción de especies en ecosistemas mixtos puede mejorar la adaptación de estos ecosistemas a los eventos de sequía es esencial para el desarrollo de nuevas estrategias de gestión. En este estudio, se investiga el impacto de la interacción de especies en el consumo de agua estimado a partir de la medida de la densidad de flujo de savia en tres especies arbóreas mediterráneas (*Quercus faginea*, *Pinus nigra* y *Pinus sylvestris*). Además, se presta un especial interés a los patrones de consumo seguidos durante la sequía estival. También se llevaron a cabo medidas de la composición de isótopos de carbono en las hojas ($\delta^{13}C$) en los mismos árboles. El descenso de la transpiración durante la sequía fue mayor para *P. sylvestris* y notablemente menor para *Q. faginea*. En el caso de *P. nigra* y *Q. faginea*, el descenso de la transpiración debido al avance de la sequía fue menor cuando la coexistencia era con especies arbóreas diferentes, especialmente si ésta era con *P. sylvestris*. Por el contrario, este descenso en *P. sylvestris* fue mayor cuando dicha especie cohabitaba con otras especies. Las

diferencias en los efectos de la sequía entre especies fueron congruentes con los valores de $\delta^{13}C$. También se mostró que durante una sequía estival, la identidad de la especie presente en los alrededores más próximos de un determinado árbol puede condicionar de forma diferente la disponibilidad hídrica y el uso del agua de las tres especies. Los resultados sugieren que las interacciones entre especies tienen un papel importante en la modulación de la respuesta propia de cada especie arbórea a la sequía. Por lo tanto, favorecer la diversidad de las especies arbóreas en esta región no parece ser sistemáticamente beneficioso en términos de disponibilidad hídrica y uso del agua para todas las especies que están interaccionando. Algunas especies sí se ven beneficiadas del carácter mixto de la masa (*P. nigra* y *Q. faginea*), pero otras no (*P. sylvestris*).

El método de medida del flujo de savia fue distinto al usado en algunos de los capítulos de la presente tesis (método de disipación térmica vs. de balance de calor en el tallo, respectivamente). Por lo que, en cierta medida, permitió la comparación cualitativa entre ambos métodos. En relación con el consumo de agua, los resultados mostraron un descenso en la transpiración tanto de *P. nigra* como de *Q. faginea* debido a la sequía (también observado en esta tesis), aunque esta disminución fue atenuada en zonas con una composición de especies mixta (Fig. A4). Sin embargo, la competencia intraespecífica exacerbó el efecto de la sequía en la transpiración en el caso de *P. sylvestris*, y puso

de relieve el importante papel de las interacciones entre especies en la modulación de la respuesta de las especies arbóreas a la sequía.

Además, se destacó la importancia de la fenología de las especies en el consumo de agua, ya que en periodos en los que *Q. faginea* todavía no tenía completamente desarrolladas las hojas, el flujo de savia fue menor que en *P. nigra*. Por el contrario, tras el desarrollo completo de las hojas el flujo de savia de *Q. faginea* fue mayor que el de *P. nigra*. Estos resultados concuerdan con los observados en el capítulo 4 de la presente tesis doctoral.

Dependiendo de la composición del bosque, también se observaron diferentes características funcionales, y diferente contenido hídrico del suelo. De este modo, *Q. faginea* tuvo una mayor disponibilidad de agua cuando coexistió con ambos pinos, mientras que en el caso de *P. nigra* fue cuando coexistió con *P. sylvestris.* Sin embargo, la composición isotópica de carbono no se vio afectada significativamente por la interacción entre las especies.

Por lo tanto, este estudio permitió conocer las disminuciones en el flujo de savia que se produjeron durante la sequía dependiendo de la especie de estudio. Además, se identificaron varios factores que condicionaban la respuesta del flujo de savia a la sequía, como la fenología de la especie, la composición de las especies o el sistema radicular de las distintas especies que componen el bosque.

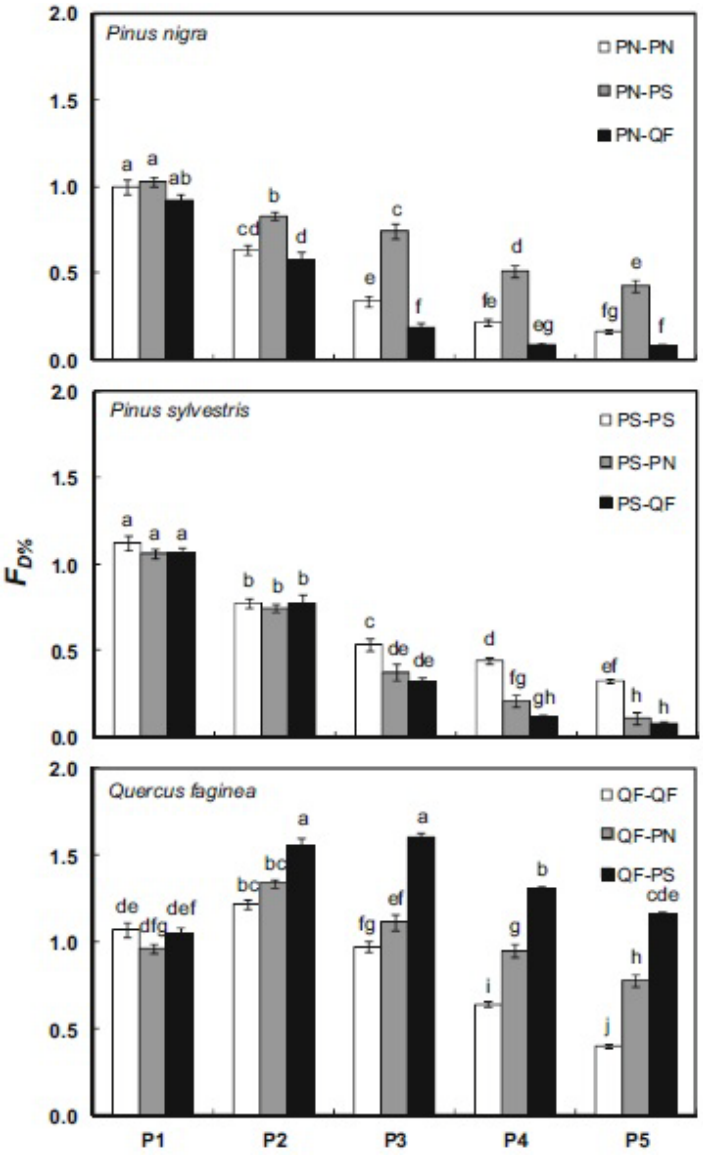

Figura A4. Media diaria de la densidad de flujo de savia relativa media ($F_{D\%}$), para cada periodo de medida (P1, P2, P3, P4 y P5) y cada nivel de interacción de especies para *Q. faginea* (QF), *P. nigra* (PN) y *P. sylvestris* (PS). Las barras verticales representan el error estándar de cada media para cada periodo de medida. Para una determinada especie, las *letras* indican diferencias significativas en $F_{D\%}$, entre periodos y niveles de composición de especies.

APÉNDICE 5: Some positive effects of the fragmentation of holm oak forests: Attenuation of water stress and enhancement of acorn production

Teresa Morán-López, **Alicia Forner**, Dulce Flores-Rentería, Mario Díaz, Fernando Valladares

Publicado en Forest Ecology and Management (2016), 370: 22-30

Los efectos de la fragmentación en la producción de bellotas están condicionados por su impacto en el estado fisiológico de las encinas durante el desarrollo de las semillas especialmente en sistemas hídricamente estresantes, como los bosques mediterráneos. Cabe esperar la formación de bordes en los bosques reduzca la competencia entre árboles, lo cual a su vez modera la escasez hídrica durante el verano, y como consecuencia, puede incrementar la producción de bellotas. Para testar estas dos hipótesis se hizo un seguimiento de la producción de bellotas y el estado hídrico del arbolado durante 2012-2014 en dos archipiélagos de bosque de encina (*Quercus ilex*) en la Península Ibérica.

La producción de bellotas y los efectos de la fragmentación no variaron entre localidades, a pesar de sus condiciones contrastadas climáticamente (el déficit hídrico acumulado desde Abril a Agosto fue un 60 % mayor en el sur). En general, los interiores de bosque mostraron una mayor proporción de árboles no productores (aprox. 50 %), mientras que los árboles de fragmentos pequeños mostraron mayores cosechas de bellotas (≥ 3 en una escala de tamaños entre 0 y 4, en aprox. 40 % de los árboles estudiados). Los resultados confirman la

expectativa que la competencia por el agua en fragmentos pequeños fue reducida, lo cual palió la escasez hídrica estival en los árboles de estudio. Esta reducción en el estrés hídrico conllevó un incremento en la producción de bellotas. En general, nuestros resultados muestran que los procesos locales como la fragmentación pueden contrarrestar diferencias climáticas entre localidades y podrían incluso anular los impactos derivados de un incremento en la aridez en las cosechas de bellotas.

En este estudio se abordó el efecto de la fragmentación y el clima en la producción de bellotas en los mismos bosques de encinas que los estudiados en el capítulo 5 de la tesis. Uno de los resultados más importantes de este trabajo, es que la fragmentación atenuó el estrés hídrico de las plantas a través de la disminución de la competencia intraespecífica, lo que a su vez generó un efecto positivo en términos de aumento en la producción de bellotas (Fig. A5). Este resultado estuvo en concordancia con los resultados obtenidos en el capítulo 5 en relación, no solo con el estado hídrico del arbolado, sino que también con el patrón de otras variables funcionales relacionadas con la capacidad de fijación de carbono del árbol. Además, permitió identificar el mecanismo por el cual el estrés hídrico era menor en encinas procedentes de fragmentos pequeños, la competencia intraespecífica. No se observó un efecto de las condiciones climáticas del lugar de estudio en la producción de bellotas ni en el efecto de la fragmentación. Por lo tanto, contrario a lo observado en los capítulos 4 y 5 de esta tesis, la aridez del

lugar de estudio no condicionó el efecto de la sequía en las especies de estudio en un carácter tan importante para la perpetuidad de la masa forestal como es la producción de semillas. En el capítulo 5 sí que se observó un efecto del lugar de estudio en rasgos funcionales como la eficiencia fotoquímica del fotosistema II medida al amanecer en los mismos encinares fragmentados. Por lo tanto, se observó que los efectos locales como la fragmentación podían contrarrestar las diferencias climáticas entre localidades e incluso llegar a anular su impacto en la producción de bellotas. En este sentido rasgos funcionales asociados al crecimiento vegetativo, podrían verse afectados por las condiciones microclimáticas de diferente manera a los rasgos reproductivos.

En conclusión, este estudio aportó una mayor comprensión de los factores que median en el estrés hídrico al que se someten las especies durante la sequía estival, y cuáles pueden ser sus consecuencias en otras variables estrechamente relacionadas con la perpetuidad de la masa forestal como es la producción de semillas.

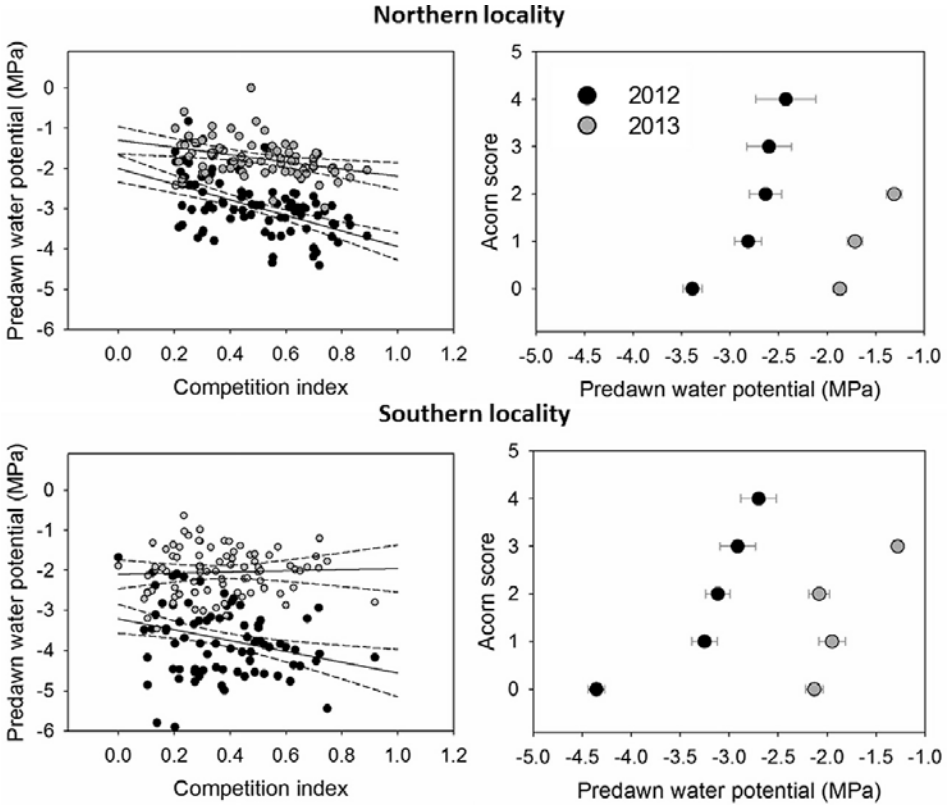

Figura A5. Izquierda: efectos de la competencia intraespecífica en los potenciales hídricos medidos al amanecer en agosto en las encinas. Las líneas continuas son las predicciones del modelo por año, y las líneas discontinuas son el intervalo de confianza del 95 %. Derecha: efectos del potencial hídrico medido al amanecer en la producción de bellotas. Las barras representan el intervalo de confianza del 95 %. Los paneles superiores e inferiores corresponden a la localidad norte y sur, respectivamente.

AFILIACIÓN DE LOS AUTORES

Alicia Forner

Laboratorio Internacional de Cambio Global (LINC-Global); Museo Nacional de Ciencias Naturales, MNCN-CSIC, Departamento de Biogeografía y Cambio Global; C/Serrano 115 bis, 28006, Madrid, España.

Ana Lázaro-Nogal

Laboratorio Internacional de Cambio Global (LINC-Global); Museo Nacional de Ciencias Naturales, MNCN-CSIC, Departamento de Biogeografía y Cambio Global; C/Serrano 115 bis, 28006, Madrid, España.

André Granier

Ecologie et Ecophysiologie Forestières, INRA; UMR 1137, 54280 Champenoux, France.

Anna Traveset

Institut Mediterrani d'Estudis Avançats, IMEDEA-CSIC; c/Miquel Marquès 21, 07190 Esporles, Mallorca, España.

Arthur Gessler

Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Zürcherstrasse 111, 8903, Birmensdorf, Switzerland.

Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), 14195, Berlin, Germany.

Bárbara Cuesta

Centro de Investigación Forestal, CIFOR-INIA, Departamento de Ecología y Genética Forestal; Carretera de la Coruña km 7,5, 28040, Madrid, España.

Charlotte Grossiord

Earth and Environmental Sciences Divisio, Los Álamos National Laboratory, Los Álamos, NM 87545, USA.

Damien Bonal

Ecologie et Ecophysiologie Forestières, INRA; UMR 1137, 54280 Champenoux, France

Dulce Y. Flores-Rentería

Universidad Nacional Autónoma de México, Facultad de Ciencias, Departamento de Ecología y Recursos Naturales; Avda. Universidad 3000, Circuito Exterior s/n, 04510 D.F. México.

Fernando Valladares

Laboratorio Internacional de Cambio Global (LINC-Global); Museo Nacional de Ciencias Naturales, MNCN-CSIC, Departamento de Biogeografía y Cambio Global; C/Serrano 115 bis, 28006, Madrid, España.

Universidad Rey Juan Carlos, Escuela Superior de Ciencias Experimentales y Tecnológicas, Departamento de Biología y Geología; c/ Tulipán s/n, E-28933 Móstoles, España.

Ismael Aranda

Centro de Investigación Forestal, CIFOR-INIA, Departamento de Ecología y Genética Forestal; Carretera de la Coruña km 7,5, 28040, Madrid, España.

Mario Díaz

Laboratorio Internacional de Cambio Global (LINC-Global); Museo Nacional de Ciencias Naturales, MNCN-CSIC, Departamento de Biogeografía y Cambio Global; C/Serrano 115 bis, 28006, Madrid, España.

Martina Pollastrini

University of Florence, Department of Agri-food and Environmental Sciences, Section of Soil and Plant Science; 50144, Florence, Italy.

Teresa Morán-López

National University of Comahue, INIBIOMA-CONICET, Ecotono laboratory; Quintral 1250, 8400 Bariloche, Río Negro, Argentina.

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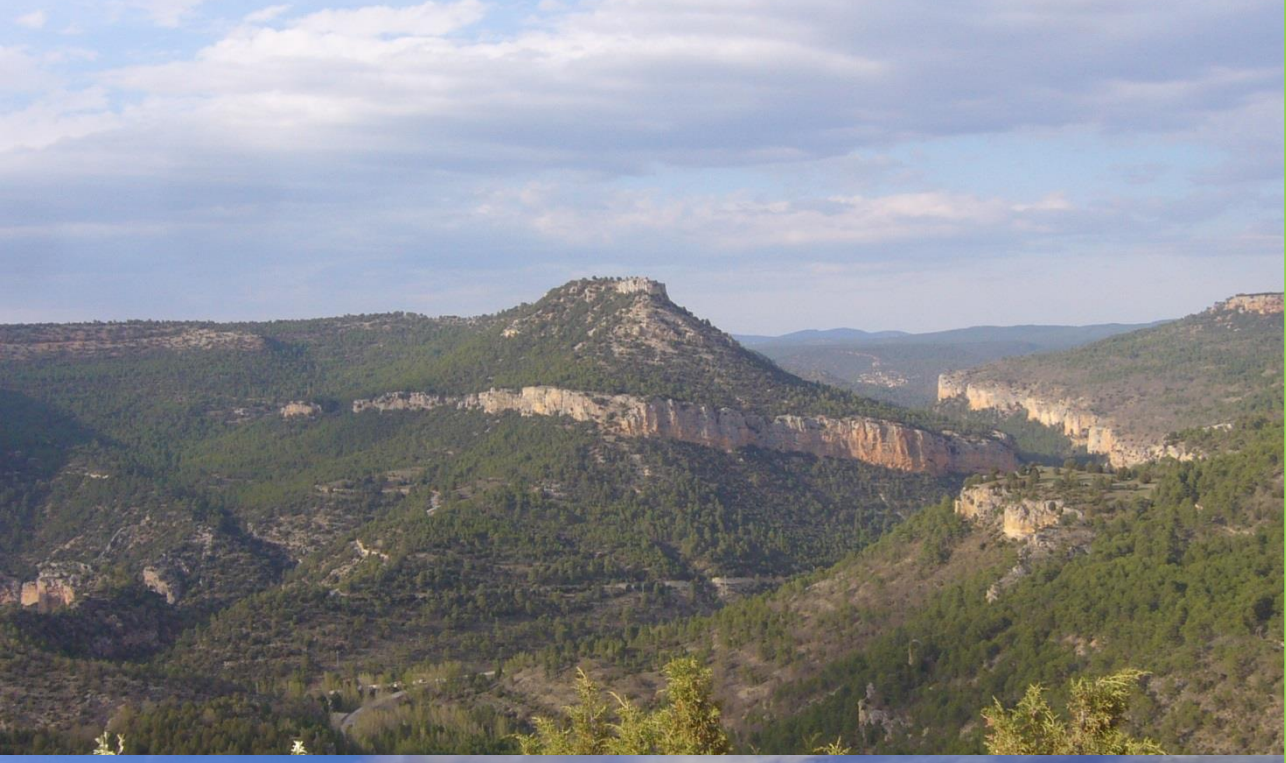
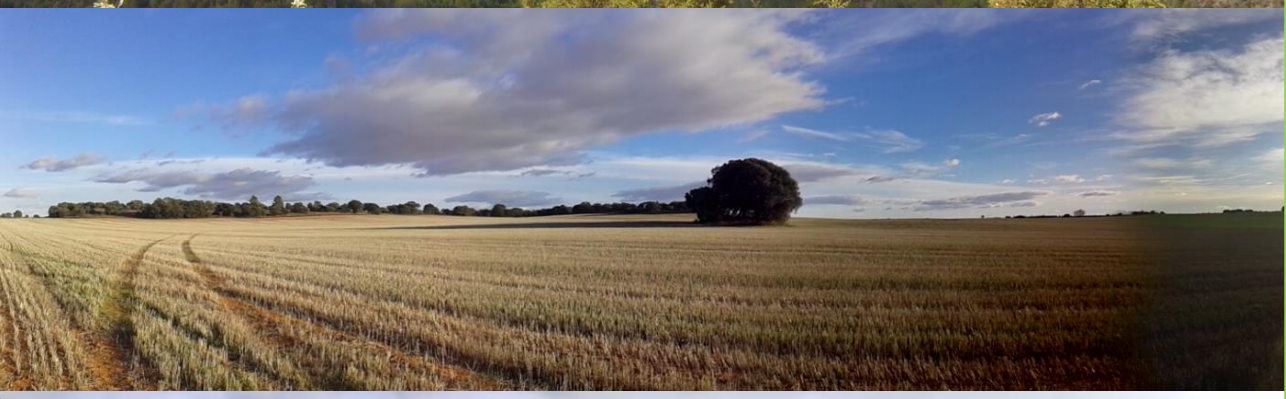
"LO ÚNICO IMPOSIBLE ES AQUELLO QUE NO INTENTAS"

Cuando creíamos que teníamos todas las respuestas, de pronto, cambiaron todas las preguntas.

MARIO BENEDETTI

Imágenes de la portada: ejemplar de *Q. ilex* antes y después de la sequía en fragmento borde, Quintanar de la Orden. Contraportada: Parque Natural del Alto Tajo, fragmento pequeño, borde e interior en Quintanar de la Orden. Author: Alicia Forner y Diego García.





Universidad Rey Juan Carlos

CSIC Consejo Superior de Investigaciones Científicas

mncn museonacionaldecienciasnaturales

INIA Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria

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