GOBIERNO
DE ESPAÑA

MINISTERIO
DE ECONOMÍA
Y COMPETITIVIDAD

INIA
Instituto Nacional de Investigación
y Tecnología Agraria y Alimentaria

Isabel Cañellas Rey de Viñas, Científica Titular del Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) y co-directora de esta Tesis Doctoral,

Hace constar:

Que el trabajo descrito en la presente memoria, titulado "Respuesta de *Pinus sylvestris* y *Quercus* spp. sub-meditarráneos al clima y la competencia a diferentes escalas: implicaciones ante escenarios de cambio climático/ *Pinus sylvestris* and sub-Mediterranean *Quercus* spp. response to climate and competition at different scales: implications under climate change scenarios", ha sido realizado por Laura Fernández de Uña bajo su dirección en el Centro de Investigación Forestal del Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CIFOR) y en la Unidad Docente de Ecología del Departamento de Ciencias de la Vida de la Universidad de Alcalá, dentro del Programa de Doctorado "Ecología, Conservación y Restauración de Ecosistemas" (D330), reuniendo todos los requisitos necesarios para su aprobación como Tesis Doctoral.

Madrid, 27 de abril de 2016

Dra. Isabel Cañellas Rey de Viñas

GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD
INIA
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria

Guillermo Gea Izquierdo, Investigador Ramón y Cajal en el Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) y co-director de esta Tesis Doctoral,

Hace constar:

Que el trabajo descrito en la presente memoria, titulado "Respuesta de *Pinus sylvestris* y *Quercus* spp. sub-meditarráneos al clima y la competencia a diferentes escalas: implicaciones ante escenarios de cambio climático/ *Pinus sylvestris* and sub-Mediterranean *Quercus* spp. response to climate and competition at different scales: implications under climate change scenarios", ha sido realizado por Laura Fernández de Uña bajo su dirección en el Centro de Investigación Forestal del Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CIFOR) y en la Unidad Docente de Ecología del Departamento de Ciencias de la Vida de la Universidad de Alcalá, dentro del Programa de Doctorado "Ecología, Conservación y Restauración de Ecosistemas" (D330), reuniendo todos los requisitos necesarios para su aprobación como Tesis Doctoral.

Madrid, 3 de mayo de 2016

Dr. Guillermo Gea Izquierdo

Universidad de Alcalá

DEPARTAMENTO DE
CIENCIAS DE LA VIDA
Edificio de Ciencias
Campus Universitario
28805 Alcalá de Henares (Madrid)
Telf. +34918854927
Fax: +34918854929
E-mail: dpto.ccvida@uah.es

MICHEL HEYKOOP FUNG-A-YOU, Profesor Titular y Director del Departamento de Ciencias de la Vida de la Universidad de Alcalá,

INFORMA:

Que el trabajo descrito en la presente memoria, titulado "Respuesta de *Pinus sylvestris* y *Quercus* spp. sub-meditarráneos al clima y la competencia a diferentes escalas: implicaciones ante escenarios de cambio climático", ha sido realizado por Dña. Laura Fernández de Uña dentro del Programa de Doctorado Ecología. Conservación y Restauración de Ecosistemas (D330), reúne todos los requisitos necesarios para su aprobación como Tesis doctoral, por acuerdo del Consejo de Departamento celebrado el día   de mayo de 2016

Alcalá de Henares,   de mayo de 2016

Universidad
de Alcalá

Departamento de Ciencias de la Vida
Unidad docente de ecología

Respuesta de *Pinus sylvestris* y *Quercus* spp. sub-meditarráneos al clima y la competencia a diferentes escalas: implicaciones ante escenarios de cambio climático

Pinus sylvestris and sub-Mediterranean *Quercus* spp. response to climate and competition at different scales: implications under climate change scenarios

Memoria presentada para optar al grado de Doctora por la
Universidad de Alcalá

Programa de Doctorado:

“Ecología, Conservación y Restauración de Ecosistemas" (D330)

Laura Fernández de Uña

Directora: Dr. Isabel Cañellas Rey de Viñas

Co-director: Dr. Guillermo Gea Izquierdo

Alcalá de Henares, Mayo de 2016

UNIVERSIDAD DE ALCALÁ, PATRIMONIO DE LA HUMANIDAD

A mi familia

Stand still. The trees ahead and the bushes beside you
are not lost. Wherever you are is called Here,
and you must treat it as a powerful stranger,
must ask it for permission to know it and be known.
The forest breathes. Listen. It answers,
I have made this place around you.
If you leave it, you may come back again, saying Here.
No two trees are the same to Raven.
No two branches are the same to Wren.
If what a tree or bush does is lost on you,
you are surely lost. Stand still. The forest knows
where you are. You must let it find you.

Lost

David Wagoner

Agradecimientos

De entre todas las personas que han contribuido en la elaboración de esta tesis doctoral quisiera agradecer especialmente a mis directores de tesis, Isabel y Guillermo, por la oportunidad que me dieron de emprender este proyecto y por su apoyo durante estos años. Me gustaría extender este agradecimiento al conjunto de investigadores del CIFOR que de una manera u otra me han prestado su ayuda, en particular a Fernando Montes, así como a Ismael Aranda por su asesoramiento en las mediciones de intercambio gaseoso y por facilitar la elaboración de los últimos capítulos con un enfoque ecofisiológico. Asimismo, quiero mostrar mi gratitud a Ángel, Estrella, Quique, Cristina y Ana por su inestimable labor en los trabajos de campo y de laboratorio, así como a Toñi y Lourdes del CISA por procesar unas muestras nada fáciles de cortar. Además, agradecerle a Borja su ayuda midiendo traqueidas (y otros asuntos) y a Chema por dejarme utilizar el microscopio para desarrollar los trabajos de xilogénesis.

I would also like to thank all the people who warmly hosted me during my different research stays along my PhD studies, without whose help this thesis would not be the way it is. Firstly, to Patrick and everyone at the WSL, particularly Loïc for kindly and patiently teaching me to process my micro-cores, as well as Diego, Stephan, Jodi, Paolo, etc. for making my stay there an incredible personal experience. Secondly, I would like to thank all the people at the Earth and Environmental Science division at LANL, specially Nate, for teaching me so much and helping me grow as a scientist, as well as for having so much faith in me. Also, to George and Zach, for being so helpful with the analysis of the samples, as well as Sanna, Henry, Turin, Adam… for being so kind during my stay. All of you, together with LANL training and security issues and the government shutdown, probably made it my most interesting research visit. Also, I would like to thank Sergio for being such a wonderful host (and person), for his weekly meetings during my stay at UQAC and for his always helpful advice even in the distance. Likewise, I would like to thank the rest of the people in the department, Hubert, Annie, Audrey, Lorena, Maxence, Françoise, Danielle, and a long etcetera for making it feel more like a family. Y un agradecimiento especial a Miguel, el andaluz más loco de Chicoutimi, por darle vida a un lugar tan frío. Also, I would like to thank Joël for giving me the chance to go to CEREGE to work with Guillermo in the final moments of my FPI scholarship. Finalmente, agradecer a Nacho, Gonzalo y Guillermo de la USC por enseñarme a procesar las muestras de xilogénesis de rebollo.

Algunos de los recuerdos más gratos y entrañables de esta etapa se los debo a mis compañeros becarios del CIFOR, con los que he tenido la suerte de compartir este viaje y gracias a los cuales me llevo no sólo conocimientos, sino también valiosas amistades, Entre ellos están mis compañeros de departamento Javi, con quien empecé y que me ha acompañado en cada paso (¡ya queda menos!), Víctor, que se fue pero no le olvidamos, Rubén, por ser la voz de la experiencia, Dani, por estar ahí, y la recién llegada Marta. También a los del departamento “enemigo”, por acogernos a Javi y a mí de recién llegados. En particular, a Paloma, por nuestras largas terapias de grupo. A Natalia, por su inestimable ayuda con el formateo y papeleo de la tesis ¡sin tu ayuda no lo habría conseguido! A Marina, por ser tan buena amiga y compañera de paseos en bici. A Quique, por tus conversaciones de fractales y demás temas extraños, las cenas de navidad no serían lo mismo sin ti. A Katha, por tu vitalidad y capacidad para aunarnos. A Gregor, por tus galletas y bizcochos, en particular por esa Sacher que triunfa allá donde la llevo. A Isa, Rose, Luis, Jesús, Ruth, Maje, Andrés, Mario… por vuestra amistad durante estos años. Sólo por vosotros ya ha merecido la pena pasar tantas horas en el INIA. Y aunque no sean becarias, no quiero olvidarme de Laura (Hernández), Isa (Dorado), e Iciar por su valioso apoyo moral, sobre todo en estos últimos meses.

También quiero hacer un reconocimiento especial a mis amigos de siempre, María, Dani, Liana, Alberto, Bea, etc. por vuestro cariño y por seguir ahí a pesar de haberme visto el pelo menos que cuando estaba en Canadá.

A mi familia, los que están y los que fueron, por inculcarme el valor del esfuerzo y apoyarme tantísimo estos años. En particular, a mis padres, por facilitarme el camino que me ha llevado hasta aquí, por apoyarme en cada paso del mismo y estar siempre ahí cuando lo he necesitado, aunque no tuviese el más mínimo sentido para vosotros. Y a mis hermanas, que siempre han sabido mantener la sonrisa incluso en mis peores momentos.

Y por último, a Gonzalo, sin cuyo apoyo esta tesis no hubiera sido posible. En lo científico, por ser la persona con la que más he discutido sobre el trabajo desarrollado en esta memoria. Pero, más importante aún, por tu apoyo, tu cariño, tus detalles, que me han dado fuerzas y han sido mi mayor apoyo durante esta etapa.

A todos, mil gracias.

Table of Contents

La presente Tesis Doctoral está escrita en formato bilingüe para cumplir los requisitos de la Mención de Doctorado Internacional

This PhD Thesis is written in a bilingual format to meet the requirements of the International Doctorate Mention

Resumen

Los escenarios de cambio climático predicen un ascenso de las temperaturas para la región mediterránea que no vendría acompañado de un aumento en las precipitaciones, así como un incremento en la frecuencia e intensidad de los episodios de sequía. Como resultado de estos cambios en las condiciones climáticas, se espera que *Pinus sylvestris* L. reduzca su rango de distribución en la región, siendo sustituido a bajas altitudes por taxones más tolerantes a la sequía como *Quercus* spp. sub-mediterráneos. El principal **objetivo** de esta tesis ha sido evaluar la respuesta fisiológica de *Pinus sylvestris*, *Quercus pyrenaica* Willd. y *Quercus faginea* Lam. a procesos de cambio global, particularmente el cambio climático, a diferentes escalas temporales, con el objetivo final de informar el desarrollo de estrategias de gestión forestal sostenible que minimicen los impactos del cambio climático en los ecosistemas forestales mediterráneos.

La tesis está estructurada en siete capítulos. El **Capítulo 1** introduce el efecto que las variables ambientales, principalmente el clima, la competencia y las concentraciones de CO_2 de la atmósfera, pueden tener en la fisiología del árbol, principalmente en el crecimiento, así como los enfoques metodológicos que pueden utilizarse para analizarlos. En el **Capítulo 2** modelizamos la relación entre el crecimiento del árbol y la interacción clima-competencia usando datos dendroecológicos y series de competencia a largo plazo de rodales de *Quercus faginea*, *Quercus pyrenaica* y *Pinus sylvestris* sometidos a diferentes regímenes de claras. Además, aplicamos estos modelos a varios escenarios de cambio climático para predecir las tendencias de crecimiento futuras de estas especies a diferentes niveles de competencia para evaluar su vulnerabilidad al cambio climático. El crecimiento descendió con el aumento de competencia, la cual explicó mayor variabilidad del crecimiento que el clima en *Q. faginea* y *P. sylvestris*.

Nuestras proyecciones de crecimiento indican que *P. sylvestris* se vería afectado más negativamente por el cambio climático que las especies de *Quercus* submediterráneos estudiadas, incluso bajo condiciones de competencia reducida.

El objetivo del **Capítulo 3** fue, primero, analizar el efecto de la competencia en la eficiencia intrínseca en el uso del agua (iWUE, por sus siglas en inglés) y el crecimiento de *Q. faginea*, *Q. pyrenaica* y *P. sylvestris*, así como comparar nuestros resultados con datos compilados en una revisión bibliográfica sobre la respuesta de iWUE derivada de anillos de crecimiento y del crecimiento a la competencia. En segundo lugar, evaluamos el efecto que la competencia, las concentraciones atmosféricas de CO_2, el clima y la edad de los árboles ejercen sobre las tendencias temporales encontradas en el crecimiento e iWUE de las tres especies estudiadas. La competencia no tuvo un efecto significativo en iWUE en ninguna de las tres especies estudiadas, mientras que las tasas de crecimiento fueron significativamente más altas bajo niveles de competencia reducidos, lo cual estaba en consonancia con la revisión bibliográfica. A largo plazo, las tres especies exhibieron una tendencia creciente significativa en iWUE debido al efecto combinado del aumento en las concentraciones de CO_2, el clima y la edad. No obstante, el crecimiento estuvo principalmente influenciado por la competencia y el clima y en la mayoría de los casos no sufrió una mejora como resultado del aumento en iWUE. Esto sugiere que los árboles responden a reducciones en la competencia principalmente a través de cambios estructurales, como el incremento en crecimiento radial, en vez de mediante ajustes en el intercambio gaseoso a nivel de hoja.

En el **Capítulo 4** el objetivo era analizar las diferencias en la fenología cambial, la fenología foliar y el intercambio gaseoso entre *Q. pyrenaica* y *P. sylvestris* en su límite de distribución altitudinal. Específicamente, evaluamos las variables ambientales que afectan al comienzo y cese de las diferentes fases de fenología cambial y foliar para cada especie, así como las relaciones entre esas fenofases y las dinámicas estacionales de intercambio gaseoso. *P. sylvestris* presentó una fenología cambial más plástica que *Q. pyrenaica*, debido principalmente a su fuerte respuesta a los déficits hídricos estivales. *Q. pyrenaica* mostró una mayor sincronización entre las fenologías cambial y foliar. Además, *Q. pyrenaica* tuvo mayor eficiencia fotosintética en el uso del nitrógeno (PNUE, por sus siglas en inglés) y conductancia estomática que *P. sylvestris* a pesar de sus potenciales hídricos foliares más bajos. Estos resultados, junto a la respuesta de la fenología cambial, podrían indicar una mayor susceptibilidad a la sequía en *P. sylvestris*.

El objetivo del **Capítulo 5** era determinar cómo responden la fenología cambial, anatomía de la madera y fenología foliar de *P. sylvestris* y *Q. pyrenaica* a sequía inducida experimentalmente en el ecotono entre las dos especies. Encontramos una finalización de la elongación del xilema más temprana bajo el tratamiento de exclusión de lluvia, particularmente en *P. sylvestris*, sin que la fenología foliar se viese afectada. Esto, junto a una menor asignación de carbono para la formación del xilema en ambas especies, sería consistente con un cambio en la distribución de recursos bajo estrés por sequía para priorizar la absorción de carbono y sumideros de carbono diferentes al xilema. No obstante, el fuerte control estomático bajo el tratamiento de exclusión de lluvia para regular los potenciales hídricos foliares sugiere que la absorción fotosintética podría verse comprometida en *P. sylvestris* por el aumento en estrés por sequía, afectando el almacenamiento de carbono a largo plazo. Por lo tanto, ambas especies podrían ser vulnerables a un aumento de estrés por sequía, aunque *P. sylvestris* presentó un mayor riesgo de mortalidad inducida por sequía.

En la discusión general (**Capítulo 6**) se contrastan los resultados encontrados en los diferentes capítulos de la tesis. Discutimos primero la

influencia del clima y la competencia en la fisiología de las especies estudiadas, particularmente en el crecimiento y la iWUE. Asimismo, analizamos las repercusiones de esas interacciones bajo escenarios de cambio climático, con un particular énfasis en las implicaciones de gestión. Finalmente, en el **Capítulo 7** presentamos las conclusiones generales derivadas de la investigación desarrollada a lo largo de esta tesis.

Summary

Climate change scenarios forecast for the Mediterranean region a rise in temperatures without a concurrent increase in precipitation, as well as increasing frequency and intensity of drought events. As a consequence of these changes in climatic conditions, *Pinus sylvestris* L. is expected to reduce its distribution range in the region, being displaced at low altitudes by more drought tolerant taxa such as sub-Mediterranean *Quercus* spp. The main **objective** of this thesis was to assess the physiological response of *Pinus sylvestris*, *Quercus pyrenaica* Willd. and *Quercus faginea* Lam. to global change processes, particularly climate change, at different temporal scales, with the ultimate goal of informing the development of sustainable forest management strategies that minimize climate change impacts on Mediterranean forest ecosystems.

The thesis is structured in seven chapters. **Chapter 1** introduces the effect that environmental variables, namely climate, competition and atmospheric CO_2 concentrations, may have on tree physiology, particularly on growth, as well as the methodological approaches that can be used to analyse them. In **Chapter 2** we modelled the relationship between tree growth and the climate-competition interaction using dendrochronological data and long-term competition series from *Quercus faginea*, *Quercus pyrenaica* and *Pinus sylvestris* stands subjected to different thinning regimes. Additionally, we applied these models to various climate change scenarios to project the future growth trends of these species at different competition levels in order to assess their vulnerability to climate change. Growth always decreased with increasing competition, which explained more growth variability than climate in *Q. faginea* and *P. sylvestris*. Our growth projections indicate that *P. sylvestris* would be more negatively affected by climate change than the

studied sub-Mediterranean *Quercus* spp., even under reduced competition conditions.

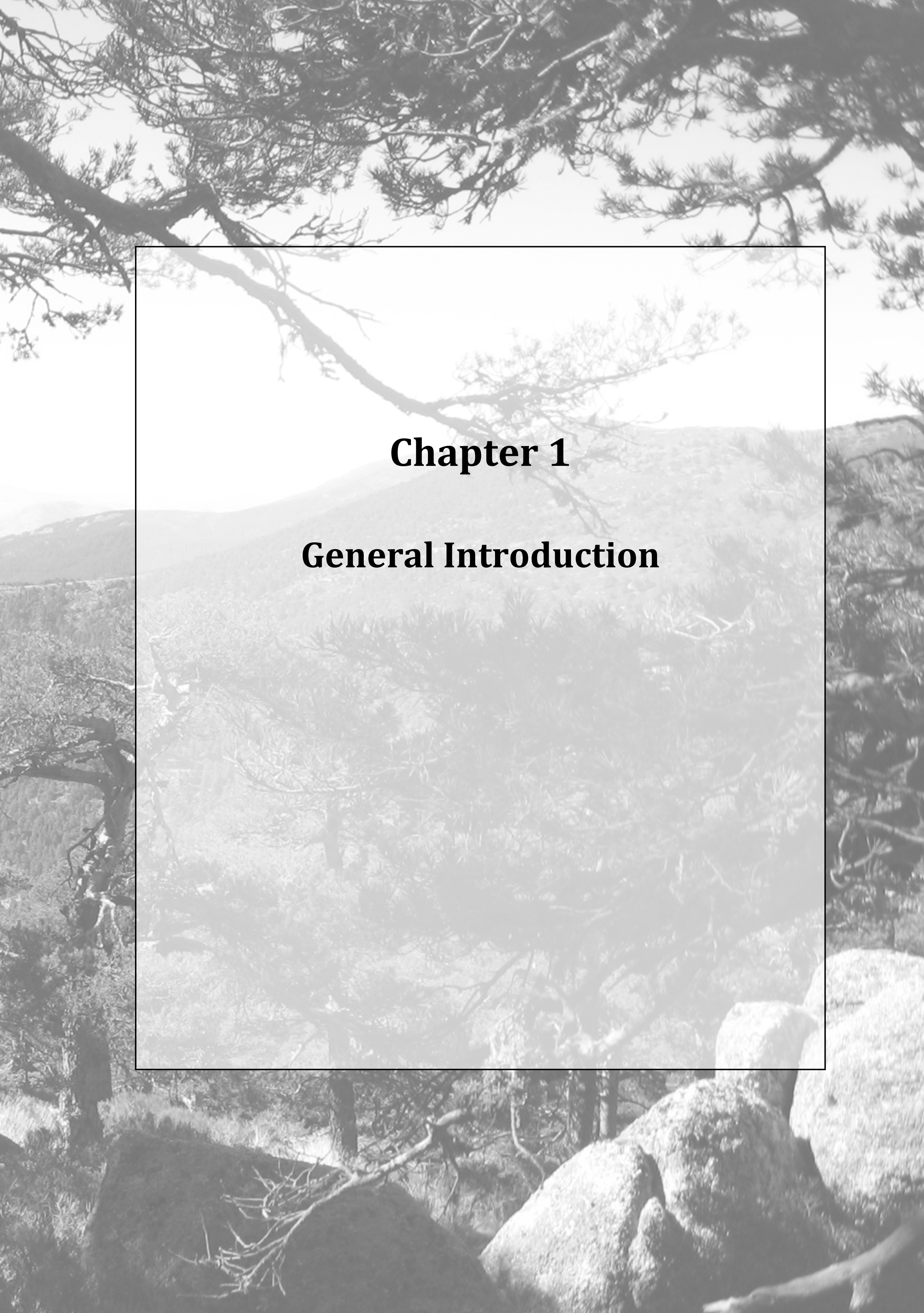
The aim of **Chapter 3** was, firstly, analysing the effect of competition on the intrinsic water-use efficiency (iWUE) and growth of *Q. faginea*, *Q. pyrenaica* and *P. sylvestris*, as well as comparing our results to compiled data from a literature review on the response of tree-ring-derived iWUE and growth to competition. Secondly, we assessed the effect that competition, atmospheric CO_2 concentrations, climate and tree age exert on the temporal trends found in the growth and iWUE of the three studied species. Competition had no significant effect on iWUE in any of the three species studied, whereas growth rates were significantly higher under low competition levels, which was consistent with the literature review. In the long term, the three species exhibited a significant increasing trend in iWUE due to the combined effect of rising CO_2 concentrations, climate and age. Growth, however, was mostly affected by competition and climate and in most cases was not enhanced as a result of increasing iWUE. This suggests that trees mainly respond to a reduction in competition through structural shifts, such as increased radial growth, rather than leaf-level gas exchange adjustments.

In **Chapter 4** we aimed to analyse the differences in cambial phenology, leaf phenology and gas exchange between *Q. pyrenaica* and *P. sylvestris* at their altitudinal distribution limit. Specifically, we assessed the environmental variables that affect the onset and cessation of the different cambial and leaf phenological phases for each species, as well as the relationships between those phenophases and seasonal gas exchange dynamics. *P. sylvestris* had a more plastic cambial phenology than *Q. pyrenaica*, mainly due to its strong response to summer water deficit. *Q. pyrenaica* showed a higher synchronization between its cambial and leaf phenology. Additionally, *Q. pyrenaica* had higher photosynthetic nitrogen

use efficiency (PNUE) and higher stomatal conductance than *P. sylvestris* despite its lower water potentials, indicating a stronger susceptibility to drought in *P. sylvestris*.

The objective of **Chapter 5** was to determine how *P. sylvestris* and *Q. pyrenaica* cambial phenology, wood anatomy and leaf phenology respond to experimental drought at the ecotone between the two species. We found an earlier cessation of xylem enlargement under the rainfall exclusion treatment, particularly in *P. sylvestris*, without an effect on leaf phenology. This, together with a lower carbon allocation to xylem formation in both species, would be consistent with a shift in the allocation of resources under drought stress to prioritize carbon uptake and carbon sinks other than xylem. Nonetheless, tighter stomatal control under the rainfall exclusion treatment to regulate leaf water potentials suggests that photosynthetic uptake in *P. sylvestris* may also be compromised by increasing drought stress, eventually affecting carbon storage in the long term. Therefore, both species could be vulnerable to increasing drought stress, with *P. sylvestris* showing a higher risk of drought-induced mortality.

In the general discussion (**Chapter 6**), the main findings of the different thesis chapters are contrasted. First, we discuss the influence of climate and competition on the physiology of the studied species, particularly on growth and iWUE. Second, we analyse the repercussions of those interactions under climate change scenarios, with a particular emphasis on the management implications. Finally, we present in **Chapter 7** the general conclusions drawn from the research performed throughout this thesis.



Chapter 1

General Introduction

Forests cover 30.6% of the global land area (FAO, 2015). As well as timber and non-wood forest products, forests provide an array of ecosystem services, including biodiversity support, soil and water protection and carbon sequestration (FAO, 2015). The conservation and sustainability of forests and, thus, the services they supply, can be gravely threatened by global environmental change processes such as land-use changes, pollution and climate change. The increase in greenhouse gases such as carbon dioxide (CO_2) has led to an increase in global temperatures in the last decades, as well as disrupted precipitation seasonal patterns (IPCC, 2013). In Mediterranean ecosystems, where water stress is already the most limiting factor for tree performance (Mediavilla & Escudero, 2003; Hernández-Santana *et al.*, 2008a), these changes in climatic forcing are expected to entail an increase in water deficit (Giorgi & Lionello, 2008; García-Ruiz *et al.*, 2011; IPCC, 2013). Altogether, these changing climatic conditions can cause alterations in, among others, growing season length, tree performance and fire, insect and disease disturbance regimes, all of which can alter forest productivity and health, leading to a widespread increase in tree mortality rates that could ultimately cause shifts in forest composition (Hughes, 2000; Thuiller *et al.*, 2005; Linderholm, 2006; McDowell *et al.*, 2008; Carnicer *et al.*, 2011; Anderegg *et al.*, 2015). Moreover, Mediterranean woodlands have been historically modified in terms of their species composition, stand structure and soil characteristics as a result of intensive natural resource exploitation (Valbuena-Carabaña *et al.*, 2010; García-Ruiz *et al.*, 2011), which could alter their ability to respond to climate change. Understanding how environmental factors, and climate in particular, drive tree physiology, phenology and overall tree performance is, therefore, essential to assess species and, thus, forest response to changing environmental conditions. Ultimately, this knowledge should be integrated into sustainable forest management strategies to minimize the potential impacts of climate change on forest ecosystems.

1.1 Leaf and xylem morphological traits across tree functional types

Trees take water and nutrients from the soil and carbon through the leaves. According to the cohesion-tension theory, water ascends under tension from the roots to the leaves along xylem conduits thanks to the negative hydrostatic pressure generated at the leaf surface as a result of water evaporation (Tyree, 1997; Hacke & Sperry, 2001). In order to balance the atmospheric water demand and soil water uptake and supply, stomata adjust leaf water potential and hydraulic conductance, therefore restraining xylem pressure to prevent cavitation and the subsequent loss in conductivity, as well as regulating the tree's gas exchange and water status (Hacke & Sperry, 2001; Meinzer *et al.*, 2009; McDowell *et al.*, 2011). Carbon assimilation is, hence, maximized within the restrictions imposed by the hydraulic system (Hernández-Santana *et al.*, 2009; Martínez-Vilalta *et al.*, 2009). Stomatal behaviour and, thus, carbon uptake is, therefore, highly dependent on leaf and xylem morphological traits, as well as environmental variables (Hacke & Sperry, 2001; Meinzer *et al.*, 2009). Consequently, trees modify different functional traits from the cell and leaf level to the tree level in order to cope with environmental stress (Bréda *et al.*, 2006; Niinemets, 2010).

Leaf traits

Leaf morphology and chemical composition can significantly affect a tree's photosynthetic performance. Indeed, leaf nitrogen content is one of the main factors controlling photosynthetic rates (Escudero & Mediavilla, 2003; Niinemets *et al.*, 2015). In this sense, photosynthetic nitrogen use efficiency (PNUE) is among the leaf traits that better describes species leaf physiology (Zheng & Shangguan, 2006). Evergreen leaves are able to photosynthesize for a longer span than deciduous leaves, which entails that they must cope with a wider environmental variability (Warren & Adams, 2004; Baldocchi *et al.*, 2010). Consequently, evergreen species have dense, thick leaves with thick-walled cells and reduced nitrogen allocation to photosynthesis, which leads to lower photosynthetic rates and PNUE than deciduous trees (Reich *et al.*, 1998; Escudero & Mediavilla, 2003; Takashima *et al.*, 2004; Warren & Adams, 2004; Zheng & Shangguan, 2006). On the other hand, winter dormancy prevents freezing damage and reduces respiration losses during periods when potential photosynthetic assimilation is low (Baldocchi *et al.*, 2010; Vitasse *et al.*, 2014). This entails, however, that deciduous trees must assimilate in a rather short period of time enough carbon to maintain their metabolism, rebuild their canopy and sapwood and replenish their carbohydrate reserves (Gallego *et al.*, 1994).

Xylem traits

Secondary xylem performs three main functions: structural support, transport of water and nutrients and storage of carbohydrates, water and defensive compounds (Cuny *et al.*, 2014). Trees generally renew part of their functional xylem every year, forming the so-called annual rings. Xylogenesis, i.e. the formation of xylem, results from a sequence of vascular cambium division and cell differentiation processes: (1) division of cambium initials and xylem mother cells; (2) xylem cell expansion; (3) cell-wall thickening, which involves the deposition of cellulose, hemicellulose, cell wall proteins and lignin to form the secondary wall; and, in the case of conductive elements, (4) programmed death, i.e. loss of cell content. This process is common to both angiosperms and gymnosperms but results in different xylem structures (Fritts, 1976; Plomion *et al.*, 2001; Delpierre *et al.*, 2016a).

Conifer’s xylem is formed exclusively by tracheids, which are overlapping dead cells usually smaller than 2 mm in diameter (Brodribb *et al.*, 2012). According to tracheid size, gymnosperm rings can be divided in earlywood, formed by thin-walled, large tracheids produced at the beginning of the growing season, and latewood, formed by thick-walled, narrow tracheids produced at the end of the season (Uggla *et al.*, 2001). Tracheids have both water transport and structural functions (Brodribb *et al.*, 2012). By contrast, the more complex angiosperm xylem is formed by fibers, which have a structural function, and vessels, specialized in water transport. Fibers are narrow and single-celled, whereas vessels are multicellular tubes up to 500 µm wide formed by dead vessel elements assembled to form pipes (Tyree & Zimmermann, 2002; Brodribb *et al.*, 2012). Depending on the arrangement of vessels along the ring, angiosperm wood may be classified as ring-porous or diffuse-porous. In ring-porous species, the majority of water transport takes place in the outermost large, highly-efficient earlywood vessels that are formed every spring due to the cavitation of previous-year earlywood vessels, whereas the smaller latewood vessels and tracheids provide water transport when the earlywood vessels are no longer functional (Granier *et al.*, 1994; Hacke & Sperry, 2001). Both tracheids and vessel elements have thick walls to withstand the negative pressure within the water column (Hacke & Sperry, 2001). Pit membranes facilitate water flow between conduits, as well as they prevent air from entering functional, water-filled conduits. Embolized, i.e. air-filled, conduits cannot conduct water and, therefore, unless they can be refilled, extensive embolism causes stomata to close (Hacke & Sperry, 2001; McDowell *et al.*, 2008; Brodribb *et al.*, 2012). Given that conifer tracheids provide both transport and structural support, gymnosperms tend to have greater hydraulic safety margins than angiosperms (Tyree & Sperry, 1988; Hacke & Sperry, 2001; Choat *et al.*, 2012). Additionally, tracheid-based xylem generally has lower maximum conducting efficiency than angiosperm vessels, leading to lower gas exchange and photosynthetic rates (Hacke & Sperry, 2001).

Xylogenesis is controlled, both in conifers and angiosperms, by an array of internal (phytohormones) and external (e.g. temperature, water availability, photoperiod) factors, which in turn control the type and amount of wood produced (Plomion *et al.*, 2001; Aloni, 2015). The hormones auxin (indole-3-acetic acid, IAA), cytokinins, gibberellins and ethylene stimulate cell division, elongation and differentiation, including programmed dead through the release of hydrolases. The synthesis of these hormones is highly influenced by temperatures (Plomion *et al.*, 2001; Uggla *et al.*, 2001; Sorce *et al.*, 2013; Aloni, 2015). Conversely, abscisic acid (ABA), which is synthesised in the shoots and roots in response to various stresses including drought, low temperature and hypoxia, inhibits growth as well as stomatal conductance and bud formation (Plomion *et al.*, 2001; Uggla *et al.*, 2001; Chaves *et al.*, 2003; Sorce *et al.*, 2013; Aloni, 2015). Thus, endogenous and exogenous drivers of tree physiology are highly interconnected. The effect of environmental factors, particularly climate, competition and CO_2, on tree physiological processes is addressed in the following sections.

1.2 Tree physiological adjustments in response to climatic variability

Due to the cyclical intra-annual climatic variability characteristic of the temperate region, trees adjust their period of maximum activity to optimize the use of available resources while minimizing the exposure to harmful conditions (Cuny *et al.*, 2012; Vitasse *et al.*, 2014; Delpierre *et al.*, 2016a). In Mediterranean mountainous ecosystems, this period of optimal conditions is reduced due to their cold winters and dry summers.

As a result of cold conditions, trees undergo a dormant period during winter, characterized by a lack of cell division, to avoid freeze damage. This dormant period can be divided in a resting stage (late autumn and winter), during which favourable environmental factors cannot terminate dormancy, and a quiescent stage (early spring), during which reactivation is controlled by favourable environmental conditions (Swidrak *et al.*, 2011). Rossi *et al.* (2008) found that mean temperatures above 8 °C are necessary to maintain metabolic processes during xylem production and differentiation, with temperatures below 4 ºC significantly limiting xylem growth, despite evergreen species may be able to photosynthesize below that threshold (Strand *et al.*, 2002). The main mechanism by which cold conditions can significantly damage tree functioning is freeze-thaw-induced embolism. If xylem sap freezes, dissolved gases form bubbles in the ice. When sap thaws, those bubbles can cause the cavitation of the conduit when negative pressure is restored. Conifer tracheids are more resistant to freeze-thaw-induced cavitation than angiosperms, explaining their dominance in northern and high-altitude environments (Hacke & Sperry, 2001; Brodribb *et al.*, 2012). Indeed, ring-porous species tend to have a delayed phenology compared to conifers or diffuse-porous species due to their higher vulnerability to freeze-thaw-induced cavitation (Wang *et al.*, 1992).

On the other hand, reduced soil water availability and high atmospheric evaporative demand can significantly constrain plant physiological processes during the Mediterranean summer. Therefore, how species cope with drought highly determines their productivity and competitive interactions (Mediavilla & Escudero, 2003; Hernández-Santana *et al.*, 2008a). The increase in temperatures without a concurrent increase in precipitation predicted for the Mediterranean region under climate change scenarios (Figure 1.1) suggests evapotranspiration rates will rise and, consequently, drought episodes are to become more frequent and severe, increasing competition for water in forest ecosystems (Giorgi & Lionello, 2008; IPCC, 2013). This increase in temperatures will entail enhanced atmospheric vapour pressure deficit (VPD), inducing higher leaf transpiration rates (McDowell *et al.*, 2008).

As a result of climatic variability, plants may face short-term (hours to days) or long-term (days to weeks) water deficits. Trees minimise water loss during short-term water deficits, whereas they cope with long-term water deficits by either shortening their life cycle to avoid dehydration or optimising the use of resources through acclimation (Chaves *et al.*, 2003).

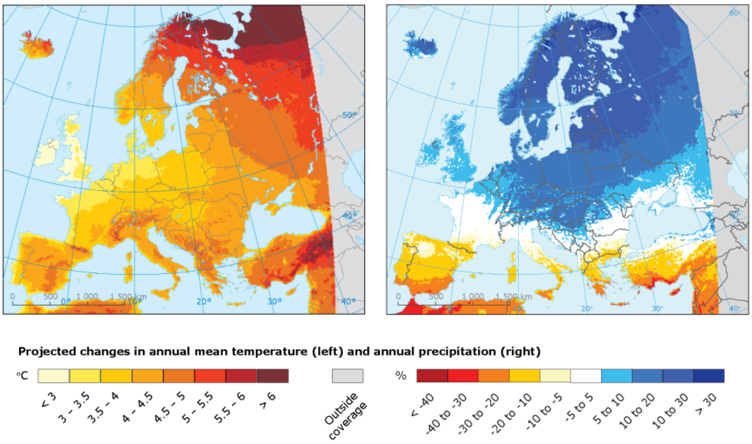


Figure 1.1. Mean temperature and precipitation projections under climate change scenarios in Europe for 2071-2100, compared to 1971-2000. Source: EURO-CORDEX initiative (http://www.euro-cordex.net/).

Water balance in the tree is controlled by the relative rates of water absorption and water loss, which are directly related to soil and leaf water deficits. These can be caused by either excessive loss of water through leaf transpiration, slow absorption of water from dry, cold or poorly aerated soil, or a combination of the two (Kramer, 1962). Water deficit coupled with high evaporative demand can induce xylem cavitation if air bubbles are aspirated into water-filled conduits, thus producing an embolized conduit. Extensive cavitation disrupts sap supply to the leaves, causing crown desiccation (Cochard *et al.*, 1996; Hacke & Sperry, 2001; McDowell *et al.*, 2008). Unlike freeze-thaw-induced cavitation, drought-induced cavitation mostly depends on pit membrane properties rather than conduit diameter (Hacke & Sperry, 2001). Drought resistance is, therefore, associated with xylem resistance to fluctuating pressure so conduit cell walls do not fail under tension (Brodribb *et al.*, 2012)

Stomatal closure is one of the earliest responses to drought, reducing sapflow and protecting the plants from extensive water loss (Cochard *et al.*, 1996; Chaves *et al.*, 2003). Stomata open and close as a result of changes in turgor of guard cells relative to epidermal cells and is regulated by ABA concentrations (Chaves *et al.*, 2003). Isohydric species generally have high vulnerability to embolism and, therefore, maintain relatively constant midday leaf water potential through early stomatal closure (McDowell *et al.*, 2008, 2011; Meinzer *et al.*, 2009). Anisohydric species, conversely, tend to be more resistant to xylem cavitation and, thus, maintain relatively open stomata and allow midday leaf water potential during drought (McDowell *et al.*, 2008, 2011; Limousin *et al.*, 2015).

Due to the non-linear relationship between photosynthetic rate (A) and stomatal conductance (g_s), water loss is more intensely constrained than carbon uptake and, therefore, as long as complete stomatal closure does not occur, most plants experience an increase in intrinsic water-use efficiency (i.e. ratio between A and g_s; iWUE) under drought (Chaves *et al.*, 2003). Additionally, trees prioritize carbon allocation to storage and defensive chemicals over growth (McDowell *et al.*, 2008, 2011). Furthermore, soil moisture deficits may reduce hydrostatic pressure and cell turgor, inhibiting cell division and expansion (Kramer, 1962; Abe *et al.*, 2003). Therefore, water availability significantly affects the seasonal timing and duration of cambial growth, the proportion of earlywood and latewood and, consequently, the width of the annual ring (Abe *et al.*, 2003; Eilmann *et al.*, 2011; Vieira *et al.*, 2015).

Ultimately, prolonged and severe drought could cause the death of the tree by hydraulic failure, carbon starvation or both (Bréda *et al.*, 2006; Breshears *et al.*, 2009; McDowell *et al.*, 2011). Although these proposed mechanisms are not free of controversy and not mutually exclusive (McDowell & Sevanto, 2010; Sala *et al.*, 2010, 2012), it is generally accepted that trees may die from hydraulic failure when extensive cavitation damage leads to the complete desiccation of the plant (McDowell *et al.*, 2008; Anderegg *et al.*, 2015). Hydraulic damage may persist and accumulate over time, increasing vulnerability to further embolism (i.e. cavitation fatigue), causing mortality even after the drought event has passed (Anderegg *et al.*, 2013, 2015). On the other hand, the carbon-starvation hypothesis, although hydraulically driven, suggests that the reduction in carbon uptake as a result of stomatal closure to prevent hydraulic failure causes the plant to starve due to the metabolic consumption of carbohydrate reserves (McDowell *et al.*, 2008). Non-structural carbohydrates, and particularly sugars, may be additionally used to maintain hydraulic transport through osmotic balance (McDowell & Sevanto, 2010; Sala *et al.*, 2012). Isohydric plants are less likely to die from hydraulic failure and, thus, more likely to die from carbon starvation due to their tighter stomatal control to avoid cavitation, whereas anisohydric plants are at a greater risk of extensive cavitation damage. Nonetheles, both isohydric and anisohydric species are susceptible to both mortality mechanisms (McDowell *et al.*, 2008). Additionally, both hydraulic failure and carbon starvation reduce, and may be exacerbated by, the tree's defensive capacity against pathogen and pest attacks (Bréda *et al.*, 2006; McDowell *et al.*, 2008, 2011).

1.3 Tree physiological adjustments in response to competition for resources

Competition is the "interaction between individuals, brought about by a shared requirement for a resource in limited supply, and leading to a reduction of the performance (e.g. survival, growth, reproduction) of at least some of the competing individuals" (Weigelt & Jolliffe, 2003). Consequently, intra-specific competition is generally stronger than inter-specific competition, as trees of the same species and, thus, with the same functional strategies, compete for equivalent resources (Tilman, 1983).

The total abundance of neighbours has been found to be the strongest driver of individual growth across different species functional types (Kunstler *et al.*, 2016). Trees may compete with their neighbours for light, water and nutrients. Trees in high-density stands may be light-limited due to shading by neighbouring trees, which reduces their photosynthetic rates and, thus, growth (Warren *et al.*, 2001; Grams *et al.*, 2007). Likewise, high-density stands have lower soil water availability as a result of higher canopy precipitation interception and stand transpiration (Aussenac & Granier, 1988; Bréda *et al.*, 1995). Consequently, trees subject to high competitive stress often present more negative water potentials and lower soil-to-leaf hydraulic conductance, stomatal conductance and photosynthetic rates than trees under reduced competition (Bréda *et al.*, 1995; Warren *et al.*, 2001; McDowell *et al.*, 2003, 2006; Sala *et al.*, 2005; Moreno-Gutiérrez *et al.*, 2011, 2012). Additionally, competition has been shown to significantly reduce growing season length (Aussenac & Granier, 1988; Bréda *et al.*, 1995; Linares *et al.*, 2009a). Altogether, these factors translate into higher growth rates when competition levels are reduced (Piutti & Cescatti, 1997; McDowell *et al.*, 2003, 2006; Martín-Benito *et al.*, 2010).

Competition can, therefore, significantly alter tree physiological responses to climate, and trees under low competition may be able to better withstand drought events than those subject to high competition stress (Piutti & Cescatti, 1997; Martín-Benito *et al.*, 2010; Sohn *et al.*, 2013, 2014). As a result of land-use changes such as fire exclusion, afforestation or the abandonment of their traditional use, Mediterranean woodlands have reached high stand densities and, therefore, high competition levels, during the last century (Vilà-Cabrera *et al.*, 2011; Gea-Izquierdo & Cañellas, 2014), which could enhance their vulnerability to climate change.

1.4 Tree physiological adjustments to rising atmospheric CO_2 concentrations

Since the pre-industrial times the atmospheric CO_2 concentrations have increased from around 280 parts per million (ppm) to a current value of ca. 400 ppm (Figure 1.2). Forests play a significant role in the global carbon cycle and, therefore, in the dynamics of atmospheric CO_2 concentrations. Elevated CO_2 has two primary effects on plant physiology. First, carbon assimilation rates may increase as a result of enhanced carboxylation and competitive inhibition of oxygenase reaction, hence reducing carbon losses through photorespiration. Second, enhanced CO_2 may induce partial stomatal closure to reduce transpiration water losses, thereby improving water use efficiency (WUE) (Huang *et al.*, 2007; Millard *et al.*, 2007; Battipaglia *et al.*, 2013). Therefore, increased CO_2 concentrations are expected to have a positive impact on tree growth as a result of increased carbon uptake per unit of water loss, process known as CO_2 fertilization (Huang *et al.*, 2007; Franks *et al.*, 2013). Plant response to increasing CO_2 concentration will depend, however, on their ability to use water and nutrients efficiently under the subsequent changing climatic conditions (Körner, 2006; Millard *et al.*, 2007; Battipaglia *et al.*, 2013). Indeed, evidence from enhanced CO_2 experiments indicates that the increase in growth in response to higher CO_2 concentrations is species dependent (Dawes *et al.*, 2011). Bader *et al.* (2010) found that CO_2 enrichment stimulated photosynthetic rates, nitrogen use efficiency and leaf non-structural carbohydrates accumulation, but did not affect leaf nitrogen concentration, leaf thickness or specific leaf area. Similarly, Bader *et al.* (2013) showed that leaf traits and radial growth were unaffected by elevated CO_2, which, however, reduced tree water consumption. Similarly, long-term studies using tree rings have also shown that the increase in iWUE has rarely translated into increased growth rates (Saurer *et al.*, 2004; Peñuelas *et al.*, 2011; Gómez-Guerrero *et al.*, 2013; Voltas *et al.*, 2013; Lévesque *et al.*, 2014). Therefore, further work is needed to elucidate the potential beneficial effect of increasing CO_2 concentrations on tree performance.

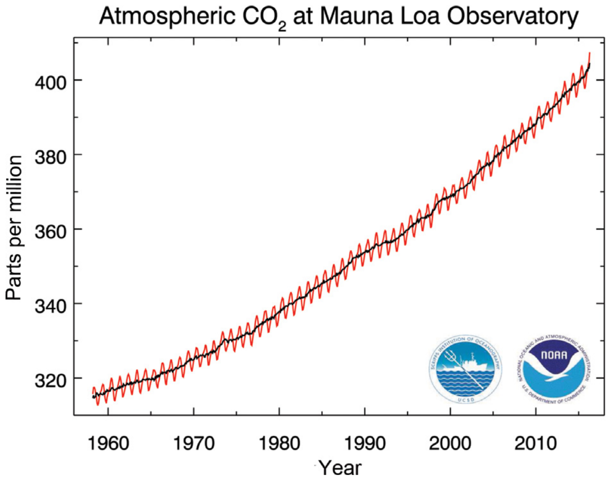


Figure 1.2. Increasing CO_2 concentrations since the mid-1950s. Source: NOAA Earth System Research Laboratory (http://www.esrl.noaa.gov/gmd/ccgg/trends/full.html)

1.5 Long and short-term approaches to study environmental impact on tree physiology

Dendrochronology is the science of dating annual rings and analysing the information they provide about the environment (Fritts, 1976). Multiple studies have used dendrochonological approaches to study growth response to climate (e.g. Andreassen et al., 2006; Andreu et al., 2007; Michelot et al., 2012; Rozas et al., 2009), competition (Bebber et al., 2004; Black and Abrams, 2003; Brudvig et al., 2011; Lorimer and Frelich, 1989; Nowacki and Abrams, 1997) or the interaction between them (Piutti & Cescatti, 1997; Gea-Izquierdo *et al.*, 2009; Linares *et al.*, 2010; Martín-Benito *et al.*, 2011; Martínez-Vilalta *et al.*, 2012). These relationships have been assessed through several modelling approaches, including linear models (Chen et al., 2010) and generalized linear models, with and without random effects (Gea-Izquierdo

& Cañellas, 2009; Martínez-Vilalta *et al.*, 2012). However, tree growth response to environmental variables is not always linear (Vaganov *et al.*, 2006; Coates *et al.*, 2009) and, therefore, non-linear modelling approaches are able to better mimic the relationship between growth and environmental variables (Evans *et al.*, 2006; Loehle, 2008; Gea-Izquierdo *et al.*, 2013). Regardless of the modelling method used, the information contained in tree-rings may be used not only to understand tree growth response to environmental variables but also to make growth projections under climate change scenarios (Chen *et al.*, 2010; Martín-Benito *et al.*, 2011; Huang *et al.*, 2013).

Due to plants discriminate against the heavier carbon isotope ^{13}C, plant material is depleted in ^{13}C in relation to air CO_2. Because plants discriminate less against ^{13}C under stress, the carbon isotope ratio ($\delta^{13}C$), through its relationship with the ratio between the intercellular and the atmospheric CO_2 partial pressures, has been widely used to study water use efficiency in plants (Farquhar & Sharkey, 1982; Farquhar *et al.*, 1982, 1989). Hence, tree ring $\delta^{13}C$ can be used to obtain information on the long-term effect of environmental variables on the tree's iWUE during the period the ring was formed (Farquhar *et al.*, 1989). Indeed, many studies have used these type of data to analyse the effect of climate (Dorado Liñán *et al.*, 2011; Cernusak & English, 2015), competition (Warren *et al.*, 2001; Brooks & Mitchell, 2011; Moreno-Gutiérrez *et al.*, 2012) and CO_2 concentrations (Saurer *et al.*, 2004; Andreu-Hayles *et al.*, 2011; Granda *et al.*, 2014; Lévesque *et al.*, 2014) on long-term trends in iWUE and their relationship to growth.

Thus, dendroecological studies can provide valuable insight into the growth response at different temporal scales, from inter-annual to multi-decadal environmental variability. However, classic dendrochronological methods can not inform about intra-annual growth dynamics (Gruber *et al.*, 2010). Cambial activity and tree-ring anatomical characteristics greatly influence tree performance and, therefore, gathering information about cambial phenology is essential to assess the plasticity of species response to different environmental conditions and, thus, make projections on their responses under climate change scenarios (Rossi *et al.*, 2006a; Gruber *et al.*, 2010; de Luis *et al.*, 2011). Xylogenesis may be studied through the frequent extraction of wood microcores during the growing season (Rossi *et al.*, 2006a). Microcores are cut into micro-sections in order to observe individual xylem cells. Additionally, micro-sections are stained and observed under visible and polarized light to differentiate among the different xylem formation phases, with glittering cell walls evidencing the presence of cellulose in the secondary cell wall and safranine-stained cell walls indicating the presence of lignine (Figure 1.3). Hence, this technique has provided significant information on the onset and cessation of xylem growth as well as on its maximum growth rates and the moment tracheids and vessels become hydraulically active (Rossi *et al.*, 2006b; Swidrak *et al.*, 2011; Gričar *et al.*, 2014; Pérez-de-Lis *et al.*, 2016). Additionally, micro-coring has also proven useful in different experimental field tests, including thinning (Linares *et al.*, 2009a; Primicia *et al.*, 2013), fertilization (Kalliokoski *et al.*, 2013) and rainfall exclusion and irrigation studies (Eilmann *et al.*, 2011; Belien *et al.*, 2012; D'Orangeville *et al.*, 2013).

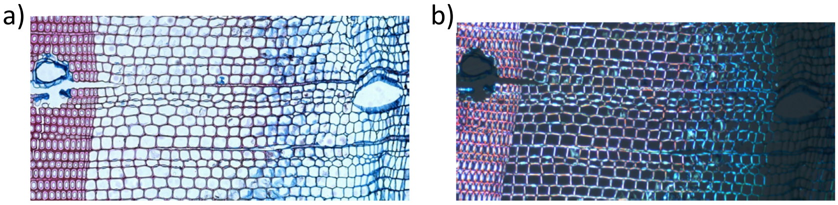


Figure 1.3. Stained micro-cores under visible (a) and polarized (b) light. Glittering cell-walls indicate the presence of cellulose and safranine-stained cell-walls (in pink) indicate the presence of lignine.

Experimental drought studies have provided a good understanding of how different aspects of tree performance respond to drought, including the above-mentioned cambial phenology (Belien *et al.*, 2012; D'Orangeville *et al.*, 2013), leaf phenology (Ogaya & Peñuelas, 2004; Pérez-Ramos *et al.*, 2010; Adams *et al.*, 2015), hydraulic traits (Anderegg *et al.*, 2013), nutrient and water use efficiency (Limousin *et al.*, 2015), carbohydrate dynamics (Dickman *et al.*, 2015) and resistance to insect attacks (Gaylord *et al.*, 2013). Therefore, this type of experiments has the potential to provide valuable insight on how trees may respond to the increasingly drier conditions predicted under climate change scenarios.

1.6 Study species

Within the Mediterranean region, mountainous areas with colder and wetter climatic conditions have served as a refuge for boreal species, which are mixed with Mediterranean taxa leading to highly biodiverse ecosystems (Ruiz-Labourdette *et al.*, 2012). However, these ecosystems could be particularly vulnerable to species loss due to the increasing temperatures predicted under climate change scenarios (Thuiller *et al.*, 2005). The higher areas of central Iberian Peninsula mountain ranges are currently covered by conifer species such as *Pinus sylvestris* L. and *Pinus nigra* J.F. Arnold, whereas the lower belt is dominated by sub-Mediterranean species such as *Quercus pyrenaica* Willd. and *Quercus faginea* Lam. (Ruiz-Labourdette *et al.*, 2012). As a result of increasing temperatures and drought stress, *P. sylvestris* is expected to reduce its current distribution range, particularly in altitude, being displaced at low elevations by sub-Mediterranean species such as *Q. pyrenaica* and *Q. faginea* (Sánchez de Dios *et al.*, 2009; Ruiz-Labourdette *et al.*, 2012). In turn, sub-Mediterranean *Quercus* species could be replaced in their lower and southern limits by more drought tolerant taxa such as *Quercus ilex* L. or *Pinus halepensis* Mill. (Sánchez de Dios *et al.*, 2009; Ruiz-Labourdette *et al.*, 2012; Rigling *et al.*, 2013).

Pinus sylvestris is a widespread Eurosiberian conifer which southernmost limit is found in the Iberian Peninsula mountain ranges, between 500 and 2000 m a.s.l. *P. sylvestris* was particularly favoured in reforestation projects carried out during the 19th and 20th centuries and currently covers around 1,140,000 ha in Spain and 16 % of the Iberian and Central Mountain Ranges (Franco Múgica *et al.*, 1998; Ruiz-Labourdette *et al.*, 2012). Its natural populations are, however, highly fragmented and disconnected, as well as genetically different from their European counterparts (Prus-Glowacki & Stephan, 1994; Franco Múgica *et al.*, 1998; Prus-Glowacki *et al.*, 2003; Ruiz-Labourdette *et al.*, 2012). As a result of increasingly drier conditions, high mortality rates have already been observed in this species, particularly at its low-elevation limit (Martínez-Vilalta & Piñol, 2002; Galiano *et al.*, 2010; Vilà-Cabrera *et al.*, 2011; Gea-Izquierdo *et al.*, 2014).

On the other hand, *Quercus pyrenaica* is a deciduous ring-porous species restricted to the transition between the temperate and the Mediterranean zones, in southwestern Europe and isolated sites in northern Morocco (Sánchez de Dios *et al.*, 2009; Ruiz-Labourdette *et al.*, 2012). In Spain it covers around 650,000 ha, being more abundant on the low belt of siliceous mountainous areas and on acid soils, although it can be found between 400 and 1600 m a.s.l. (do Amaral Franco, 1990). This species is well adapted to sub-Mediterranean climatic conditions, partially owing to its late budburst, that protects it from early-spring frosts, and the pilosity of its leaves, which reduces water losses during summer droughts (Valbuena-Carabaña *et al.*, 2008). Similarly, *Quercus faginea* is a deciduous sub-Mediterranean ring-porous species found in the Iberian Peninsula and northwestern Africa between 500 and 1500 m a.s.l., which, unlike *Q. pyrenaica*, prefers basic soils (do Amaral Franco, 1990). This species is characterized by a high variability in leaf morphology, with varying degrees of sclerophylly depending on site xericity (do Amaral Franco, 1990).

Given their resprouting ability, both *Q. pyrenaica* and *Q. faginea* were traditionally managed as coppices or coppices with standards for fuel, wood and charcoal production, which favoured vegetative regeneration and the proliferation of multiple-stemmed clumps (Cañellas *et al.*, 1996, 2004; Valbuena-Carabaña *et al.*, 2008). Since the 1960's, however, the stands of these two species have reached high densities with little structural diversity as a result of the abandonment of their traditional use (Cañellas *et al.*, 1996, 2004; Valbuena-Carabaña *et al.*, 2008). Overaged coppice stands are, consequently, suffering growth stagnation (Cañellas *et al.*, 1996, 2004; Corcuera *et al.*, 2006; Salomón *et al.*, 2016), which could increase their vulnerability to climate change.

1.7 Study sites

Six sites representative, both in terms of their composition and their climatic conditions, of the dominant woodlands encountered within the Iberian Peninsula mountain ranges were selected for the development of the research presented in this thesis: Barriopedro (BP; *Quercus faginea*), Navasfrías (NA; *Q. pyrenaica*), Rascafría (RA; *Q. pyrenaica*), Duruelo (DU; *Pinus sylvestris*), Neila (NE; *P. sylvestris*) and Valsaín (VA; *Q. pyrenaica* - *P. sylvestris*) (Figure 1.4). The former five were used to develop the research detailed on Chapters 2 and 3. These sites were located at even-aged, monospecific stands part of the INIA's network of permanent thinning monitoring plots. Plots were established between 1968 and 2004 in order to increase tree productivity in *P. sylvestris* stands and transform *Quercus* spp. stands into high forests. At each stand, 770-1600-m^2 plots were randomly assigned either a thinning treatment or left unaltered for control purposes, with at least one repetition per treatment. Thinning from below (i.e. thinning that removes the smallest trees in the stand) was applied the year of plot establishment and periodically to maintain low competition levels. Diameter at breast height of all trees was measured in all plots every 4-10 years since plot establishment and, therefore, tree density and plot basal area were periodically monitored at each site. These series of tree-to-tree competition proxies provided an extraordinary opportunity to study the long-term relationship between growth and the climate-competition interaction. Indeed, the lack of long-term competition series has often limited the study of the effect of competitive interactions on climate-growth relationships, with most studies relying on the comparison of competition classes or single-measurement competition values (Piutti & Cescatti, 1997; Gea-Izquierdo *et al.*, 2009; Linares *et al.*, 2010).

Site Valsaín was used to carry out the work described in Chapters 4 and 5. This stand was located at the transition zone between *Pinus sylvestris*- and *Quercus pyrenaica*-dominated woodlands. This site is found at *P. sylvestris* local low-elevation xeric limit, where this species has been suggested to be threatened by recent climatic changes (Gea-Izquierdo *et al.*, 2014). Unlike the previously-described mono-specific sites, work at this stand allowed us to study the response to climate in two functionally different species under equal climatic conditions. Additionally, in April 2012, a rainfall exclusion experiment was established at this site to assess the effect of drought on *P. sylvestris* and *Q. pyrenaica* in depth.

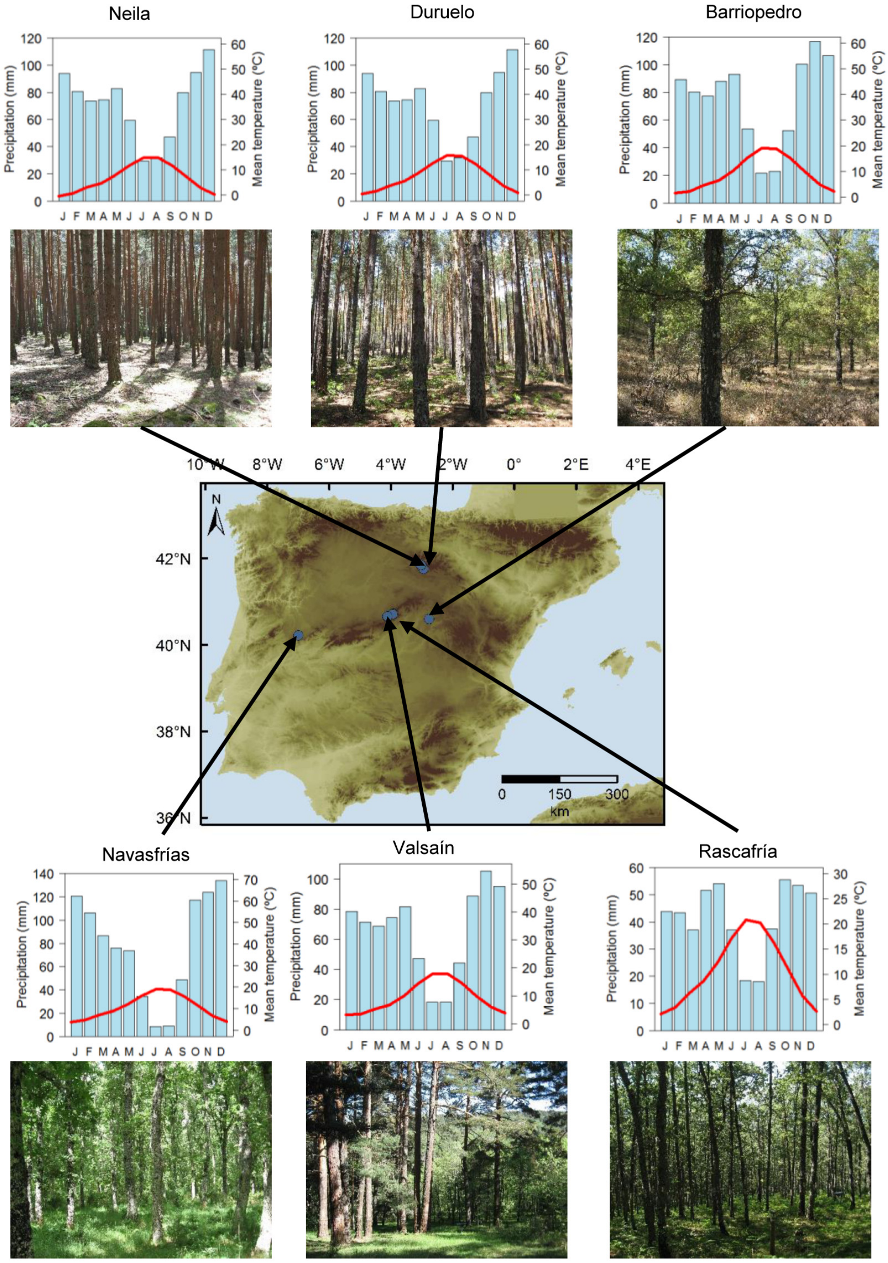


Figure 1.4. Location and climatic conditions of the study sites

1.8 Thesis objectives and outline

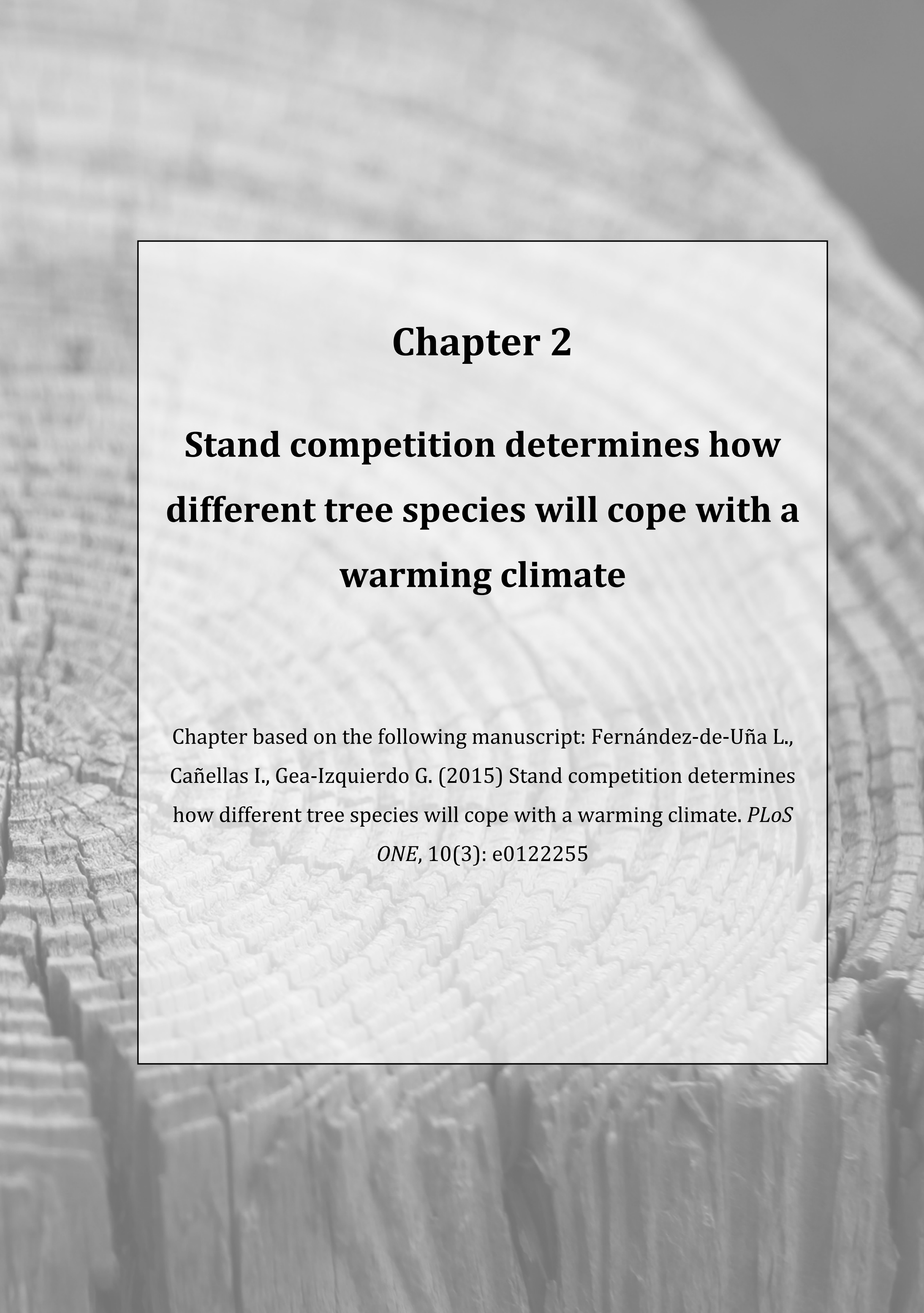
The main objective of the research developed during this thesis was to assess the physiological response of *Pinus sylvestris*, *Quercus pyrenaica* and *Quercus faginea* to global change processes, particularly climate change, at different temporal scales from both a stand- and a tree-level perspective. This research was performed with the ultimate goal to inform the development of sustainable forest management strategies in order to maximize the mitigation of climate change impacts on Mediterranean forest ecosystems. The thesis is structured in seven chapters, including the present Chapter 1 General Introduction. Chapters 2 through 5 describe the different research studies carried out within this thesis. Chapter 6 contains the General Discussion and Chapter 7 presents a set of general conclusions derived from the above-mentioned work.

More specifically, in Chapter 2 we model the relationship between tree growth, climate and competition using dendrochronological data and long-term competition series from *Quercus faginea*, *Quercus pyrenaica* and *Pinus sylvestris* stands subjected to different thinning regimes. Additionally, we apply these models to various climate change scenarios to project the future growth trends of these species at different stand competition levels in order to assess their vulnerability to climate change and future performance.

In Chapter 3 we firstly aimed to analyse the effect of competition on the intrinsic water-use efficiency and growth of *Q. faginea*, *Q. pyrenaica* and *P. sylvestris* and compare our results to compiled data from a systematic literature review on the response of tree-ring-derived iWUE and growth to competition. Secondly, we assess the differential effect that competition, atmospheric CO_2 concentrations, climate and age exert on the temporal trends found in the growth and iWUE of the three studied species.

The aim of Chapter 4 is to compare the cambial phenology, leaf phenology, leaf traits and gas exchange of *Q. pyrenaica* and *P. sylvestris* at their ecotone. More specifically, we aimed to assess the environmental variables that affect the onset and cessation of the different cambial and leaf phenological phases for each species, as well as the relationships between those phenophases and seasonal gas exchange dynamics.

The objective of Chapter 5 is to determine how *P. sylvestris* and *Q. pyrenaica* will respond to the increasing drought stress predicted under climate change scenarios by analysing the response of these two functionally contrasting species in terms of their cambial phenology, wood anatomy and leaf phenology to experimental drought at the altitudinal limit between the two species.



Chapter 2

Stand competition determines how different tree species will cope with a warming climate

Chapter based on the following manuscript: Fernández-de-Uña L., Cañellas I., Gea-Izquierdo G. (2015) Stand competition determines how different tree species will cope with a warming climate. *PLoS ONE*, 10(3): e0122255

Resumen

Las interacciones planta-planta influyen en cómo los bosques lidian con el clima y contribuyen a modular la respuesta de las diferentes especies ante futuros escenarios climáticos. En este trabajo analizamos las relaciones funcionales entre crecimiento, clima y competencia en *Pinus sylvestris*, *Quercus pyrenaica* y *Quercus faginea* para investigar cómo la competencia a nivel de rodal modifica la sensibilidad de los bosques al clima y simular cómo las tasas de crecimiento anuales de estas especies de diferente tolerancia a la sequía cambiarían a lo largo del siglo XXI. Se modelizaron datos dendroecológicos de rodales sometidos a claras usando un novedoso enfoque no-lineal multiplicativo para minimizar sesgos relacionados con la asunción general de que existe una relación lineal entre covariables y mimetizar mejor las relaciones biológicas implicadas. El crecimiento siempre decreció exponencialmente con el aumento en competencia, la cual explicaba más variabilidad en el crecimiento que el clima en *Q. faginea* y *P. sylvestris*. El efecto de la precipitación fue asintótico en todos los casos, mientras que la relación entre crecimiento y temperatura alcanzó un máximo a partir del cual el crecimiento descendía con temperaturas más cálidas. Nuestras proyecciones de crecimiento indican que *P. sylvestris*, la especie menos tolerante a la sequía, se verá afectada más negativamente por el cambio climático que los robles sub-mediterráneos estudiados. El crecimiento medio de *Q. faginea* y *P. sylvestris* decrecería bajo todos los escenarios de cambio climático evaluados. No obstante, el crecimiento de *P. sylvestris* descendería sin importar el nivel de competencia, mientras que este descenso se vería compensado bajo niveles reducidos de competencia en *Q. faginea*. Por el contrario, el crecimiento de *Q. pyrenaica* se mantendría similar a las tasas actuales, excepto bajo el escenario más cálido. Nuestros modelos dan información sobre la naturaleza de las interacciones específicas para cada especie entre clima y competencia y conllevan implicaciones importantes

para la gestión. Asumiendo que el crecimiento individual está directamente relacionado con el rendimiento del árbol, aquellos árboles bajo baja competencia soportarían mejor las condiciones más cálidas predichas bajo escenarios de cambio climático, aunque de manera variable dependiendo de la especie. Por tanto, podría ser conveniente aplicar claras siguiendo una regla exponencial para asegurar la conservación a largo plazo de bosques mediterráneos con alta densidad, particularmente en sitios limitados por la sequía.

Abstract

Plant-plant interactions influence how forests cope with climate and contribute to modulate species response to future climate scenarios. We analysed the functional relationships between growth, climate and competition for *Pinus sylvestris*, *Quercus pyrenaica* and *Quercus faginea* to investigate how stand competition modifies forest sensitivity to climate and simulated how annual growth rates of these species with different drought tolerance would change throughout the 21st century. Dendroecological data from stands subjected to thinning were modelled using a novel multiplicative nonlinear approach to overcome biases related to the general assumption of a linear relationship between covariates and to better mimic the biological relationships involved. Growth always decreased exponentially with increasing competition, which explained more growth variability than climate in *Q. faginea* and *P. sylvestris*. The effect of precipitation was asymptotic in all cases, while the relationship between growth and temperature reached an optimum after which growth declined with warmer temperatures. Our growth projections indicate that the less drought-tolerant *P. sylvestris* would be more negatively affected by climate change than the studied sub-Mediterranean oaks. *Q. faginea* and *P. sylvestris* mean growth would decrease under all the climate change scenarios assessed. However, *P. sylvestris* growth would decline regardless of the competition level, whereas this decrease would be offset by reduced competition in *Q. faginea*. Conversely, *Q. pyrenaica* growth would remain similar to current rates, except for the warmest scenario. Our models shed light on the nature of the species-specific interaction between climate and competition and yield important implications for management. Assuming that individual growth is directly related to tree performance, trees under low competition would better withstand the warmer conditions predicted under climate change scenarios but in a variable manner depending on the species. Therefore, thinning following an exponential rule may be

desirable to ensure long-term conservation of high-density Mediterranean woodlands, particularly in drought-limited sites.

2.1 Introduction

Changes in forest productivity (Sarris *et al.*, 2007), tree phenology (Misson *et al.*, 2011) and species distribution, both in latitude (Thuiller *et al.*, 2005) and altitude (Lenoir *et al.*, 2008), have been observed as a result of climate change (Hughes, 2000). Its long-term effects can be, however, modulated by other factors, such as CO_2 and nitrogen fertilization (Hughes, 2000; Quinn Thomas *et al.*, 2009) or stand dynamics affected by natural disturbances, forest management, and inter-tree relationships. Competition, both above- and belowground, decreases individual radial growth as a result of reduced resource availability, more negative water potentials and decreased photosynthetic rates and stomatal conductance (Bréda *et al.*, 1995; McDowell *et al.*, 2003; Martín-Benito *et al.*, 2010). Consequently, competition affects the variability of the tree growth response to climate (Piutti & Cescatti, 1997; Gea-Izquierdo *et al.*, 2009; Linares *et al.*, 2010; Martín-Benito *et al.*, 2010).

Dendrochronological methods provide us with long series of growth data with an annual resolution. These datasets can be used to analyse forests' response to spatio-temporal changes in climate. However, due to a lack of long-term competition series, there are few studies that have been able to profit from the valuable long time span of this type of data to investigate the effect of the interaction between competition and climate on tree growth. Consequently, most studies addressing the effect of competition on the growth response to climate have analysed this relationship by comparing different competition classes or thinning regimes (Piutti & Cescatti, 1997; Gea-Izquierdo *et al.*, 2009; Linares *et al.*, 2010), whereas fewer studies explicitly modelled growth as a function of both climate and competition (Martín-Benito *et al.*, 2011; Martínez-Vilalta *et al.*, 2012). Thus, there is still much need to understand the underlying relationship between competition and climatic variability because these studies have traditionally simplified the growth response to environmental variables and assumed a linear relationship between them. Nonetheless, the physiological response to environmental forcing, and hence the growth response, is not linear (Canham *et al.*, 2006; Vaganov *et al.*, 2006; Fichtner *et al.*, 2012; Gea-Izquierdo *et al.*, 2013). Concretely, the functional response to a specific environmental variable has either a sigmoid form, when it increases until reaching a saturation state, such as the response of photosynthetic rates to light (Vaganov *et al.*, 2006; Landsberg & Sands, 2011), or a bell-shaped form, when the response to the environmental factor presents an optimum or optimal range, e.g. the effect of nitrogen on growth (Vaganov *et al.*, 2006; Landsberg & Sands, 2011). Because nonlinear approaches can empirically model the biological mechanisms that control the relationship between growth and the interaction between the different environmental variables (Ni *et al.*, 2002; Vaganov *et al.*, 2006), they have greater power to predict growth under past and future climatic conditions (Ni *et al.*, 2002; Tolwinski-Ward *et al.*, 2010; Gea-Izquierdo *et al.*, 2013).

In the Mediterranean region, drought is a key ecological factor determining plant performance and species distribution (Andreu *et al.*, 2007; Sarris *et al.*, 2007). Climate change scenarios forecast rising temperatures with stable or even decreasing precipitation, further increasing the frequency and intensity of drought events (IPCC, 2007). Within the region, mountainous areas with colder and more humid climates have served as a refuge for boreal species. These species mingle with Mediterranean taxa increasing overall biodiversity (Ruiz-Labourdette *et al.*, 2012). Consequently, Mediterranean mountainous ecosystems could be particularly vulnerable to species loss under climate change scenarios (Thuiller *et al.*, 2005). The lower belt of the mountains of the central Iberian Peninsula is currently dominated by sub-Mediterranean species such as *Quercus pyrenaica* Willd. and *Quercus faginea* Lam., whereas the higher areas with colder, more continental climates are covered by conifer species such as *Pinus sylvestris* L. and *Pinus nigra* J.F.

Arnold (Ruiz-Labourdette *et al.*, 2012). This distribution has been, however, highly influenced by different cycles of deforestation and reforestation in historical times (Franco Múgica *et al.*, 1998). *P. sylvestris* was particularly favoured in reforestation during the 19th century and currently covers 16.2% of the Iberian and Central Mountain Ranges (Franco Múgica *et al.*, 1998; Ruiz-Labourdette *et al.*, 2012). Nevertheless, this Eurosiberian species finds its southwestern distribution limit in the Iberian Peninsula mountain ranges, where its natural populations are highly fragmented (Franco Múgica *et al.*, 1998; Ruiz-Labourdette *et al.*, 2012). Consequently, as a result of climate change, *P. sylvestris* is expected to reduce its current distribution range, particularly in altitude, being displaced at low elevations by sub-Mediterranean species such as *Q. pyrenaica* and *Q. faginea* (Sánchez de Dios *et al.*, 2009; Ruiz-Labourdette *et al.*, 2012; Rigling *et al.*, 2013). These two species are only found in the transition between the temperate and the Mediterranean zones and together cover 30.3% of the above-mentioned mountain ranges (Sánchez de Dios *et al.*, 2009; Ruiz-Labourdette *et al.*, 2012). They were traditionally managed as coppices or coppices with standards for firewood extraction, which favoured vegetative regeneration (Cañellas *et al.*, 1996, 2004; Valbuena-Carabaña *et al.*, 2008). As a result of the abandonment of their traditional use, the stands of these two species have reached high densities with little structural diversity (Cañellas *et al.*, 1996, 2004; Valbuena-Carabaña *et al.*, 2008), which could increase their vulnerability to climate change. *Q. faginea* has a higher drought tolerance than *Q. pyrenaica*, and, although both species are better adapted to drought than *P. sylvestris*, they withstand lower water potentials than Mediterranean evergreen species such as *Quercus ilex* L. (Corcuera *et al.*, 2002; Duursma *et al.*, 2008; Eilmann *et al.*, 2009; Montserrat-Martí *et al.*, 2009). Consequently, with climate change, sub-Mediterranean *Quercus* species could be replaced in their lower and southern limits by more drought tolerant taxa (Sánchez de Dios *et al.*, 2009; Ruiz-Labourdette *et al.*, 2012; Rigling *et al.*, 2013).

Given the large area covered by these species in Mediterranean mountain ranges, understanding how *P. sylvestris*, *Q. pyrenaica* and *Q. faginea* stands will respond to changing climatic conditions is essential to assess which management practices would minimize the potentially adverse ecological and socio-economic impacts and ensure their conservation. This study first aims to describe and simulate the relationship between tree growth, climate and competition via a biologically meaningful nonlinear approach, using dendrochronological data and long-term competition series from stands subjected to different thinning regimes. Second, we apply these models to various climate change scenarios to project the future growth trends of these species at different stand competition levels to assess their vulnerability to climate change. Specifically, we were interested to analyse how competition modifies the growth response to climate. We hypothesized that the net effect of climate upon growth would be limited by a competition scale-dependent relationship and that the effect of the interaction between climatic factors and competition on growth would depend on the functional characteristics of the target species, particularly its drought-tolerance.

2.2 Materials and methods

Study sites

We selected different locations for each of the study species within the INIA network of long-term thinning experimental plots: Barriopedro (BP) for *Q. faginea* (QUFG); Navasfrías (NA) and Rascafría (RA) for *Q. pyrenaica* (QUPY); and Duruelo (DU) and Neila (NE) for *P. sylvestris* (PISY; Figure 2.1). The site characteristics are detailed in Table 2.1. All plots were located in even-aged, monospecific, naturally regenerated stands representative of the dominant woodlands currently found within the region. *Quercus* spp. stands were traditionally managed as coppice forests. Multiple stems per tree were common at the *Q. faginea* site, whereas the trees at the *Q. pyrenaica* sites generally had only one stem. At each site, 770-1600-m^2 plots were marked and randomly either assigned a thinning treatment (light thinning – 15-25% plot basal area [BA] reduction –, moderate thinning – 35% BA reduction–, or heavy thinning – up to 50% BA reduction) or left unaltered for control purposes, with at least one repetition per treatment. Thinning from below (i.e. thinning that removes the smallest trees in the stand) was performed the year of plot establishment (Table 2.1) and in approximately 10-year rotation periods. Diameter at breast height (DBH) of all trees was measured in all plots every 4-10 years since plot establishment.

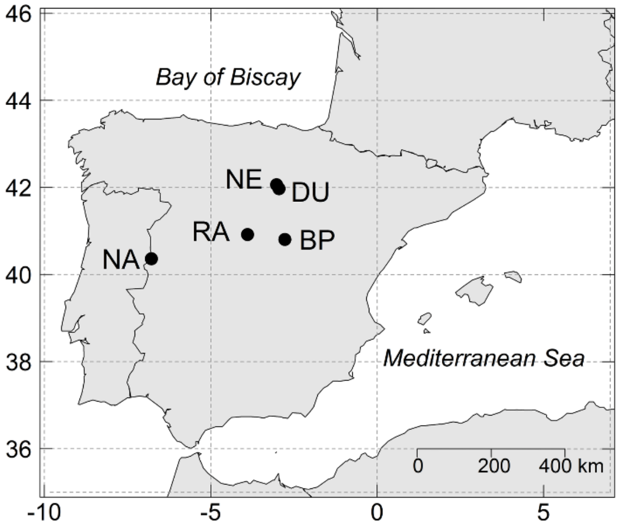


Figure 2.1. Map of the Iberian Peninsula with the location of the study sites. BP: Barriopedro (*Q. faginea*); NA: Navasfrías (*Q. pyrenaica*); RA: Rascafría (*Q. pyrenaica*); DU: Duruelo (*P. sylvestris*); NE: Neila (*P. sylvestris*).

Table 2.1. Site characteristics. Age: Average cambial age at breast height in 2014; Est. year: Year of plot establishment (i.e. year in which plots were marked and the first thinning was applied); Altit.: Altitude; C: Control; L: Light thinning; M: Moderate thinning; H: Heavy thinning; Expos.: Exposure; Prec.: Mean annual precipitation; Temp.: Mean annual temperature; DBH: diameter at breast height; Plot BA: Plot basal area. Average diameter is that of the most recent inventory and includes the standard deviation. Average DBH, plot BA and density show the minimum and maximum values per plot for each site.

Site	Species	Age	Est. year	N plots	Altit. (masl)	Soil type	Expos.	Slope (%)	Prec. (mm)	Temp. (ºC)	Stand type	Treatments	Average DBH (mm)	Plot BA (m^2/ha)	Density (trees/ha)
BP	*Q. faginea*	59	1980	21	860	Haplic calcisol	W	0-35	505	10.5	Xeric	C, L, M, H	88±22-123±28	2.4-19	675-4090
NA	*Q. pyrenaica*	64	2004	9	895	Haplic umbrisol	W	0-10	927	10.7	Mesic	C, L, M	188±51-199±49	15-28	430-1100
RA	*Q. pyrenaica*	53	1994	8	1400	Haplic umbrisol	SE	30	790	9.4	Mesic	C, L, M, H	133±47-161±39	17-40	765-5490
DU	*P. sylvestris*	78	1968	8	1200	Haplic umbrisol	NW	15	860	7.3	Mesic	C, L, H	208±61-288±52	18-56	440-2870
NE	*P. sylvestris*	71	1972	9	1340	Haplic umbrisol	NE	15-20	860	6.6	Mesic	C, L, M	242±66-301±67	35-75	350-4600

Dendrochronological data

At each plot, 20 trees with diameters in the first inventory (i.e. before thinning was applied) above the plot's average were selected and sampled between 2010 and 2012. In trees with multiple stems (such as the case of control plots at the *Q. faginea* site), the largest stem was sampled. Two cores were sampled from each tree at 1.3 m height, with a total of 1100 trees from 55 plots. Cores were air dried, mounted and sanded and measured with a LINTAB measuring table (Rinntech, Heidelberg, Germany) with an accuracy of 0.01 mm. Tree ring series were visually and statistically crossdated with the software TSAP (Rinntech, 2003), using the statistics Gleichläufigkeit (Glk), t-value and the crossdating index (CDI). Crossdating was finally verified with COFECHA (Holmes, 1983).

Ring-width data were transformed into basal area increments (BAI) in mm^2/year. BAI series were averaged per tree and single tree BAI series were in turn averaged per plot to obtain 55 plot BAI chronologies. Average plot chronologies included only those years for which at least five individual tree series existed. BAI series were used because, with the exception of the first few years of increasing juvenile growth, they minimize the tree-size and age effects while conserving both the high and low frequency signal in the tree ring series (Biondi & Qeadan, 2008). Because prewhitening BAI chronologies to eliminate autocorrelation also removed the short-term growth variations due to changes in competition that we aimed to model, we used raw BAI chronologies as the dependent variable in our models.

Competition data

Data from the periodic plot inventories were used to build annual chronologies of tree density and basal area (in stems/ha and m^2/ha, respectively), which were used as proxies of competition at the plot level as they reflect the degree of crowding in the stand (Gea-Izquierdo & Cañellas, 2009; Martínez-Vilalta *et al.*, 2012). Competition series ranged between 9 and 42 years long. We assumed that mortality between inventories occurred gradually in such a way that the annual changes were equal to the difference between inventories divided by the number of years between them. Harvested trees were added to the estimated natural mortality of the year of thinning when this was performed.

Climate data

Climate data were obtained from the Spanish Meteorological Agency (AEMET), the Peñalara Natural Park Research and Management Centre, Barriopedro site's meteorological station (in operation from 1980 to 1991), Herrera et al. (2012) and the CRU TS 3.10 dataset (Mitchell and Jones 2005; accessed through the KNMI explorer http://climexp.knmi.nl/get_index.cgi). We considered the climatic data from the weather station located closest to each study site (at 3-19 km away) and at a similar altitude to be the same as in our plots. Missing data were estimated using linear regressions between the data from that reference station and data from the closest weather stations and, when this was not possible, with data from Herrera et al. (2012) or CRU. Because Neila did not have a station nearby at the same altitude, we used for that site the same precipitation data as for Duruelo, correcting temperature data with a lapse rate of 0.5 ºC/100 m (Kirchner *et al.*, 2013). Monthly and seasonal precipitation, mean temperatures, mean maximum temperatures and mean minimum temperatures were used for the study. Months were pooled as follows: Annual (January - December), Hydrological year (October of previous year - September of current year), Growing season (April-September), Spring (March-May), Summer (June-August), MJJ (May-July), JJ (June-July), Autumn (September-November) and Winter (December of previous year-February of current year).

Data analysis

We calculated Kendall rank correlation coefficients between BAI and the various precipitation and temperature variables to narrow down the covariates that were to be tested in the models. We used nonparametric tests because BAI data were not normally distributed. We also explored the data visually to detect non-linear relationships between covariates. The three species differed in the variables triggering a maximum response in growth, and therefore we calibrated a model per species.

We modelled growth using a nonlinear multiplicative approach (Canham *et al.*, 2006), which allows modelling the nonlinear relationships between variables and investigating the interactions among them, as well as including the effect of the most strongly limiting factor. Growth was estimated as a function of the maximum potential growth (MG; BAI in mm^2/year), i.e. the potential tree growth when all the environmental variables are at their optimum, multiplied by functions of tree size, competition and climatic variables (temperature and precipitation) with values enclosed between 0 and 1 (Canham *et al.*, 2006). Because age was highly correlated with tree size (Kendall's τ coefficient=0.55-0.83), this variable was not included in the model. Therefore, the general form of our model was:

$$BAI = MG \cdot f_1(Size) \cdot f_2(Competition) \cdot f_3(Precipitation) \cdot f_4(Temperature) + \varepsilon \quad [2.1]$$

where $f_i(x)$ are unitless functions (modifiers) representing the functional relationships between growth and the different covariates and ε is the random error.

Several functions per covariate were compared to choose the function that best fitted the data (Table 2.2):

(1) $f_1(Size)$: The effect of size (mean annual diameter of the trees forming the chronology in mm) followed a logistic function (function 1 in Table 2.2), and thus increased up to an asymptote.

(2) *f*$_2$*(Competition)*: As suggested empirically by our data (Figure 2.2) and expressed in the literature (Piutti & Cescatti, 1997; Canham *et al.*, 2006), the relationship between BAI and competition followed a decreasing curve. Thus, we compared different formulations to represent this response, including the modified Gaussian, negative exponential and negative potential functions (functions 2 through 4 in Table 2.2). For PISY, we allowed the parameters in the competition function to vary with site because the competition curves from DU and NE were parallel (Figure 2.2). This was most likely a result of different site characteristics, such as soil fertility, not explained by our model.

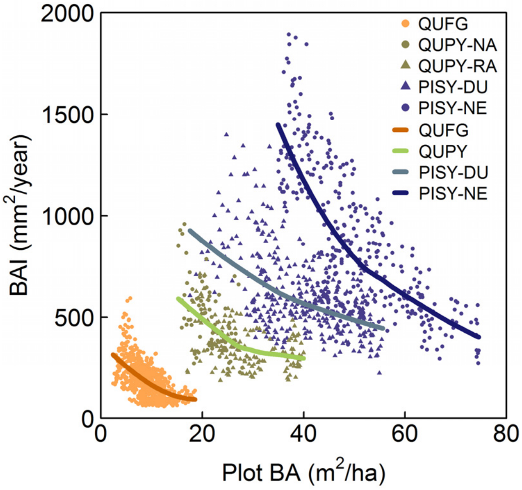


Figure 2.2. BAI (mm^2/year) plotted against stand basal area (BA) per site. The relationship between competition and growth follows a negative exponential form in all sites, as shown by locally weighted polynomial regression (LOESS smoother) lines.

(3) *f*$_3$*(Precipitation)*: The relationship between growth and precipitation was expected to be represented by an increasing monotonic function with an asymptote (Vaganov *et al.*, 2006; Gea-Izquierdo *et al.*, 2013). Nonetheless, to ensure that growth did not decrease with increasing precipitation after an optimum, we compared the logistic function with a modified Gaussian (functions 1 and 2 in Table 2.2).

(4) *f_4(Temperature)*: We expected temperatures to present an optimum or optimal range at which trees perform best (Vaganov *et al.*, 2006; Gea-Izquierdo *et al.*, 2013). Consequently, we compared logistic, modified Gaussian, log-normal and modified Laplace functions (functions 1, 2, 5 and 6 in Table 2.2) to cover a variety of shapes reflecting different ecological responses to temperature.

Table 2.2. Functions assessed, where *x* may be size, competition, precipitation or temperature variables, and f(x) ~ [0, 1].

Function ID	Function type	Function general expression	Variables
1	Logistic	$f(x)=\dfrac{1}{1+\left(\dfrac{x}{a}\right)^{b}}$	Size Precipitation Temperature
2	Modified Gaussian	$f(x)=e^{-0.5\left(\frac{x-a}{b}\right)^{c}}$	Competition Precipitation Temperature
3	Exponential	$f(x) = ae^{bx}$	Competition
4	Potential	$f(x) = a^{bx}$	Competition
5	Log-normal	$f(x) = e^{-0.5\left(\frac{\log\left(\frac{x}{a}\right)}{b}\right)^{2}}$	Temperature
6	Modified Laplace	$f(x) = e^{\left(-\frac{\lvert x-a\rvert}{0.5}\right)^{b}}$	Temperature

Models were fitted using maximum likelihood. Model parameters, including MG and individual function parameters, were estimated using the global optimization algorithm ‘simulated annealing’ (Goffe *et al.*, 1994). Various combinations of response functions and covariates preselected on the exploratory analysis were compared to select the covariate and response function that exhibited the strongest relationship with growth for each modifier in equation 1 (i.e. one for competition, one for precipitation and one for temperature). The best model was selected based on the models' log-likelihood, Akaike Information Criterion (AIC), adjusted coefficient of determination (R^2) and root mean square error (RMSE) (Johnson & Omland, 2004). A model was considered to have a significantly higher explanatory power than another when the difference in AIC between models (ΔAIC) was equal to or greater than 2 (Burnham & Anderson, 2002). Because data and residuals were heteroscedastic and presented positive kurtosis, a Gamma probability density function was used to fit ε in the models.

Growth projections under different climatic and competition scenarios

To project growth at the various studied sites by applying the selected best models, we used climatic scenarios of monthly minimum and maximum temperatures and precipitation of the study sites obtained from the University of Cantabria (http://www.meteo.unican.es/en/projects/estcena). Projections used the general circulation model ECHAM5 for the IPCC emission scenarios A1B, A2 and B1 (IPCC, 2007). Since climate predictions were made for 20 x 20 km grids, data were adjusted to the study sites when necessary using the overlapping period 2001-2011, common to both the data used to calibrate the models and the future predictions.

We projected two types of scenarios based on tree size. First, we projected the growth of the studied stands throughout the 21st century, increasing tree diameter based on the BAI predicted for previous years (hereafter dynamic projections). Second, we assessed the changes in annual growth that a given size class would suffer as a result of climate and competition, for which we used the same diameter per size class throughout the simulation period (hereafter constant-diameter projections). We estimated BAI for two different size classes per species: 100 and 150 mm-diameter for *Q. faginea*, 150 and 200 mm for *Q. pyrenaica* and 200 and 300 mm for *P. sylvestris*. We considered two competition scenarios: heavy thinning (HT) and control (C). For the dynamic projections, in the heavy thinning scenario we presumed that competition would be kept steady at the same level as at present through forestry practices. Because under natural conditions competition rates are expected to increase up to a maximum level at which rates remain constant through self-thinning (Pretzsch & Biber, 2005), for the control scenario we assumed that competition levels would increase over time at rates similar to those measured in the most recent years. We estimated for each site a specific asymptote reflecting constant competition rates based on observed trends and maximum competition levels found in National Forest Inventory data. For the constant-diameter projections, we kept competition levels constant at the same levels as in the present for both the control and heavy thinning scenarios. Climate and competition projections were introduced in the selected models to predict growth throughout the 21st century. For a better assessment of the long-term trends, a smoothing spline with a 50% frequency cut-off at 30 years was applied to the simulated growth series.

All analyses were carried out with R version 2.13.1 (R Core Team, 2014). For BAI calculations and future growth projection smoothing we used the package “dplR” (Bunn, 2008), while models were fitted using the package “likelihood” (Murphy, 2012).

2.3 Results

Descriptive statistics of the studied 55 tree ring chronologies can be found in Table S1. *Q. faginea* had the slowest growth, followed by *Q. pyrenaica*. Growth rates were lower at the driest sampled site for each species (Table 2.1 and Table S2.1 in Supporting information). Trees were relatively young, with chronologies ranging between 54 and 86 years in length (Table S2.1).

Growth model

The variance explained by the models (R^2) ranged from 55% for *Q. pyrenaica* to 78% for *Q. faginea* and *P. sylvestris* (Table 2.3 and Figure 2.3). The effect of size on growth was stronger on *Q. pyrenaica* and *P. sylvestris*, whereas most of the growth variability of *Q. faginea* was explained by competition. For *Q. faginea* and *P. sylvestris*, the effect of competition on growth was stronger than that of climatic variables, whereas *Q. pyrenaica* growth was more influenced by temperature than by competition (Table 2.3). Of the two climatic variables assessed, temperature had a stronger influence on both *Quercus* species growth than precipitation, whereas *P. sylvestris* responded more strongly to the latter (Table 2.3). For all species, plot BA better reflected the effect of competition changes in the stand than tree density. The relationship between growth and competition followed a negative exponential function in all cases (function 3 in Table 2.2; Figures 2.2 and 2.4). *Q. pyrenaica* and *P. sylvestris* responded similarly to competition, whereas *Q. faginea* suffered stronger decreases in growth with increasing competition (Table 2.4 and Figure 2.4).

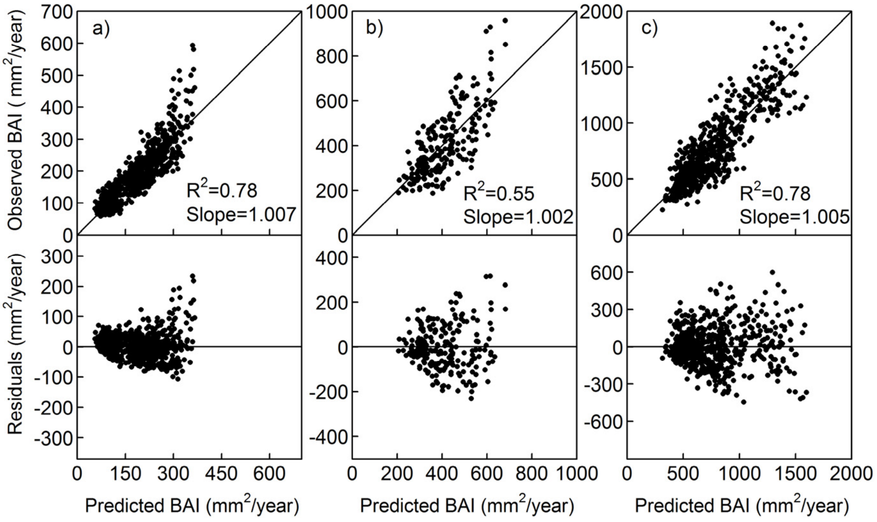


Figure 2.3. Observed vs. Predicted BAI and Residual plots for the models for (a) *Quercus faginea*, (b) *Quercus pyrenaica* and (c) *Pinus sylvestris*.

Table 2.3. Effect of adding one more environmental variable to the nonlinear model. Comp: basal area for all species; Prec: P_{Hyd} for *Q. faginea*, P_{Spr} for *Q. pyrenaica* and P_{MJJ} for *P. sylvestris*; Temp: $Tmax_{Spr}$ for *Q. faginea* and *Q. pyrenaica* and $Tmax_{Hyd}$ for *P. sylvestris*; LL: Log-likelihood; ΔAIC: difference between the AIC of the model being considered and the AIC of the complete model (Size·Comp·Prec·Temp); R^2: Adjusted coefficient of determination; RMSE: Root mean square error; N par: Number of parameters in the model.

	QUFG					QUPY					PISY				
Model	**LL**	**ΔAIC**	**R^2**	**RMSE**	**N par**	**LL**	**ΔAIC**	**R^2**	**RMSE**	**N par**	**LL**	**ΔAIC**	**R^2**	**RMSE**	**N par**
Size	-3830.6	925.4	0.11	84.1	4	-1390.4	95.7	0.24	129.2	4	-4783.5	719.0	0.39	250.3	4
Size·Prec·Temp	-3787.6	847.4	0.20	79.7	8	-1354.9	33.2	0.45	110.1	8	-4593.1	346.3	0.63	195.0	8
Size·Comp	-3459.1	184.5	0.70	48.6	5	-1375.7	68.3	0.35	119.4	5	-4524.0	203.9	0.70	175.3	6
Size·Comp·Prec	-3422.3	114.9	0.73	46.2	7	-1356.2	33.6	0.46	109.4	7	-4454.8	69.7	0.76	159.3	8
Size·Comp·Temp	-3406.0	82.4	0.75	45.0	7	-1342.5	6.2	0.53	102.3	7	-4486.3	132.6	0.73	166.6	8
Size·Comp·Prec·Temp	-3362.9	0	0.78	42.1	9	-1337.2	0	0.55	100.1	9	-4417.9	0	0.78	152.7	10

Table 2.4. Values and support intervals of the parameters for the different models. MG: maximum growth (mm^2); Size_a and Size_b: parameters of the size function (logistic), which represent the half saturation point and the scale of the function, respectively; Comp_a and Comp_b: parameters of the competition function (negative exponential), which represent the intercept and the shape of the function, respectively; Prec_a and Prec_b: parameters of the precipitation function (logistic); Temp_a and Temp_b: parameters of the temperature function (modified Laplace), which represent the optimum temperature and the scale of the function, respectively.

Parameter	**QUFG**	**QUPY**	**PISY**	
			DU	**NE**
MG	1055.2 (1044.1, 1065.7)	1965.9 (1908.9, 2006.5)	5546.8 (5491.3, 5602.3)	
Size_a	83.8 (83.8, 83.8)	227.5 (220.7, 234.3)	205.7 (203.6, 207.8)	
Size_b	-5.26 (-5.74, -4.92)	-1.48 (-1.66, -1.32)	-2.09 (-2.27, -1.95)	
Comp_a	1	1	0.6660 (0.6526, 0.6726)	1
Comp_b	-0.1217 (-0.1243, -0.1181)	-0.0191 (-0.0206, -0.0178)	-0.0209 (-0.0214, -0.0203)	
Prec_a	221.0 (211.3, 229.9)	87.7 (85.0, 89.5)	39.0 (36.3, 41.4)	
Prec_b	-2.13 (-2.31, -1.99)	-20.27 (-32.16, 12.96)	-1.63 (-1.79, -1.52)	
Temp_a	16.1 (16.1, 16.4)	13.3 (13.0, 13.5)	14.0 (14.0, 14.0)	
Temp_b	0.0312 (0.0274, 0.0337)	0.0393 (0.0334, 0.0482)	0.0701 (0.0638, 0.0745)	

The relationship between growth and the climatic variables also followed the same functional form for the three species, although the precipitation and temperature variables triggering this response were species-specific. The logistic function best captured the relationship between precipitation and growth (function 1 in Table 2.2), indicating that the growth response reached an asymptote with increasing moisture availability, whereas the modified Laplace (function 6, a distribution with a single maximum located at a sharply pointed peak) worked best for temperatures (Figure 2.4). This function suggests that growth increased exponentially up to an optimum, after which growth decreased exponentially with increasing temperatures. The characteristics of this growth response to temperature were similar for both *Quercus* species, whereas changes in growth with temperature were steeper in *P. sylvestris*. For the analysis of the climatic variables affecting growth, only the final climate variables selected for the models are given due to the large number of variables tested. *Q. faginea* responded more strongly to precipitation of the hydrological year (P_{Hyd}) and spring maximum temperatures ($Tmax_{Spr}$), whereas *Q. pyrenaica* responded to spring precipitation (P_{Spr}) and maximum temperatures ($Tmax_{Spr}$). *P. sylvestris* growth was mostly related to May-July precipitation (P_{MJJ}) and maximum temperatures of the hydrological year ($Tmax_{Hyd}$).

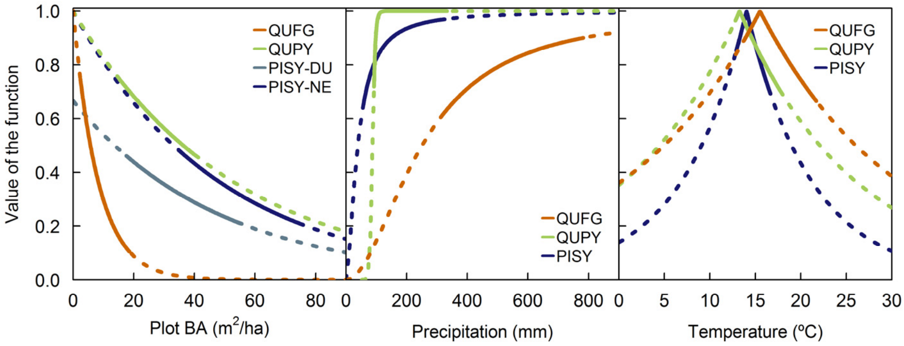


Figure 2.4. Comparison of the different functional relationships between growth and the various environmental variables as fitted in the models for each of the studied species. Solid lines represent the range for which the models were calibrated, whereas dotted lines represent extrapolations of the models. Precipitation and Temperature are P_{Hyd} and $Tmax_{Spr}$, repectively, for *Q. faginea*, P_{Spr} and $Tmax_{Spr}$ for *Q. pyrenaica*, and P_{MJJ} and $Tmax_{Hyd}$ for *P. sylvestris*. To ease the comparison among species of the different relationships, growth is shown on a relative scale for each species, with 1 representing the species-specific maximum growth.

Growth projections under climate change scenarios

According to the dynamic projections made by our models for the study sites, the forecasted climate change scenarios would affect *P. sylvestris* more negatively than the oaks studied. *Q. faginea* growth would experience a decrease in control plots under all climatic scenarios, whereas it would remain constant under the heavy thinning scenario, except for the A2 scenario, which forecasts higher temperature increases (IPCC, 2007) (Figure 2.5). *P. sylvestris* growth would significantly decrease under all size, climatic and competition scenarios assessed at both study sites (Figures 2.5 and 2.6). Conversely, *Q. pyrenaica* growth would increase during the 21st century under the A1B and B1 scenarios in RA, the *Q. pyrenaica* site located at a higher, colder location, whereas in NA this increase would only occur under reduced competition conditions, with stable or slightly decreasing growth trends in control plots (Figure 2.5). Nonetheless, the constant-diameter projections indicate that these increasing growth trends are due to the positive effect of increasing tree size and not a response to future climate, because they predict that both *Q. pyrenaica* and *Q. faginea* tree productivity would remain constant or slightly decrease during the 21st century for all size-classes assessed (Figure 2.6). Under the A2 scenario, however, the models predict a decrease in growth under both competition levels for all sites (Figures 2.5 and 2.6).

2.4 Discussion

Species-specific nonlinear interaction between competition and climate

Our nonlinear model succeeded in capturing the functional response of growth to climate, as well as the nonlinear interaction between climatic variables and stand competition. Precipitation had a positive effect on growth, as expected in water-limited Mediterranean ecosystems (Sarris *et al.*, 2007; Gea-Izquierdo *et al.*, 2009; Gómez-Aparicio *et al.*, 2011; Moreno-Gutiérrez *et al.*, 2012). However, this positive effect is nonlinear and ultimately saturates and reaches an asymptote (Vaganov *et al.*, 2006; Gea-Izquierdo *et al.*, 2013). This indicates that in the study areas moisture availability constrains growth up to a species-specific threshold, after which precipitation never reaches high enough levels to limit growth (Rozas & García-González, 2012). In contrast, the response to temperature was positive up to an optimum, after which tree growth is limited by increasing temperatures (Vaganov *et al.*, 2006; Gea-Izquierdo *et al.*, 2013). This is consistent with the response of photosynthetic rates to temperatures, which also shows an optimum as a result of Rubisco inactivity at low temperatures and stomatal closure with increasing water stress at high temperatures (Webb *et al.*, 1974; Farquhar & Sharkey, 1982; Hikosaka *et al.*, 2006).

The growth response of each species to climate depends on the species' ecological requirements, particularly its drought tolerance. According to our model, the growth response to temperature was similar in both *Quercus* species, both in terms of the shape of the function and the strength of the effect, which was more intense than that of precipitation. In contrast, the effect of precipitation was stronger than that of temperature on *P. sylvestris*, although its response function to temperature showed that this species has higher sensitivity to temperature changes than do the other two species. This species has its southernmost and, thus, dry distribution limit in the Iberian Peninsula and is less drought tolerant than the studied *Quercus* spp. (Eilmann *et al.*, 2009; Rigling *et al.*, 2013), which would explain its stronger dependence on precipitation. The nonlinear response to climatic variables and the species-specific precipitation and temperature thresholds identified by our models improve our understanding of how species will respond under climate change. The assessment of the functional relationships between growth and climatic forcing can therefore improve the reliability of models using long-term dendroecological data. Hence, nonlinear approaches are able to overcome modelling shortcomings resulting from assumptions of linearity such as the 'divergence problem' observed in the temperature-tree growth relationship (D'Arrigo *et al.*, 2008; Loehle, 2008).

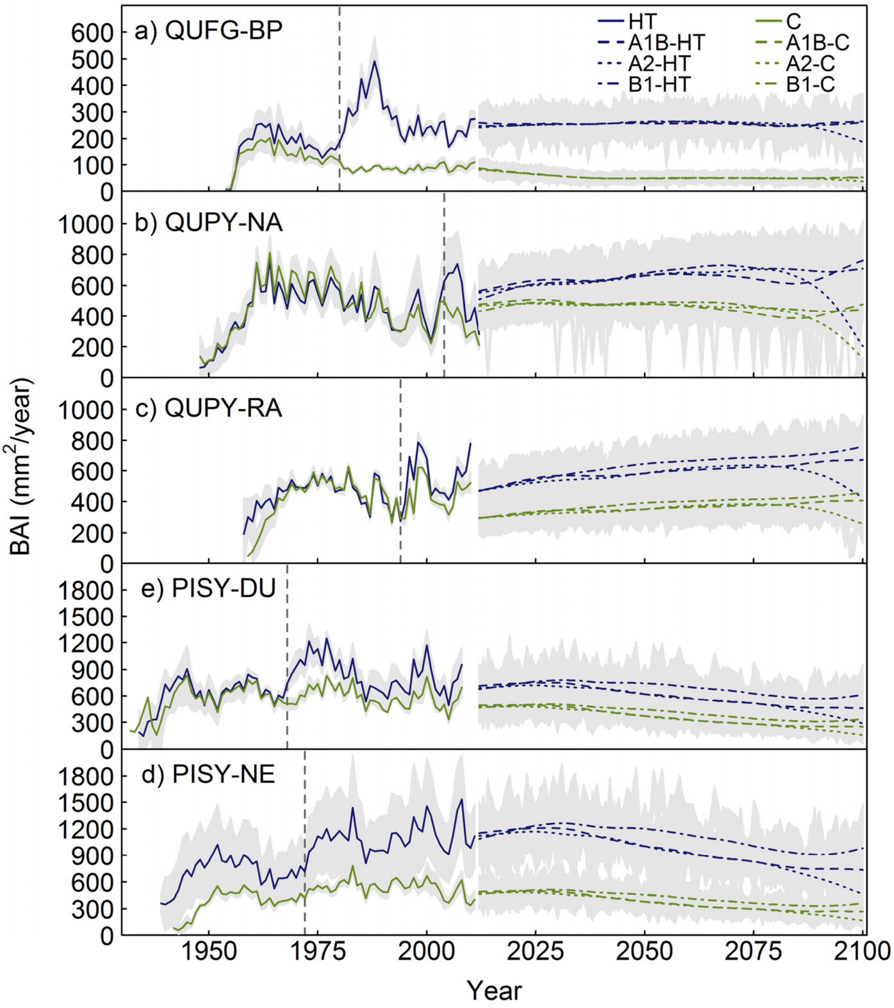


Figure 2.5. Mean chronologies and dynamic growth projections under different competition (Control, C, and Heavy thinning, HT) and climate change (A1B, A2 and B1) scenarios for the different study sites. (a) Barriopedro (*Q. faginea*); (b) Navasfrías (*Q. pyrenaica*); (c) Rascafría (*Q. pyrenaica*); (d) Duruelo (*P. sylvestris*); and (e) Neila (*P. sylvestris*). Grey dashed vertical lines indicate the time when plots were established and thus, the years from which data were used to calibrate the models, which do not include increasing juvenile growth. Shading represents the confidence intervals, calculated as the mean±standard deviation.

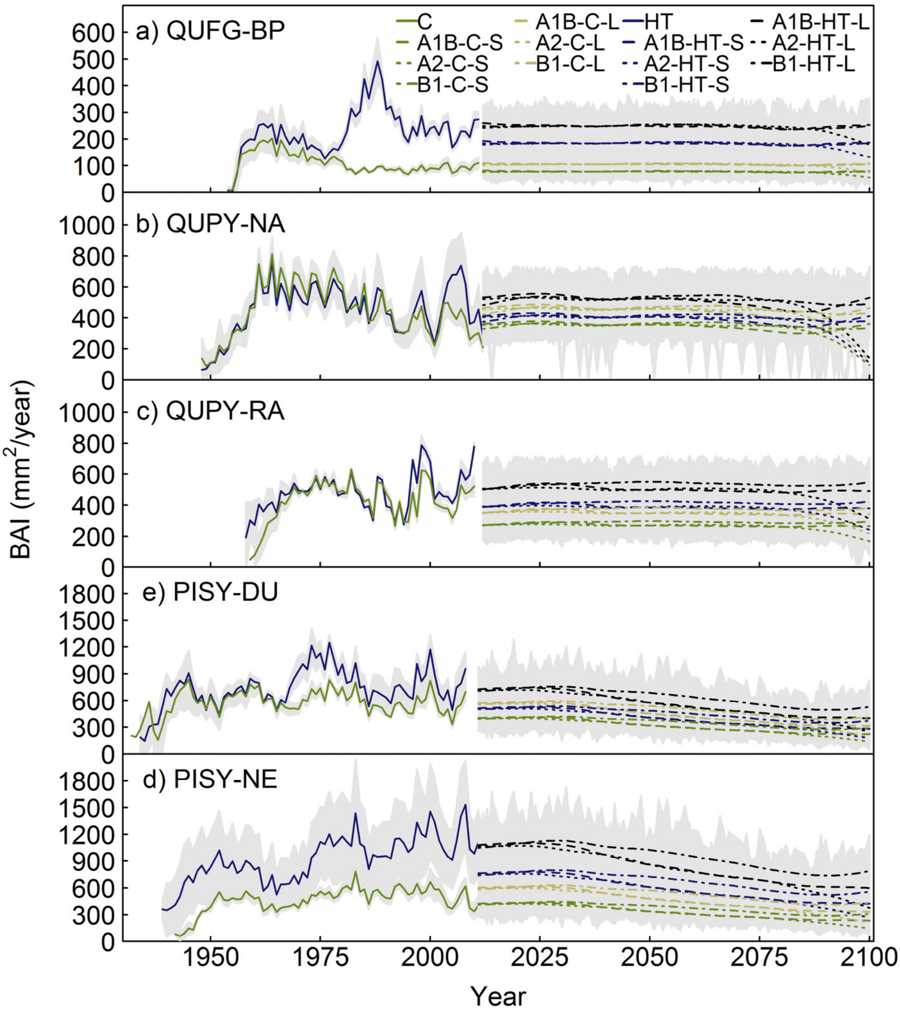


Figure 2.6. Mean chronologies and constant-diameter growth projections for the various size classes under different competition and climate change scenarios for the different study sites. (a) Barriopedro (*Q. faginea*); (b) Navasfrías (*Q. pyrenaica*); (c) Rascafría (*Q. pyrenaica*); (d) Duruelo (*P. sylvestris*); and (e) Neila (*P. sylvestris*). Competition scenarios: Control (C) and Heavy thinning (HT). Climate scenarios: A1B, A2 and B1. Size classes: S (100 mm for QUFG, 150 mm for QUPY and 200 mm for PISY) and L (150 mm for QUFG, 200 mm for QUPY and 300 mm for PISY). Shading represents the confidence intervals, calculated as the mean±standard deviation.

In spite of the substantial influence of climate on growth, the effect of competition was, however, dominant over that of climate for *Q. faginea* and *P. sylvestris*, as shown for other species (Linares *et al.*, 2010; Gómez-Aparicio *et al.*, 2011; Vayreda *et al.*, 2012). The functional relationship between growth and competition followed a negative exponential function in all species. However, the responses of *Q. pyrenaica* and *P. sylvestris* to competition were similar in amplitude, while *Q. faginea* suffered stronger growth constraints with increasing competition. *Q. faginea* trees presented multiple stems which shared the same root system, and this characteristic caused high competition levels. After thinning, only one stem per tree remained on low competition plots. These single-stem trees most likely profited from larger root systems, which together with the more xeric conditions of this site, could have amplified the effect of competition on this species compared with the other two. This reflects the strong interaction between competition and climatic stress, which our model was able to capture thanks to its multiplicative nature. A proper characterization of this interaction is crucial to understand the combined effect of these two abiotic factors on the response of forests to climate change. Consequently, assuming that growth is a proxy for species performance and, therefore, that reduced growth rates imply enhanced vulnerability, the sustainability of these stands will depend on the species-specific nonlinear interaction between competition intensity and long-term climate forcing, with individuals subject to high competition levels being less likely to survive enhanced xericity (Bigler *et al.*, 2004; Galiano *et al.*, 2010; Linares *et al.*, 2010; Gómez-Aparicio *et al.*, 2011).

Growth projections under different climate and competition scenarios. Implications for future management

Most studies on growth projections under climate change scenarios have neglected the importance of competition (Chen *et al.*, 2010; Huang *et al.*, 2013), despite the evidence indicating its role in altering tree growth response to climate (Piutti & Cescatti, 1997; Gea-Izquierdo *et al.*, 2009; Linares *et al.*, 2010; Gómez-Aparicio *et al.*, 2011), and thus, the need to modify growth projections under future climate scenarios (Martín-Benito *et al.*, 2011). Our models predicted a reduction of tree growth in stands with high competition levels for *Quercus faginea* and *Pinus sylvestris*, whereas this reduction would be minimized under low competition levels. Multiple studies have recorded the negative effect of high temperatures and drought on tree physiology and growth (Bréda *et al.*, 2006; McDowell *et al.*, 2008), as well as the positive effect of reduced competition for resources on tree performance, particularly under xeric conditions (Bréda *et al.*, 1995; McDowell *et al.*, 2003; Martín-Benito *et al.*, 2010; Moreno-Gutiérrez *et al.*, 2012). Therefore, the predicted decreasing future growth trends and highly reduced growth rates could forecast enhanced tree mortality rates (Bigler *et al.*, 2004; Gea-Izquierdo *et al.*, 2014), particularly under high competition levels. Several studies have already observed an increase in mortality, consistent with self-thinning dynamics, in certain species of the Mediterranean region, including *P. sylvestris*, as a result of increased stand competition, rising temperatures and drought episodes (Martínez-Vilalta & Piñol, 2002; Vayreda *et al.*, 2012; Ruiz-Benito *et al.*, 2013). Increased mortality, together with reduced regeneration (Kouba *et al.*, 2012; Benavides *et al.*, 2013) and decreased growth, could lead to the decline of these stands and their substitution by better adapted species, as predicted by species distribution models (Sánchez de Dios *et al.*, 2009; Ruiz-Labourdette *et al.*, 2012). Our models suggest that *P. sylvestris* growth would be more negatively affected by climate change than that of the two more drought-tolerant sub-Mediterranean *Quercus* spp. studied. This could forecast a displacement in altitude of *Pinus sylvestris* in favour of *Quercus pyrenaica*, for which our models predicted an increase in growth for the site located at a higher elevation, which is consistent with species distribution simulations in the literature (Sánchez de Dios *et al.*, 2009; Ruiz-Labourdette *et al.*, 2012).

Nonetheless, under the warmest climate scenario, *Q. pyrenaica* growth would also decline at both sites, indicating that, due to the nonlinear nature of the temperature-growth relationship, the forecasted temperature increase may become limiting for this species growth too (Gea-Izquierdo *et al.*, 2013).

Nevertheless, the mean growth rates predicted under decreased competition for both *Quercus* species indicate that applying competition reductions similar to those assessed with our models could mitigate the potential negative effects of climate change upon growth for trees suffering from stand densification. In contrast, *P. sylvestris* would suffer a reduction in growth even under the low-competition scenario, indicating high vulnerability to increasing temperatures. Therefore, heavier thinning intensities than those applied today may be necessary to maintain this species' tree growth. Because the negative effect of competition declines exponentially with decreasing competition, species-specific thresholds below which growth could be optimised without substantially reducing stand density must be identified, as intense thinning may have detrimental effects on stand productivity and sustainability (Valbuena-Carabaña *et al.*, 2008; Camarero *et al.*, 2011). These thresholds could also be site-dependent, particularly at the edges of each species distribution, where competitive stress may be strengthened due to the nonlinear interaction between competition and climate.

Despite their value for adaptive management, growth projections must be assessed with caution, because there are many inherent uncertainties associated with the extrapolation of models outside their calibration range. One of their main limitations is the impossibility of incorporating potential species acclimation to changing climatic conditions. The nonlinear nature of the models can, however, partially offset this uncertainty because they most likely capture the functional growth response to environmental variability. Moreover, we did not cover the whole climatic range of the studied species.

Therefore, these models could still be improved by calibrating them with data from a broader climatic gradient (Gea-Izquierdo *et al.*, 2013), and thus, extend their applicability to the entire distribution range of each of the studied species. This can be particularly relevant for *Q. faginea*, for which we only had one site and for which, therefore, our ability to assess climatic variability was most likely more reduced than that for the other two species. Nonetheless, as long series of competition levels are not readily available, our multispecies approach using stand competition series from long-term experimental plots is particularly valuable.

Conclusions

Our nonlinear models highlighted the species-specific nature of the growth response to climate and its interaction with competition. These models overcome several limitations of classic linear approaches. Moreover, they shed light on factors that contribute to better understanding of instabilities in the growth response to climate and could be used to detect climatic thresholds of species performance. As a result of this nonlinear interaction, trees under low competition will better withstand the warmer conditions predicted under climate change scenarios, particularly for the two *Quercus* species studied. Competition will most likely be naturally maintained at sustainable levels through self-thinning (Pretzsch & Biber, 2005). However, this process can be accelerated to reduce the vulnerability of the remaining trees to drought through thinning. In light of our results, plot basal area reductions as intense as or even heavier than the ones currently applied may be necessary to prevent stand growth stagnation, particularly in drought-limited sites. However, the assessment of the species-specific competition thresholds should follow an exponential rule, as shown by our models. Although thinning is already commonly applied in *P. sylvestris* stands, it still needs to be further developed and implemented for the studied *Quercus* spp. Because proactive approaches are more likely to avoid or reduce damage than reactive ones as they enhance the stand's resilience, it is important to adapt management in time to prevent possible forest decline and to ensure long-term conservation of high-density Mediterranean woodlands.

2.5 Acknowledgements

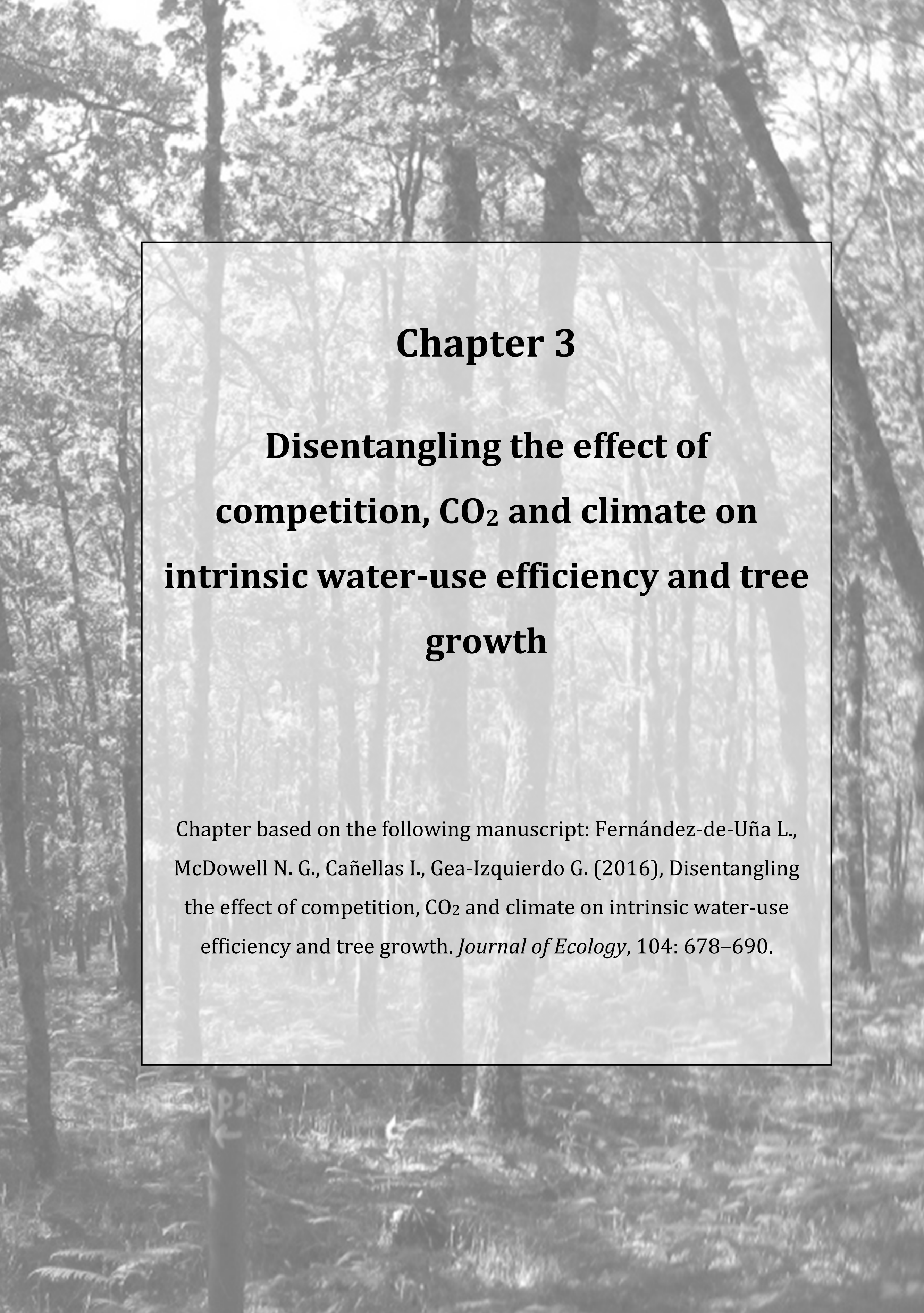
The authors thank AEMET and University of Cantabria for the data provided for this work (Spain02 dataset, http://www.meteo.unican.es/datasets/spain02; and climate projections under IPCC scenarios), as well as the Peñalara Natural Park Research and Management Centre for their climate data. We would also like to thank all the technicians for their field and laboratory assistance.

2.6 Supporting information

Table S2.1. Characteristics of the complete tree ring chronologies averaged per treatment (Treat.). C: Control; L: Light thinning; M: Moderate thinning; H: Heavy thinning; RW: Ring-Width; Rbar: Interseries correlation; EPS: Expressed Population Signal; MS: Mean sensitivity, AR1: Mean autocorrelation. N trees indicates the total number of trees used to build the chronologies and N cores the number of cores used, thus, it does not include those that were discarded.

Site	Treat.	N chron.	N trees [N cores]	Mean length (years)	RW (mm)		Rbar	EPS	MS	AR1
					Mean	Std. Dev				
BP	C	4	84[163]	58	0.68	0.53	0.591	0.993	0.210	0.808
	L	5	102[198]	57	0.74	0.45	0.656	0.985	0.218	0.737
	M	6	120[240]	58	0.89	0.51	0.778	0.981	0.222	0.726
	H	6	120[240]	58	0.96	0.49	0.753	0.980	0.202	0.747
NA	C	3	60[120]	68	1.45	0.85	0.633	0.986	0.256	0.791
	L	3	58[116]	71	1.47	0.83	0.672	0.988	0.238	0.790
	M	3	60[119]	69	1.43	0.74	0.710	0.984	0.249	0.743
RA	C	2	40[79]	54	1.34	0.83	0.486	0.988	0.237	0.814
	L	2	40 [78]	54	1.30	0.74	0.527	0.987	0.227	0.811
	M	2	40[80]	54	1.35	0.67	0.584	0.984	0.225	0.752
	H	2	40[79]	61	1.52	0.77	0.588	0.984	0.224	0.753
DU	C	3	62[124]	86	1.26	0.69	0.701	0.979	0.229	0.758
	L	2	40[80]	82	1.35	0.75	0.718	0.986	0.224	0.777
	H	3	60[118]	79	1.45	0.64	0.711	0.977	0.217	0.765
NE	C	3	58[117]	72	1.49	0.78	0.639	0.983	0.194	0.811
	L	3	60[120]	80	1.78	0.74	0.606	0.971	0.175	0.807
	M	3	60[120]	80	1.86	0.77	0.655	0.978	0.181	0.803


33
2
34



Chapter 3

Disentangling the effect of competition, CO_2 and climate on intrinsic water-use efficiency and tree growth

Chapter based on the following manuscript: Fernández-de-Uña L., McDowell N. G., Cañellas I., Gea-Izquierdo G. (2016), Disentangling the effect of competition, CO_2 and climate on intrinsic water-use efficiency and tree growth. *Journal of Ecology*, 104: 678–690.

Resumen

Los escenarios de cambio climático predicen un aumento de las temperaturas en la región mediterránea, lo que podría aumentar la vulnerabilidad a la sequía de los ecosistemas forestales. Los efectos a largo plazo del clima en el rendimiento del árbol pueden ser, no obstante, modulados por factores ambientales como la competencia y el incremento en las concentraciones de CO_2 de la atmósfera. En este studio evaluamos el efecto simultáneo de la competencia, el clima y las concentraciones de CO_2 en la eficiencia intrínseca en el uso del agua (iWUE, por sus siglas en inglés) derivada de los anillos de crecimiento y los incrementos de área basimétrica (BAI, por sus siglas en inglés) de especies con diferente tolerancia a la sequía: dos frondosas mediterráneas (*Quercus faginea* Lam. y *Quercus pyrenaica* Willd.) y una conífera (*Pinus sylvestris* L.). Además, dado que la competencia puede ser gestionada para mitigar el efecto del aumento en el estrés hídrico, examinamos más a fondo la influencia de esta variable en iWUE y crecimiento usando datos compilados de la literatura, proporcionando la primera revisión sobre la respuesta de iWUE a la competencia. La competencia no tuvo un efecto significativo en iWUE en ninguna de las tres especies estudiadas mientras, como era de esperar, las tasas de crecimiento fueron significativamente más altas bajo niveles reducidos de competencia. Estos resultados fueron consistentes con la revisión bibliográfica, la cual mostró que raramente ocurren cambios en iWUE en respuesta a cambios en competencia, apoyando la hipótesis de que el intercambio gaseoso a nivel de hoja tiende a ser un rasgo homeostático. A largo plazo, las tres especies exhibieron una tendencia ascendente significativa en iWUE debida al efecto combinado de las concentraciones de CO_2 ascendentes, el clima y la edad. El crecimiento, sin embargo, se vio mayoritariamente afectado por la competencia y el clima y en la mayor parte de los casos no aumentó como resultado del incremento en iWUE. Por lo tanto, independientemente de su

respuesta funcional a la sequía, los árboles responden a una reducción en competencia principalemente mediante cambios estructurales como el aumento en crecimiento radial en vez de mediante ajustes en el intercambio gaseoso a nivel de hoja. El CO_2 y el clima son, por tanto, los principales causantes de la variabilidad en iWUE, en vez de la competencia. En consecuencia, si la sequía inducida por el aumento de temperaturas llega a ser limitante, reducir la competencia por los recursos puede no compensar el efecto negativo del aumento en estrés hídrico en la fisiología del árbol y podría ocurrir un declive en el crecimiento sin un efecto de fertilización por CO_2.

Abstract

Climate change scenarios forecast rising temperatures for the Mediterranean region, which could enhance vulnerability to drought stress in forest ecosystems. The long-term effects of climate forcing on tree performance can be, however, modulated by other environmental factors, such as competition and rising atmospheric CO_2 concentrations. We assessed the concomitant effect of competition, climate and CO_2 concentrations on the tree-ring $\delta^{13}C$-derived intrinsic water-use efficiency (iWUE) and basal area increments (BAI) of species with different drought tolerance: two Mediterranean deciduous species (*Quercus faginea* Lam. and *Quercus pyrenaica* Willd.) and one conifer (*Pinus sylvestris* L.). Additionally, given that competition may be managed to mitigate the effect of increasing drought stress, we further examined the influence of this variable on iWUE and growth using data compiled from the literature, providing the first review on the response of iWUE to competition. Competition had no significant effect on iWUE in any of the three species studied, whereas, as expected, growth rates were significantly higher under low competition levels. This was consistent with the literature review, which showed that shifts in iWUE with competition changes are rare; supporting the hypothesis that leaf-level gas exchange tends to be a homeostatic trait. In the long term, the three species exhibited a significant increasing trend in iWUE due to the combined effect of increased CO_2 concentration, climate and age. Growth, however, was mostly affected by competition and climate and in most cases was not enhanced as a result of the increase in iWUE. Therefore, regardless of their functional response to drought, trees respond to reduced competition through structural shifts such as increased radial growth rather than leaf-level gas exchange adjustments. CO_2 and climate are, therefore, the main drivers of iWUE variability, rather than competition. Thus, if temperature-induced drought becomes limiting, reducing competition for resources may not offset the detrimental effect of

increasing drought stress on tree physiology and growth decline may occur without a CO_2 fertilization effect.

3.1 Introduction

Global environmental change involves changes in the physical and biogeochemical environment, either caused by natural processes or influenced by human activities such as fossil fuel consumption or land-use changes. One of the most widely acknowledged environmental challenges is climate change, caused by increasing atmospheric concentrations of greenhouse gases such as carbon dioxide (CO_2) (IPCC, 2013). In the Mediterranean region, the predicted increase in temperatures without a concurrent increase in precipitation suggests evapotranspiration rates will rise and, consequently, drought episodes are to become more frequent and severe, increasing competition for water in forest ecosystems (Bréda *et al.*, 2006; Giorgi & Lionello, 2008; IPCC, 2013; Williams *et al.*, 2013). Drought causes a decrease in soil water potential, which, among other physiological mechanisms, reduces trees' photosynthetic carbon uptake and, thus, growth, as a result of stomatal closure to prevent hydraulic failure (McDowell *et al.*, 2008). Subsequently, trees' defensive capacity against stressors is reduced, increasing their vulnerability to further drought stress, frost damage or pathogen and pest attacks (Bréda *et al.*, 2006; McDowell *et al.*, 2008, 2011; Eilmann *et al.*, 2010). Consequently, prolonged and severe drought could ultimately cause the death of the tree by carbon starvation, hydraulic failure or both (Bréda *et al.*, 2006; Breshears *et al.*, 2009; McDowell *et al.*, 2011).

In contrast, CO_2 availability positively influences photosynthetic rates and, therefore, increasing atmospheric CO_2 concentrations have caused a wide-spread enhancement in water-use efficiency (WUE) (Saurer *et al.*, 2004; Peñuelas *et al.*, 2011; Franks *et al.*, 2013; Voltas *et al.*, 2013). Despite its expected positive impact on tree growth as a result of increased carbon uptake per unit of water loss (Huang *et al.*, 2007; Franks *et al.*, 2013), enhanced WUE has, however, seldom translated into an increase in growth rates (Saurer *et al.*, 2004; Peñuelas *et al.*, 2011; Gómez-Guerrero *et al.*, 2013;

Voltas *et al.*, 2013; Lévesque *et al.*, 2014). Increased WUE as a result of rising CO_2 concentrations could rather improve trees' ability to withstand dry conditions, unless drought induces stomatal closure, cancelling out the CO_2 fertilization benefit (Franks *et al.*, 2013).

Following land-use changes such as fire exclusion, afforestation or the abandonment of their traditional use, Mediterranean woodlands have reached high stand densities during the last century (Vilà-Cabrera *et al.*, 2011; Gea-Izquierdo & Cañellas, 2014). Low-density stands have higher soil water availability as a result of lower canopy precipitation interception and stand transpiration (Aussenac & Granier, 1988; Bréda *et al.*, 1995), as well as reduced competition for other resources such as light and nutrients (Warren *et al.*, 2001). Consequently, trees under low competition often present less negative water potentials and higher soil-to-leaf hydraulic conductance, stomatal conductance and photosynthetic rates than trees subject to high competitive stress (Bréda *et al.*, 1995; Warren *et al.*, 2001; McDowell *et al.*, 2003, 2006; Moreno-Gutiérrez *et al.*, 2011, 2012). This, together with longer growing seasons observed under reduced competition conditions (Aussenac & Granier, 1988; Bréda *et al.*, 1995), translates into higher growth rates (Piutti & Cescatti, 1997; McDowell *et al.*, 2003, 2006; Martín-Benito *et al.*, 2010). Therefore, competition alters tree physiological responses to climate, and trees under low competition may be able to better withstand drought than those at high competition levels (Piutti & Cescatti, 1997; Martín-Benito *et al.*, 2010; Sohn *et al.*, 2013, 2014).

The different synergistic and opposing effects that environmental variables exert on plant performance make predictions of how trees will respond to changing environmental conditions difficult. Tree rings are a valuable tool to study the long-term effect of environmental variables on growth, and their carbon stable isotope ratio ($\delta^{13}C$) can provide an insight in the tree's intrinsic water-use efficiency (iWUE) during the period the ring was formed (Farquhar *et al.*, 1989). Several studies have used these data to analyse the effect of climate and competition (Brooks & Mitchell, 2011; Moreno-Gutiérrez *et al.*, 2012) or climate and CO_2 concentrations (Andreu-Hayles *et al.*, 2011; Lévesque *et al.*, 2014) on tree iWUE and growth, whereas fewer have aimed to disentangle the effects of climate, competition and CO_2 concentrations together (Linares *et al.*, 2009b; Martín-Benito *et al.*, 2010). Moreover, tree factors such as age can interact with how these environmental variables affect tree physiology (Voelker *et al.*, 2006; Resco *et al.*, 2011). Most research on the relationship between environmental factors and tree-ring-derived iWUE and growth has focused on conifer species (Warren *et al.*, 2001; Andreu-Hayles *et al.*, 2011; Lévesque *et al.*, 2014), whereas deciduous species have often been neglected (Di Matteo *et al.*, 2010; Granda *et al.*, 2014). Since competition dynamics can be managed to minimize the potentially negative effect of climate change on tree physiology, we firstly aimed to analyse the effect of competition on the intrinsic water-use efficiency and growth of two deciduous species and one conifer commonly found in Mediterranean mountains: *Quercus faginea* Lam., *Quercus pyrenaica* Willd. and *Pinus sylvestris* L. Additionally, in order to deepen the understanding of how this variable affects tree performance, we compared our results to compiled data from a systematic literature review on the response of tree-ring-derived iWUE and growth to competition, providing the first review on the matter. Secondly, we assessed the differential effect that competition, atmospheric CO_2 concentrations, climate and age exert on the temporal trends found in the growth and iWUE of the three studied species. We hypothesise that the physiological response of each species to the assessed environmental variables will depend on species-specific functional traits related to drought tolerance.

3.2 Materials and Methods

Study sites

Different even-aged, monospecific stands within a network of thinning experimental sites in Spain were used for the study (Table 3.1). At each site, plots of 770-1600 m^2 size were marked and either thinned or left unaltered as controls (Figure 3.1). Thinning from below (i.e. the smallest trees in the stand were removed) was applied the year of site establishment and periodically thereafter to maintain low competition levels in order to transform *Quercus* spp. stands into high forests and increase tree productivity in *P. sylvestris* stands. Diameter at breast height (DBH) of all trees was measured every 4-10 years after experimental site establishment. Additionally, tree height, crown diameter (North-South and East-West diameters) and crown base height of those trees selected for isotope analysis were measured in early 2014. Crown diameters were used to calculate crown projected area.

Table 3.1. Site characteristics for each study site. BP: Barriopedro; NA: Navasfrías; RA: Rascafría; DU: Duruelo; NE: Neila; Coord.: Coordinates; N plots: Number of plots within each site; Est. year: Experimental site establishment year, i.e. year in which first thinning was applied; Age: Cambial age in 2014; Elev.: Elevation; Exp.: Exposure; Prec.: Mean annual precipitation; Temp.: Mean annual temperature; BA: mean plot basal area in control plots since establishment; BA reduct.: plot basal area reduction in thinned plots compared to control plots.

Site	Species	Coord. (º)	N plots	Est. year	Age	Elev. (masl)	Exp.	Slope (%)	Prec. (mm)	Temp. (ºC)	BA (m^2/ha)	BA reduct.
BP	*Q. faginea*	40.80 N, 2.78 W	10	1980	59	860	W	0-35	505	10.5	11.6	65%
NA	*Q. pyrenaica*	40.36 N, 6.79 W	6	2004	64	895	W	0-10	927	10.7	24.6	33%
RA	*Q. pyrenaica*	40.92 N, 3.89 W	4	1994	53	1400	SE	30	790	9.4	38.1	53%
DU	*P. sylvestris*	41.97N, 2.95W	6	1968	78	1200	NW	15	860	7.3	47.0	43%
NE	*P. sylvestris*	42.06N, 3.02W	6	1972	71	1340	NE	15-20	860	6.6	60.6	24%

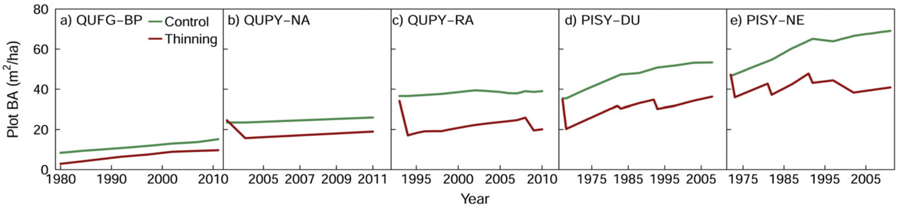


Figure 3.1. Plot basal area trends sce first inventory for control and thinned plots at each site. QUFG-BP: *Quercus faginea* (site BP); QUPY-NA: *Q. pyrenaica* (site NA); QUPY-RA: *Q. pyrenaica* (site RA); PISY-DU: *P. sylvestris* (site DU); PISY-NE: *P. sylvestris* (site NE). Note the different time scales for each site.

Growth data

At each site, two cores were sampled between 2010 and 2012 from 20-23 trees per plot. Selected trees had similar diameters in the first inventory to ensure the initial tree sizes were comparable between treatments. A total of 646 trees from 32 plots were finally used for the study. Ring-width series were measured with a LINTAB measuring table (Rinntech, Heidelberg, Germany) with a precision of 0.01 mm, visually cross-dated and statistically verified with the softwares TSAP (Time Series Analysis and Presentation; Rinntech, 2003) and COFECHA (Holmes, 1983). Ring-width data were converted to basal area increments (BAI; cm^2 year^{-1}) and individual BAI series were averaged per tree. In order to calculate BAI for those cores that did not reach the pith of the tree, we estimated the missing radius based on tree diameter, growth in the earliest observed years and last ring curvature. BAI series were used because, unlike ring width series and with the exception of the first years of increasing juvenile growth rates, they minimize the effect of tree size and age on annual growth trends while retaining the high and low frequency signals contained in the tree ring series (Biondi & Qeadan, 2008).

Tree-ring isotope data

We assessed intrinsic water-use efficiency (iWUE), which represents the ratio between photosynthetic assimilation (A) and stomatal conductance (g_s), through the carbon isotope ratio ($\delta^{13}C$) of tree rings. Two plots per treatment were randomly selected at each site, except for *Quercus faginea*, which was only represented by one site (BP) and, therefore, we selected four plots per treatment at that site. At each selected plot, an additional core was extracted from five of the trees previously cored for growth analysis to run the isotope analysis. In order to study the short- and long-term effects of thinning on tree's response to drought, we selected three dry years (i.e. more than one standard deviation below each site's average) based on hydrological year precipitation data and site establishment date, one before thinning was applied and two after thinning. Site NA was an exception, because plots were established in 2004 and, thus, only one dry year after thinning could be selected. We also sampled the two subsequent years to each of those dry years, in such way that we sampled three periods (two for site NA) and nine years per tree (six for site NA), with a total of 121 trees and 1040 rings being processed.

Cores were air-dried and tree rings were dated, separated with a scalpel and ground with a mixer mill MM 300 (Retsch, Haan, Germany). We used whole-wood tissue from annual rings rather than latewood or cellulose because (1) selecting dry years involved small sample size, difficulty to separate earlywood and latewood and, in some cases, lack of latewood in *Quercus* spp. trees, particularly under high competition levels; and (2) different studies have reported similar $\delta^{13}C$ values in cellulose and whole-wood (e.g., McCarroll & Loader, 2004; Sohn *et al.*, 2013). Tree-ring samples of 0.1-0.4 mg were analysed for $\delta^{13}C$ on a MAT 253 isotope ratio mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) coupled to a Costech elemental analyser (Costech Analytical Technologies Inc., Valencia, CA, USA) at Los Alamos National Laboratory. $\delta^{13}C$ results were calibrated using International Atomic Energy Agency (IAEA) standards C3 (cellulose, $\delta^{13}C = -24.91‰$), C6 (sucrose, $\delta^{13}C = -10.8‰$) and C8 (oxalic acid, $\delta^{13}C = -18.3‰$). An internal standard (NIST 1547 Peach Leaves) was used to assess analytical drift and precision. The average analytical precision was 0.06‰.

In order to remove the effect of the variability in source air $\delta^{13}C$ ($\delta^{13}C_a$) during the growing season (April through September) on $\delta^{13}C$, isotopic data were transformed to discrimination (Δ; Farquhar *et al.*, 1982):

$$\Delta \approx \frac{\delta^{13}C_a - \delta^{13}C}{1+\delta^{13}C} \qquad [3.1]$$

$\delta^{13}C_a$ data were obtained from NOAA Earth System Research Laboratory (NOAA- ESRL, http://www.esrl.noaa.gov/gmd/dv/data/). Δ can also be estimated as a function of the growing season CO_2 concentration in air (c_a), the intercellular CO_2 concentration in the plant (c_i), the CO_2 fractionation due to diffusion in air (a; 4.4 ‰) and the fractionation due to carboxylation by Rubisco and PEP carboxylase (b; 27 ‰) (Farquhar *et al.*, 1982):

$$\Delta = a + (b-a)\frac{c_i}{c_a} \qquad [3.2]$$

where c_a data were also obtained from NOAA- ESRL. The use of Δ inherently includes the impact of mesophyll CO_2 fractionation during mesophyll conductance (Seibt *et al.*, 2008; Bickford *et al.*, 2010). Possible changes in leaf size and structure and, therefore, in mesophyll conductance and their effect on c_i could not be measured and, thus, were assumed to be constant along the study period. c_i was derived from equation 3.2. In order to be able to compare between sites at different elevations, we transformed CO_2 concentrations into CO_2 partial pressures to calculate the differences between the air CO_2 partial pressure (p_a) and the plant CO_2 partial pressure (p_i) (p_a-p_i). Partial pressures for each year analysed were calculated as follows (Hultine & Marshall, 2000; McDowell *et al.*, 2010):

$$p = c\frac{101.325}{e^{(z/29.3)/T_K}} \qquad [3.3]$$

where p is either p_a or p_i, c is CO_2 concentration (either c_a or c_i), z is the site altitude and T_K the mean temperature (in Kelvin) of the growing season of the year the ring was formed. p_a-p_i was used as a proxy of intrinsic water-use efficiency, as it constitutes the numerator in δ^{13}C-derived iWUE calculations (Farquhar *et al.*, 1989).

Competition data

Harvest and diameter data from the periodic inventories were used to estimate annual plot basal area series (in m^2/ha), which were used as long-term proxies of competition since they reflect well the degree of crowding in the stand and, thus, tree-to-tree competition. In order to calculate plot basal area annual values for the years between inventories, competition changes were assumed to occur gradually. Thus, annual increments were calculated as the change in competition between two inventories divided by the number of years between them. The corresponding annual increment was added to the previous-year plot basal area value. When thinning was applied, harvested trees were added to the estimated mortality of that year. Competition data for the years before thinning was applied were estimated based on the changes inventoried at the control plots in the first 10 years after plot establishment.

Climate data

Precipitation and mean, maximum and minimum temperature data were obtained from the Spanish Meteorological Agency (AEMET), the meteorological station at site BP (active from 1980 to 1991), the Peñalara Natural Park Research and Management Centre, Herrera *et al.* (2012) and the University of East Anglia Climate Research Unit (CRU) time-series dataset CRU TS 3.10 (Harris *et al.*, 2014; accessed through the KNMI explorer http://climexp.knmi.nl/). We considered the closest meteorological station to each study site, located in all cases within a 19-km radius, to be the reference station for that site. In order to estimate missing data, we used linear regressions between data from each reference station and data from the closest stations, Herrera *et al.* (2012) or CRU TS 3.10 (Harris *et al.*, 2014), depending on data availability. Site NE did not have a nearby station at the same altitude and, therefore, we used site DU's climatic data to estimate that site's data due to their proximity (*c.* 13 km). Precipitation was considered to be the same at both sites, whereas a temperature lapse rate of 0.5 ºC/100 m (Kirchner *et al.*, 2013) was used to estimate temperatures. Months were pooled in the following periods: Annual (January - December), Hydrological year (October of previous year - September of current year), Growing season (April - September), Winter (December of previous year - February of current year), Spring (March - May), Summer (June - August) and Autumn (September - November). All the study sites suffered summer drought to some extent and mean annual temperatures increased since the 1970s at an average rate of 0.014-0.058 ºC per year (0.6-2.4 ºC net increase).

Literature review of the effect of competition on Δ and growth

In order to further assess the effect of competition, we compared Δ and BAI data from a literature data review that included ten studies from different climatic regions, corresponding to eight different conifers and only one deciduous species. To our knowledge, these are all of the existing studies that directly analyse the effect of competition on both carbon discrimination and growth. Δ data were used instead of iWUE because they were the most commonly reported data in all the studies assessed. If only $\delta^{13}C$ data were reported we transformed them to Δ. When several thinning levels were assessed on the same study, we only compared the lowest and highest competition levels reported to harmonize all studies.

Data analysis

We first tested differences in tree growth, iWUE, height and crown size between control and thinned plots for each site. Second, we assessed the presence of temporal trends in iWUE and BAI. Finally, in order to assess which environmental variables drove those temporal trends, data were pooled by species to analyse the relationship between the response variables (iWUE and BAI) and environmental variables (plot basal area, CO_2 concentration, temperature and precipitation) and age. For all the tests performed, data normality was initially tested using normal probability plots and frequency histograms and further checked through the residuals of the fitted models. The significance level used in all tests was 0.05.

Because data were normally distributed and measures were repeated within individuals, differences between treatments in iWUE were assessed using linear mixed models (LMMs) with a random intercept to account for correlation within trees and a first-order autocorrelation structure in the error matrix to account for temporal correlation (Zuur *et al.*, 2009). Since growth data were not normally distributed, differences between treatments were assessed using non-parametric Kruskal-Wallis one-way analysis of variance, when only one measure per tree was taken (i.e. height and crown area), and generalized mixed models with a Gamma distribution, an inverse link function, a random intercept associated with tree and a first-order autocorrelation structure (GLMMs; Zuur *et al.*, 2009), when we analysed several repeated measures within individuals (i.e. BAI). The fixed effect variables included in both the iWUE LMMs and BAI GLMMs were Treatment, to assess differences between control and thinned plots, and the interaction 'Treatment × Year', to assess whether or not differences existed in specific years. Additionally, average values per treatment for our sites were pooled with the data obtained from the literature. LMMs and GLMMs with a random intercept associated with site were used, respectively, to analyse Δ and BAI data.

Temporal trends in iWUE and BAI were also assessed through LMMs and GLMMs. In this case, an identity link function was applied in BAI GLMMs. BAI trends were assessed since 1970 to avoid juvenile growth. Time period (i.e. three-year periods used in the isotopic analysis) and Year were used as the fixed factors to assess temporal trends in iWUE LMMs and BAI GLMMs, respectively. A random intercept to account for correlation within trees and a first-order autocorrelation structure were also included in the models.

Finally, we tested for each species the effect of the environmental variables (plot basal area, CO_2 concentration, temperature and precipitation) and age on iWUE and BAI by fitting models containing different combinations of these variables. LMMs with a random intersect associated with tree and a first order autocorrelation structure were used to model iWUE and growth as a function of environmental variables. Since BAI data were not normal, a square root was applied to normalize those data. The Akaike Information Criterion (AIC) was used to assess the information contained in each LMM fitted. For a given response variable, lower values of AIC indicate a better fit. A model was considered superior when the difference in AIC (ΔAIC) between models was equal to or greater than 2 (Burnham & Anderson, 2002). Temperature and precipitation variables were preselected through Kendall rank correlation coefficients. Additionally, we calculated the variance inflation factor (VIF) of the model containing all the response variables (Plot basal area + CO_2 concentrations + Temperature + Precipitation + Age) to assess collinearity. VIF values larger than 10 indicate high collinearity among variables (Dormann *et al.*, 2013). Since data presented high collinearity between CO_2 and Age (Table S3.1 in Supporting Information), sequential regression was used to create a new variable

(ResAge) which resulted from the residuals of the lineal model of Age as a function of CO_2 (Dormann *et al.*, 2013). CO_2 was used as the main variable due to the expected changes in iWUE with increasing height and, therefore, age, were not strong enough to have caused the changes in iWUE alone (Table S3.2 in Supporting Information).

All analyses were carried out with R version 3.1.1 (R Core Team, 2014). LMMs were fit with the package “lme4” (Bates *et al.*, 2014) and GLMMs were fit with the package “MASS” (Venables & Ripley, 2002). Multiple mean comparisons (Tukey contrasts) for the interaction ‘Treatment × Year’ were performed with the package “multcomp” (Hothorn *et al.*, 2008).

3.3 Results

Effect of competition on tree growth and intrinsic water-use efficiency

BAI increased immediately after thinning for all species (Figure 3.2). Growth rates reached a maximum 4-10 years after thinning and remained significantly higher than those of control plots thereafter, even during dry years (Table 3.2 and Figure 3.2), since plot basal area remained lower in thinned plots until present. Although growth differences existed before thinning in sites BP and NE, they became much stronger after thinning (Table 3.2 and Figure 3.2). BAI was inversely correlated with plot basal area in all species (Figure 3.3a and Figure S3.1 in Supporting Information). No significant differences in tree height were, however, found between treatments, except for sites BP and NE where trees under low competition were significantly taller (Table 3.2). Conversely, for all species and sites, except for site NA, which was the last site established, trees under low competition had significantly larger crown area than those in control plots (Table 3.2).

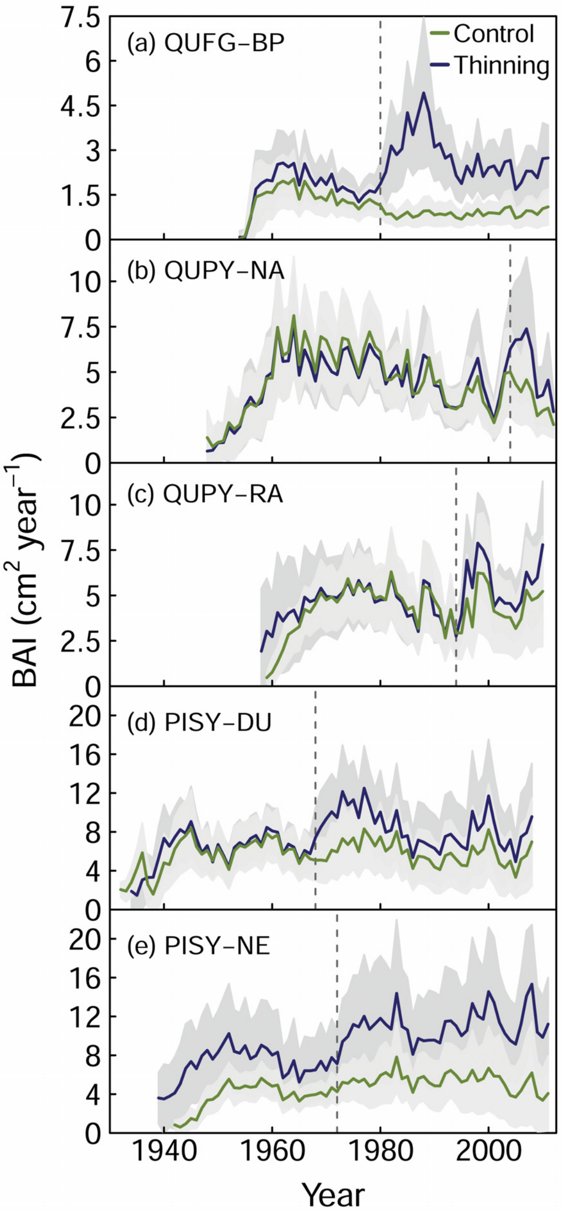


Figure 3.2. Control and thinning basal area increment (BAI) chronologies for the different study sites. QUFG-BP: *Quercus faginea* (site BP); QUPY-NA: *Q. pyrenaica* (site NA); QUPY-RA: *Q. pyrenaica* (site RA); PISY-DU: *P. sylvestris* (site DU); PISY-NE: *P. sylvestris* (site NE). Grey dashed vertical lines indicate the time when plots were established. Shades represent the confidence intervals, calculated as Mean ± Standard deviation, for control (light grey) and thinning (dark grey) chronologies.

Table 3.2. Mean (standard deviation) values of the different growth variables measured for control (C) and thinned (T) plots for each site. BAI BT and BAI AT refer to mean basal area increments before (BT) and after (AT) the first thinning was applied. Height and crown measurements were performed in 2014. Significant differences between treatments per site at the 0.05 level are marked in bold.

	BP		**NA**		**RA**		**DU**		**NE**	
Variable	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**
BAI BT	**1.46**	**1.90**	4.76	4.63	3.61	3.75	4.84	5.76	**4.15**	**7.41**
(cm^2 year^{-1})	**(0.79)**	**(0.91)**	(2.57)	(2.41)	(1.97)	(2.23)	(2.80)	(2.69)	**(1.72)**	**(4.30)**
BAI AT	**0.88**	**2.75**	**3.60**	**5.32**	**3.09**	**4.59**	**5.01**	**7.64**	**5.35**	**11.10**
(cm^2 year^{-1})	**(0.43)**	**(1.41)**	**(2.02)**	**(3.20)**	**(2.53)**	**(2.54)**	**(3.35)**	**(4.45)**	**(3.28)**	**(5.65)**
Height	**6.2**	**7.0**	16.4	15.5	13.1	12.5	18.0	17.9	**23.9**	**25.9**
(m)	**(1.1)**	**(0.9)**	(1.1)	(1.6)	(1.9)	(2.0)	(1.3)	(1.2)	**(1.9)**	**(1.1)**
Crown area	**3.5**	**7.4**	13.2	15.4	**7.2**	**11.2**	**14.2**	**26.2**	**5.1**	**22.8**
(m^2)	**(2.1)**	**(3.2)**	(3.3)	(8.5)	**(2.6)**	**(3.9)**	**(5.4)**	**(10.1)**	**(2.7)**	**(9.7)**

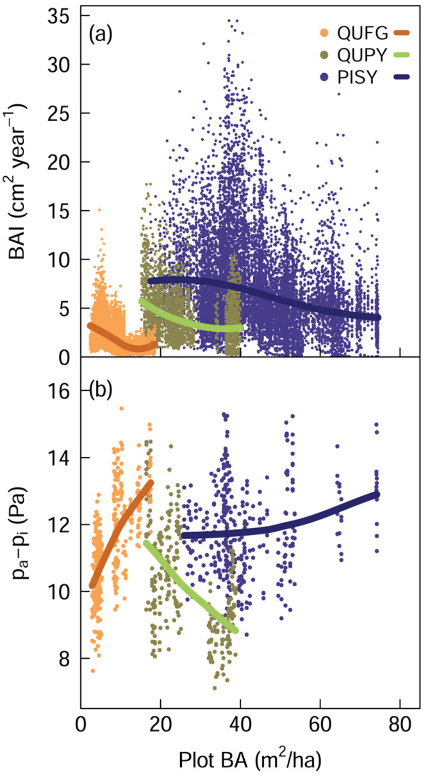


Figure 3.3. Relationship between plot basal area (BA) and basal area increments (BAI) (a) and p_a-p_i (b) for the different species: *Quercus faginea* (QUFG), *Quercus pyrenaica* (QUPY) and *Pinus sylvestris* (PISY). Locally-weighted polynomial regression (LOESS smoother) lines show average trends per species.

No significant differences between treatments were found in p_a-p_i and, thus, in iWUE, not even during the driest years (Figure 3.4). Nevertheless, when all data were pooled to analyse the direct relationship between iWUE and competition, p_a-p_i showed an increasing trend with increasing plot basal area for *Q. faginea*, whereas it decreased for *Q. pyrenaica* and remained constant for *P. sylvestris* (Figure 3.3b).

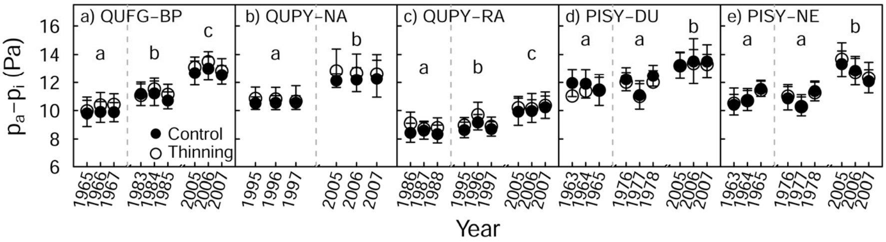


Fig. 3.4. p_a-p_i by treatment at each sampled date and site. QUFG-BP: *Quercus faginea* (site BP); QUPY-NA: *Q. pyrenaica* (site NA); QUPY-RA: *Q. pyrenaica* (site RA); PISY-DU: *P. sylvestris* (site DU); PISY-NE: *P. sylvestris* (site NE). The first year of each three-year period is the selected drought year. Grey dashed vertical lines separate pre- and post-treatment periods. There were no significant differences due to treatment. Significant differences over time among the time periods assessed are indicated with letters.

A significant effect of competition on growth was found in all the studies assessed in the literature review (Table 3.3 and Figure 3.5). Conversely, 60% of the studies detected no differences in Δ between competition regimes, regardless of the species identity, time period, thinning level or climatic conditions assessed (Table 3.3). Despite the results of individual studies, when all data were pooled together there was no significant effect of competition on Δ (d.f.: 463), whereas reduced competition levels consistently enhanced BAI (*P*<0.001, d.f.: 591; Figure 3.5).

Table 3.3. Comparison of the characteristics of different studies analysing the effect of competition on tree-ring δ^{13}C content and growth. Differences in discrimination ($\Delta(\Delta)$) and basal area increments (ΔBAI) between low-competition and high-competition plots were calculated as Thinned - Control. Significant differences marked in bold. EW: earlywood; LW: latewood; TR: tree ring.

Reference	**Species**	**Site**	**High competition**	**Low competition**	**Climate**	**Component**	**$\Delta(\Delta)$ (‰)**	**ΔBAI (cm^2/year)**
Brooks *et al.* (2011)	*Pseudotsuga menziesii* (Mirb.) Franco	Vancouver Island, BC, Canada	4500 trees/ha	66 % of initial BA	1160 mm, 8.9 °C	EW and LW cellulose	0.18	**6**
DiMatteo *et al.* (2010)	*Quercus cerris* L.	Southern Tuscany, Italy	3181 trees/ha	682 trees/ha	906 mm, 14.4 °C	TR whole-wood	**0.94**	**1.27**/0.1[*]
Martín-Benito *et al.* (2010)	*Pinus nigra* Arn.	Central Spain	1600 trees/ha	1020 trees/ha	663 mm, 11.3 °C	TR cellulose	-0.95	**2.54**
McDowell *et al.* (2003)	*Pinus ponderosa* Douglas ex C. Lawson	Black Butte, OR, USA	31.47-60.00 m^2/ha	8.45-12.23 m^2/ha	360 mm	TR cellulose	**0.96**	**19.83**
McDowell *et al.* (2006)	*Pinus ponderosa* var. *scopulorum* (Engelm.) E. Murray	Coconino National Forest, AZ, USA	45 m^2/ha	7 m^2/ha	564 mm, 6.0 °C	TR whole-wood	**0.19**/ -0.37[†]	**26.56**
Moreno-Gutiérrez *et al.* (2012)	*Pinus halepensis* Mill.	Murcia, Spain	770-1150 trees/ha	< 20 trees/ha	288 mm, 19 °C	LW cellulose	0.05	**4.53**
Powers *et al.* (2010)	*Pinus resinosa* Ait.	Chippewa National Forest, MN, USA	35-45 m^2/ha	16 m^2/ha	—	TR whole-wood	-0.27	**20-30%**
Sohn *et al.* (2012)	*Picea abies* (L.) H.Karst.	Göggingen, Germany	400 trees/ha	200 trees/ha	780 mm, 7.5 °C	EW and LW whole-wood	-0.53	**15.32**
Sohn *et al.* (2013)	*Picea abies* (L.) H.Karst.	Göggingen, Germany	1100 trees/ha	200 trees/ha	780 mm, 7.5°C	EW and LW whole-wood	-0.27	**24.6**
		Freising, Germany	1500 trees/ha	200 trees/ha	790 mm , 7.9°C			
Warren *et al.* (2001)	*Pinus pinaster* Ait.	South-western Australia	750 trees/ha	250 trees/ha	978 mm	Seasonal cellulose	**0.75**[‡]	**24**
	Pinus radiata D. Don							**21**

[*] Differences in BAI after coppicing and after thinning; [†] Differences in Δ for the period 5-12 years after thinning and 12 years after thinning and onwards; [‡] Treatment differences in δ^{13}C

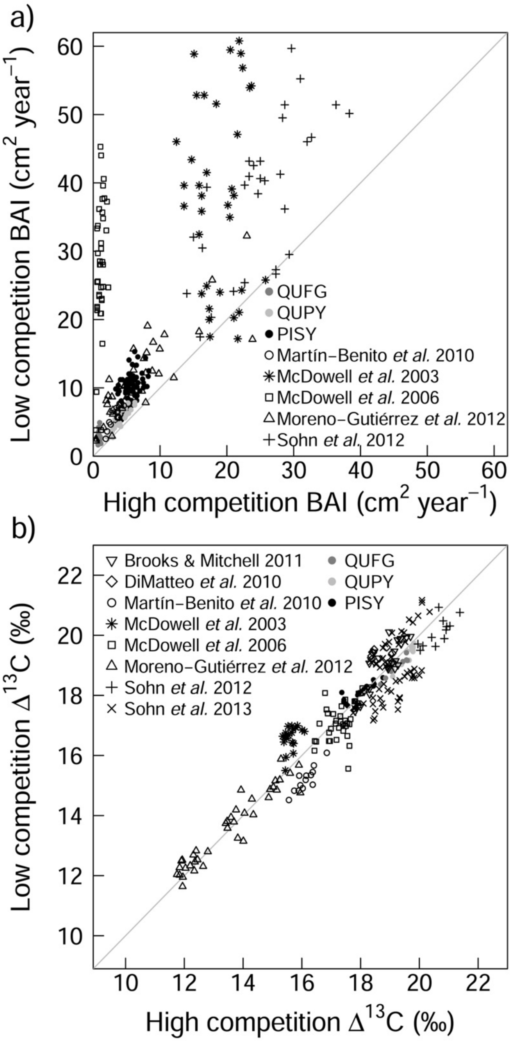


Figure 3.5. High competition (control plots) vs. low competition (thinned plots) BAI (a) and Δ (b) for our (filled dots) and other studies.

Effect of environmental variables on tree growth and intrinsic water-use efficiency

A significant increasing time trend in p_a-p_i was observed in both treatments and all species and sites, particularly notable for the 2005-2007 period (compared to the first period; Figure 3.4). Conversely, growth trends since the 1970s differed between treatments. Control plots had decreasing growth trends ($P<0.001$; range of degrees of freedom – d.f.: 1609-3443) in all sites except in NE, which had a non-significant increasing trend (d.f.: 2377; Figure 3.2). Growth trends in thinned plots in the last 40 years were, however, highly modulated by thinning events (Figures 3.1 and 3.2). These short-term growth changes were contained in a positive multi-decadal growth trend in sites BP, NE and RA ($P<0.004$, d.f. ranging between 1596 and 4916). In sites NA and DU, however, BAI in thinned plots followed a negative trend (non-significant for site DU, d.f. ranging between 2332 and 2509) (Figure 3.2).

BAI and p_a-p_i response to environmental variables differed among species (Tables 3.4 and 3.5 and Figures S3.1 and S3.2 in Supporting Information). According to the best models fitted for each species (Table 3.4 and Figure S3.3 in Supporting information), *Q. faginea* and *P. sylvestris* growth responded to all the variables assessed (competition, CO_2, precipitation, temperature and age), whereas *Q. pyrenaica* growth was more intensely influenced by competition and climate. Conversely, *Quercus pyrenaica* p_a-p_i responded more strongly to CO_2 and age than to climate or competition, whereas *P. sylvestris* p_a-p_i was also influenced by temperature and *Q. faginea pa-pi* was more influenced by CO_2 and precipitation (Table 3.5 and Figure S3.4 in Supporting information).

Additionally, to better separate the effect of CO_2 from that of climate on the temporal trends found in intrinsic water-use efficiency, changes in p_i, p_i/p_a ratios and p_a-p_i with increasing CO_2 concentrations were interpreted following Saurer *et al.* (2004) (Figure 3.6). Constant p_i and, thus, decreasing p_i/p_a, with increasing p_a indicates a significant improvement in iWUE, stronger than that expected as a result of increasing p_a. Conversely, constant p_i/p_a ratios indicate a modest increase in iWUE. Finally, constant p_a-p_i indicates that p_i increased as much as p_a and, therefore, no iWUE enhancement occurred (Saurer *et al.*, 2004). *Q. faginea* p_i increased with rising CO_2 concentrations, whereas p_i/p_a slightly decreased. In *Q. pyrenaica,*

p_i remained relatively constant after an initial increase, while p_i/p_a decreased. *P. sylvestris* p_i increased with rising CO_2 concentrations, whereas p_i/p_a remained constant. For all species, p_a-p_i increased with CO_2 concentrations (Figure 3.6).

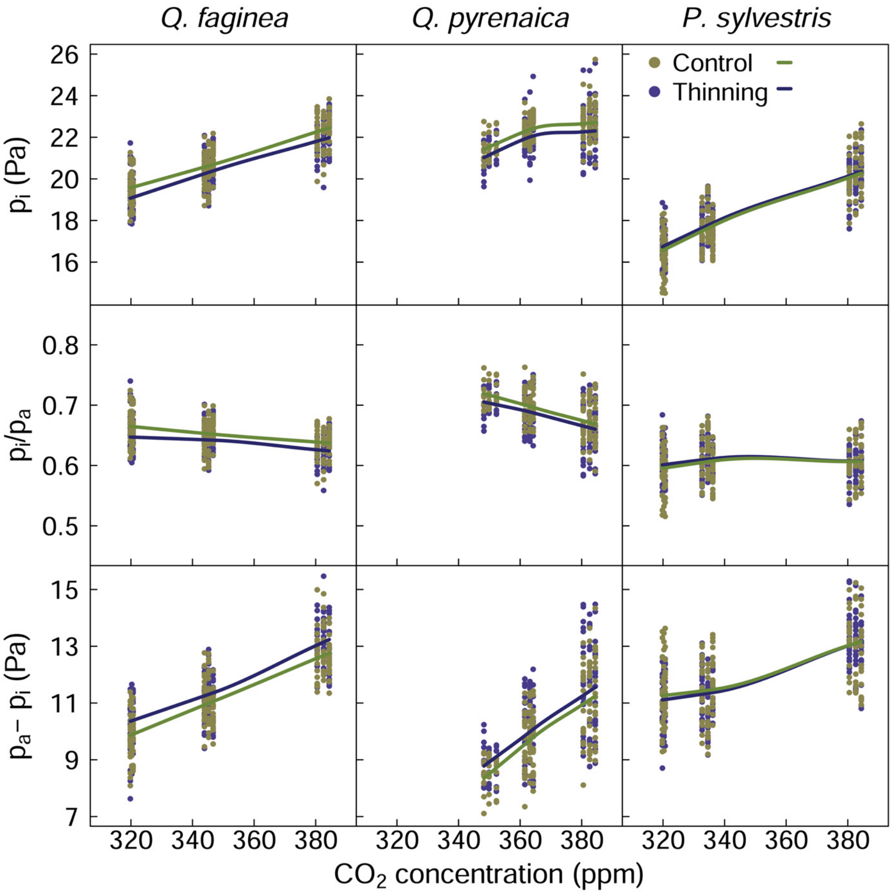


Figure 3.6. Changes in p_i, p_i/p_a and p_a-p_i with increasing atmospheric CO_2 concentrations for *Quercus faginea*, *Quercus pyrenaica* and *Pinus sylvestris*. Lines represent locally weighted polynomial regressions (LOESS smoother).

Table 3.4. Differences in the Akaike Information Criterion (ΔAIC) of the transformed-BAI linear mixed models. ΔAIC was calculated as the difference between the AIC of the model being assessed and the AIC of the model with the lowest AIC (i.e. the model with ΔAIC = 0). The best models are marked in bold. resAge: residuals of the regression of Age=f(CO_2); Comp: Competition; Prec: precipitation of the current growing season for *Q. faginea* and *P. sylvestris* and precipitation of the previous autumn in *Q. pyrenaica*; Temp: mean temperature of the previous autumn for *Q. faginea* and *P. sylvestris* and mean temperature of the previous spring in *Q. pyrenaica*.

Model fixed effects	**N par**	***Q. faginea***	***Q. pyrenaica***	***P. sylvestris***
Competition	5	363.96	398.67	1757.14
CO_2	5	388.04	407.58	1922.55
Precipitation	5	179.06	258.82	967.98
Temperature	5	259.41	33.72	1228.48
resAge	5	288.66	407.98	1873.26
Comp + Prec + Temp	7	99.21	**0**	131.89
Comp + CO_2+ Prec + Temp	8	9.85	1.55	109.25
Comp + Prec + Temp + ResAge	8	45.72	0.71	2.39
Comp + CO_2 + Prec + Temp + ResAge	9	**0**	2.62	**0**

3.4 Discussion

Competition modifies growth but not iWUE regardless of species functional characteristics

Direct gas exchange measurements have shown that both photosynthetic rates (A) and stomatal conductance (g_s) may improve under low competition levels (Sala *et al.*, 2005; McDowell *et al.*, 2006). If we consider growth as a proxy of carbon uptake (McDowell *et al.*, 2003), both our results and the literature review confirm this pattern, as for all the species analysed BAI increased as a result of reduced competition (Table 3.3 and Figure 3.5) and showed a negative relationship with competition levels (Figure 3.3). On the contrary, no significant differences between treatments were found in intrinsic water-use efficiency for any of the three species, not even during the driest years (Figure 3.4). Given this lack of statistical differences between treatments, which consistently had different plot basal area levels, and that none of the best p_a-p_i models included competition as a covariate, the observed p_a-p_i trends with plot basal area (Figure 3.3) were likely caused by collinearity with other factors that also changed over time, such as CO_2 concentrations, temperatures or tree age, and not by competition. Confirming our results, most studies on the effect of competition on iWUE have not found differences between competition levels (Table 3.3). Conversely, McDowell *et al.* (2003, 2006) and Di Matteo *et al.* (2010) found increases in Δ after thinning, whereas Warren *et al.* (2001) found a decrease. Powers *et al.* (2010) did not find significant differences between thinning treatments in $\delta^{13}C$ but observed a negative relationship with plot basal area. Nonetheless, where differences were found, these were generally short-lasting (McDowell *et al.*, 2006; Powers *et al.*, 2010; Brooks & Mitchell, 2011) or occurred in particularly dry years (McDowell *et al.*, 2006; Sohn *et al.*, 2013, 2014). Despite the different short-term outcomes of the above-mentioned studies, the common trend (Figure 3.5) indicates no general effect of competition on iWUE and an evident effect on growth, thus supporting the hypothesis that leaf-level gas exchange tends to be a homeostatic trait, in such way that there is an equilibrium between tree structural parameters, such as sapwood area and leaf area, and leaf-level physiological parameters, such as iWUE (Ehleringer, 1993; McDowell *et al.*, 2006).

Changes in iWUE depend on the balance between photosynthetic rate and stomatal conductance, which can be differentially affected by competition levels. Thinning may increase soil water availability through reduced root competition and canopy rainfall interception, thus decreasing drought stress, increasing g_s and reducing intrinsic water-use efficiency (McDowell *et al.*, 2003, 2006; Di Matteo *et al.*, 2010; Brooks & Mitchell, 2011; Sohn *et al.*, 2014). On the other hand, thinning may reduce competition for light and nutrients such as nitrogen, which may increase A and, hence, trees' iWUE

(Warren *et al.*, 2001; Linares *et al.*, 2009b; Brooks & Mitchell, 2011). Therefore, the effect of reduced competition on intrinsic water-use efficiency depends on the strength that the different factors exert on A and g_s, making iWUE change only under conditions when water, nutrient or light availability are particularly limiting and drive differential changes in A or g_s. This would explain the heterogeneity in the results from different studies with a prevalence of no competition effect.

Table 3.5. Differences in the Akaike Information Criterion (ΔAIC) of the p_a-p_i linear mixed models. ΔAIC was calculated as the difference between the AIC of the model being assessed and the AIC of the model with the lowest AIC (i.e. ΔAIC = 0). If a model had a ΔAIC<2 and fewer parameters than the best model, that model was selected instead. Selected models are marked in bold. resAge: residuals of the regression of Age=f(CO_2); Comp: Competition; Prec: precipitation of the previous year's winter for *Q. faginea*, precipitation of the previous autumn in *Q. pyrenaica* and precipitation of the current winter for *P. sylvestris*; Temp: mean temperature of the hydrological year for *Q. faginea*, mean temperature of the previous summer in *Q. pyrenaica* and mean temperature of the current summer for *P. sylvestris*.

Model fixed effects	**N par**	***Q. faginea***	***Q. pyrenaica***	***P. sylvestris***
Competition	5	100.59	78.17	232.34
CO_2	5	12.03	7.33	110.63
Precipitation	5	164.35	82.09	181.16
Temperature	5	184.82	80.04	86.50
resAge	5	201.07	72.03	243.91
CO_2 + Prec	6	**0.44**	9.20	70.78
CO_2 + resAge	6	14.00	**0.87**	112.44
Comp + CO_2 + Prec	7	1.31	9.73	72.05
Comp + CO_2 + resAge	7	15.12	1.99	113.32
CO_2 + Prec + Temp	7	0	7.50	6.70
CO_2 + Temp + resAge	7	14.51	0	**0.95**
Comp + CO_2 + Prec + Temp	8	0.72	8.18	8.43
Comp + CO_2 + Temp + resAge	8	15.67	1.40	2.93
CO_2 + Prec + Temp + resAge	8	1.97	1.03	0
Comp + CO_2 + Prec + Temp + resAge	9	2.70	2.53	1.97

Additionally, stomatal conductance partially depends on the ratio between foliage area and sapwood area (Whitehead *et al.*, 1984; McDowell *et al.*, 2006). We found significantly higher crown size and radial growth in trees in thinned stands compared to control trees, which is consistent with shifting carbon allocation to increase leaf area and sapwood area in trees under low competition (Bréda *et al.*, 1995; Misson *et al.*, 2005; McDowell *et al.*, 2006; Sohn *et al.*, 2012). This has been observed both in isohydric and anisohydric species, indicating no dependence on the functional response to drought. A strong linear relationship between foliage area and sapwood area has been widely observed (Whitehead *et al.*, 1984; Mencuccini *et al.*, 1994). Increased foliage area enhances crown-level carbon uptake. Larger sapwood area implicates enhanced conductive capacity and stem water storage capacity, which allows trees to better withstand dry periods (Pineda-García *et al.*, 2013). Several studies have also found less negative water potentials in trees under low competition regardless of the effect on iWUE (Warren *et al.*, 2001; McDowell *et al.*, 2003, 2006; Moreno-Gutiérrez *et al.*, 2012), further supporting that trees under low competition levels have higher resistance to drought (Sohn *et al.*, 2012, 2013). Moreover, other structural changes, such as root expansion, may be expected as a result of decreased competition. Therefore, our site-specific results together with the compiled analysis showing little effect of competition on Δ but a significant effect on growth (Figure 3.5) indicate that, given a long-term increase in resources such as those derived from reduced competition, trees undergo structural changes such as increased sapwood or leaf area rather than changing leaf-level efficiency, which is in agreement with the theory of homeostatic maintenance of gas exchange (Ehleringer, 1993; Marshall & Monserud, 1996; McDowell *et al.*, 2006; Limousin *et al.*, 2009). Moreover, as root and foliar area expand, water stress per unit leaf area may eventually increase in thinned stands, in such way that gas exchange is not significantly different from control stands

(Aussenac & Granier, 1988), further explaining the lack of differences observed in Δ between treatments (McDowell *et al.*, 2006; Sohn *et al.*, 2012).

iWUE and growth response to CO_2 is modulated by climate

An increasing temporal trend in iWUE has been observed globally, but, similar to our multiple-site study, this has rarely resulted in a growth increase (Saurer *et al.*, 2004; Maseyk *et al.*, 2011; Peñuelas *et al.*, 2011; Franks *et al.*, 2013; Voltas *et al.*, 2013; Granda *et al.*, 2014; Lévesque *et al.*, 2014). Our study sites were located in areas with low nitrogen deposition (de Vries & Posch, 2011) and, hence, nitrogen fertilization is unlikely to be the cause of the increase in iWUE (Leonardi *et al.*, 2012). This trend was also unaffected by the competition level (Figure 3.4) and, therefore, it is most likely due to rising CO_2, age or increasing drought stress, as observed in other studies (Andreu-Hayles *et al.*, 2011; Peñuelas *et al.*, 2011; Granda *et al.*, 2014; Lévesque *et al.*, 2014). These three variables have simultaneously increased in recent years and, thus, their temporal effects can be confounding. *Q. pyrenaica* and *P. sylvestris* p_a-p_i were significantly influenced by age, whereas age did not affect *Q. faginea* iWUE (Table 3.5). Age may have an influence on iWUE through changes in micro-environmental conditions during the tree's life or through physiological changes that occur as a result of changes in the tree's structure, such as shifts in hydraulic conductance with increasing tree height (Bert *et al.*, 1997; Peñuelas *et al.*, 2008; Leavitt, 2010; Resco *et al.*, 2011). An increase in $\delta^{13}C$ with age known as the "juvenile effect" has been observed due to the absorption of $\delta^{13}C$-depleted CO_2 or shading (Bert *et al.*, 1997; Peñuelas *et al.*, 2008; Leavitt, 2010; Resco *et al.*, 2011). This, however, is unlikely to be the cause of the increasing trend in iWUE observed in our study because we avoided the innermost rings and, due to the structure of the studied stands, sampled trees were not in the understory. The effect of age in iWUE could be, conversely, attributable to enhanced hydraulic stress due to height increases (Monserud & Marshall, 2001;

McDowell *et al.*, 2005). This would explain the lack of an age effect on *Quercus faginea*, as this species' trees barely increased in height during the study period. Nonetheless, because the mean height increment between the first and the last inventory was only 1.2 m for *Q. pyrenaica* and 8.5 m for *P. sylvestris*, which would have only caused minor changes in Δ (Table S3.2 in Supporting information; Monserud & Marshall 2001; McDowell, Licata & Bond 2005), the effect of age observed in *Q. pyrenaica* and *P. sylvestris*, even though significant, is probably modest compared to that of other factors, such as CO_2 concentrations or climate.

All species p_a-p_i was strongly influenced by CO_2 (Table 3.5 and Figure 3.6). Atmospheric CO_2 concentrations may affect iWUE by increasing carbon assimilation, reducing stomatal conductance to prevent water loss or both (Huang *et al.*, 2007). Additionally, stomatal conductance and, therefore, carbon uptake and iWUE, are strongly influenced by temperature and precipitation, as these are the main drivers of evapotranspiration rates and vapour pressure deficit. Accordingly, the iWUE of *Q. faginea* and *P. sylvestris* and the growth of all species were highly influenced by climatic factors (Tables 3.4 and 3.5). Therefore, the effect of increasing CO_2 concentrations on iWUE depends, among other factors such as age (Bert *et al.*, 1997; Voelker *et al.*, 2006; Peñuelas *et al.*, 2008; Resco *et al.*, 2011), on how the subsequent increase in temperatures impacts tree physiology. If temperatures are favourable and water availability is not limiting, trees could profit from the increase in atmospheric CO_2 concentrations by increasing A at a constant or decreasing g_s, which may increase radial growth (Granda *et al.* 2014; but see Bader *et al.* 2013 for a lack of response in growth). However, if temperature-induced drought becomes limiting to plant physiology, increases in iWUE are likely due to reduced stomatal conductance to avoid water loss rather than to enhanced carbon assimilation in response to increasing CO_2 concentrations (Andreu-Hayles *et al.*, 2011; Maseyk *et al.*, 2011; Bader *et al.*, 2013; Voltas *et al.*, 2013). This is translated into constant or even reduced growth rates, as it has been observed in this and other studies (Saurer *et al.*, 2004; Andreu-Hayles *et al.*, 2011; Peñuelas *et al.*, 2011).

Enhanced evapotranspiration rates driven by higher temperatures may increase tree-to-tree competition for resources, causing a further reduction in growth (Ackerly & Bazzaz, 1995; Linares *et al.*, 2009b). If competition is reduced, either artificially or by natural causes, trees, rather than changing their iWUE, mostly undergo structural changes such as increased growth and sapwood area, as shown in this and other studies (Figure 3.5; Marshall & Monserud 1996; McDowell *et al.* 2006), which may increase their chances of surviving drought (Sohn *et al.*, 2012, 2013; Pineda-García *et al.*, 2013). Nonetheless, if reduced competition is not able to offset the limiting effect of temperature-induced drought on tree physiology, a decline in growth rates may still follow (Fernández-de-Uña *et al.*, 2015) and, therefore, CO_2 fertilization may not occur. Therefore, the CO_2 and temperature impacts on iWUE are substantially larger than any impact of competition, supporting previous results that suggest thinning impacts trees primarily through shifts in sapwood and leaf area rather than shifts in gas exchange per unit leaf area (Marshall & Monserud 1996; McDowell *et al.* 2006).

3.5 Acknowledgements

The authors would like to thank the funding from the Spanish Ministry of Economy and Competitiveness for the projects AGL2013-46028R and AGL2010-21153-C02 and the associated PhD scholarship for L.F.U., the Madrid Regional Government for the projects S2013/MAE-2760 and REGENFOR, as well as funding from EUFORINO and the Department of Energy, Office of Science, to N.G.M. The authors also thank AEMET and the University of Cantabria for the data provided for this work (Spain02 dataset, http://www.meteo.unican.es/datasets/spain02), as well as the Peñalara Natural Park Research and Management Centre for their climate data. Finally, we would like to thank A. Bachiller, E. Garriga, G. Perkins, Z. R Vance and E. Viscasillas for their field and laboratory assistance.

3.6 Supporting information

Table S3.1. Variance inflation factors (VIF) of each variable in the full models containing either Age or the residuals of Age against CO_2 (ResAge). VIF>10 indicate high collinearity

	BAI			**p_a-p_i**		
	QUFG	**QUPY**	**PISY**	**QUFG**	**QUPY**	**PISY**
	Competition + CO_2 + Precipitation + Temperature + Age					
Competition	5.44	1.08	1.24	4.61	1.33	1.66
CO_2	68.34	7.63	35.214	178.80	8.81	38.46
Temperature	1.20	1.30	1.014	2.51	1.25	2.75
Precipitation	1.10	1.31	1.01	1.90	1.53	2.54
Age	74.62	7.70	34.75	172.39	9.33	35.04
	Competition + CO_2 + Precipitation + Temperature + ResAge					
Competition	5.44	1.08	1.24	4.61	1.33	1.66
CO_2	5.47	2.49	1.33	5.03	2.16	1.81
Temperature	1.20	1.30	1.01	2.51	1.25	2.75
Precipitation	1.10	1.31	1.01	1.90	1.53	2.54
ResAge	1.21	2.47	1.08	1.02	1.50	1.08

Table S3.2. Maximum change in discrimination expected as a result of changes in height assuming an average decrease in Δ of 0.0186‰ per meter increase in height (Monserud & Marshall, 2001) compared to the actual changes occurred between the first and last periods. BP: Barriopedro; NA: Navasfrías; RA: Rascafría; DU: Duruelo; NE: Neila

	Potential changes in Δ (‰)	**Actual changes in Δ (‰)**
BP – *Q. faginea*	-0.044	-0.69
NA – *Q. pyrenaica*	-0.0465	-0.79
RA – *Q. pyrenaica*	-0.117	-0.41
DU – *P. sylvestris*	-0.199	+0.28
NE – *P. sylvestris*	-0.212	+0.12

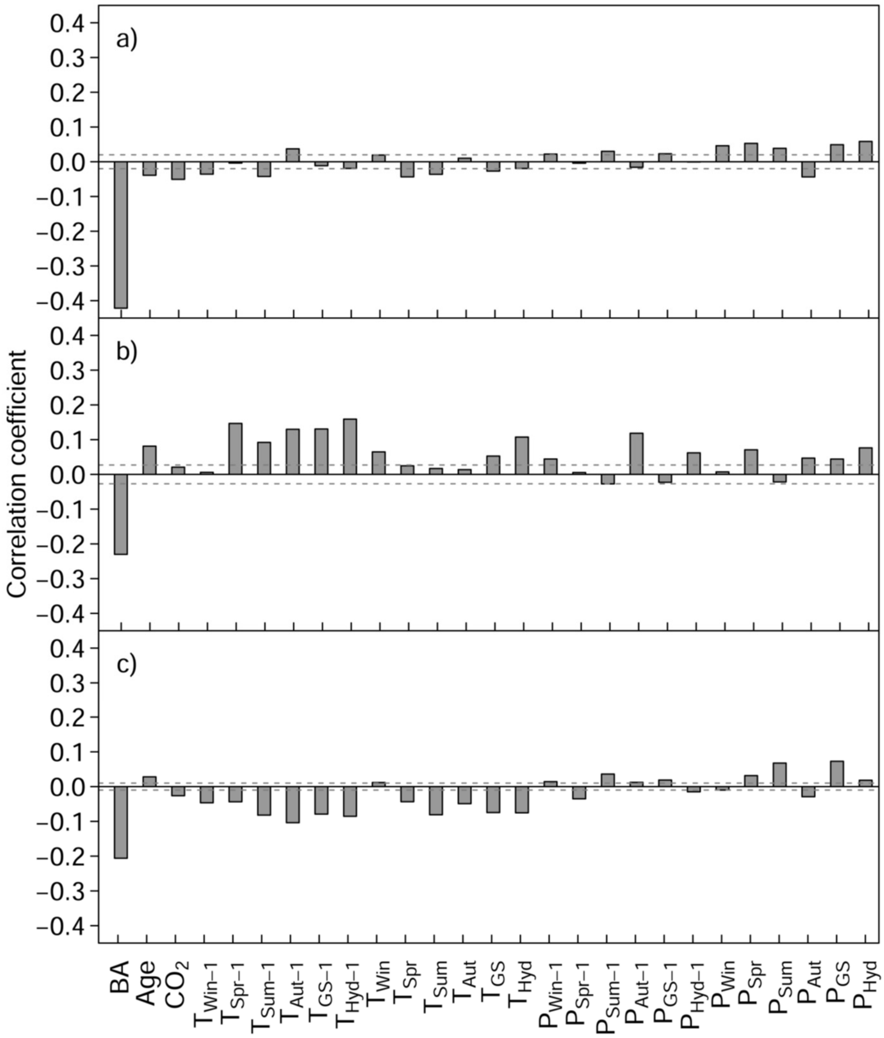


Figure S3.1. Kendall's τ correlation coefficients between BAI and age and environmental variables (plot basal area – BA –, CO_2 concentrations and seasonal temperatures –T– and precipitation – P – of the current and previous year – -1) in (a) *Quercus faginea*, (b) *Quercus pyrenaica* and (c) *Pinus sylvestris*. Dashed horizontal lines indicate significance at the 0.05 level.

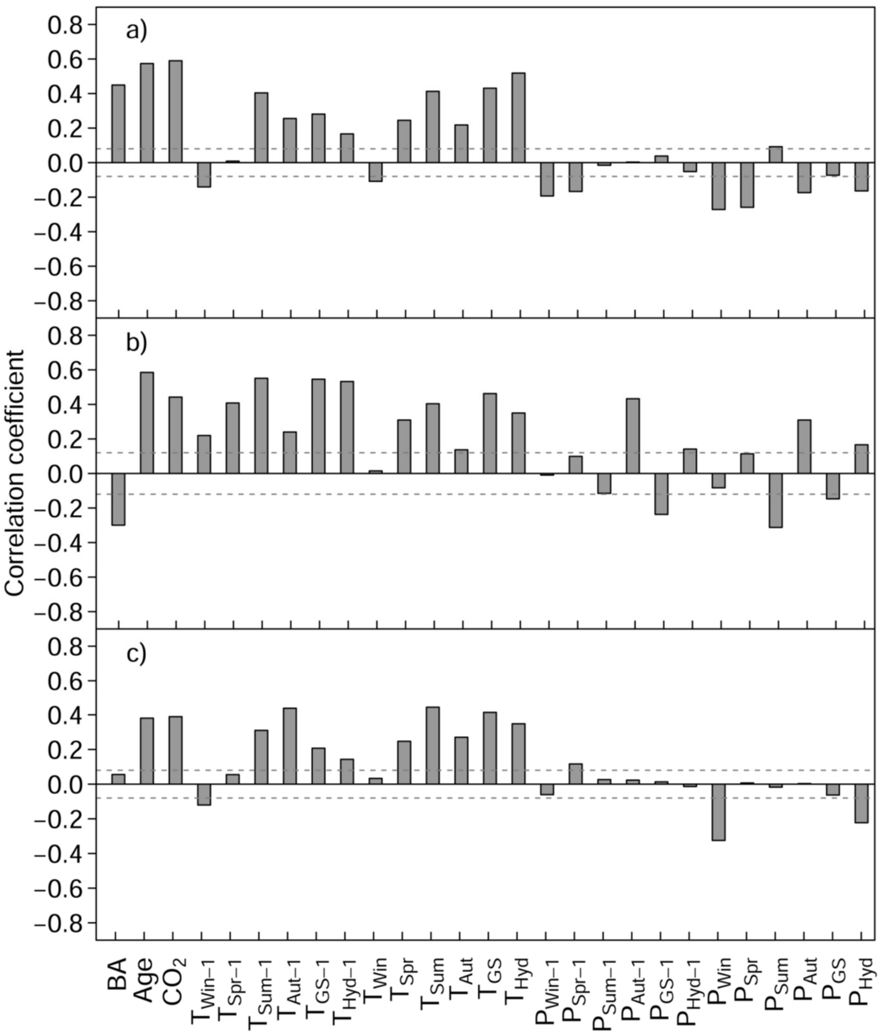


Figure S3.2. Kendall's τ correlation coefficients between p_a-p_i and age and environmental variables (plot basal area – BA – , CO_2 concentrations and seasonal temperatures –T– and precipitation – P – of the current and previous year – -1) in (a) *Quercus faginea*, (b) *Quercus pyrenaica* and (c) *Pinus sylvestris*. Dashed horizontal lines indicate significance at the 0.05 level.

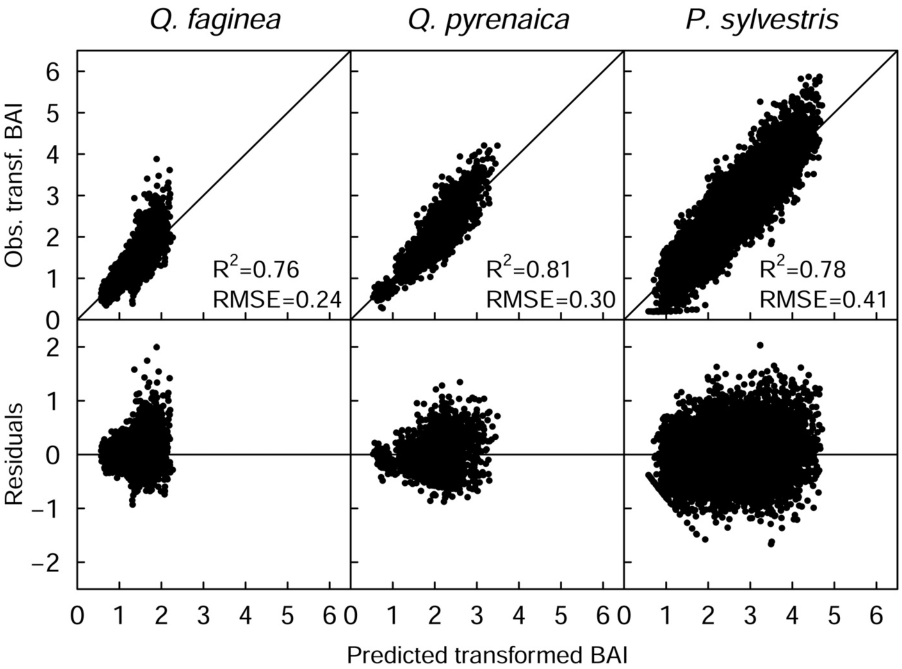


Figure S3.3. Observed vs. Predicted transformed basal area increments (BAI) and Residual plots of the models for *Quercus faginea*, *Quercus pyrenaica* and *Pinus sylvestris*. RMSE: Root-mean-square error

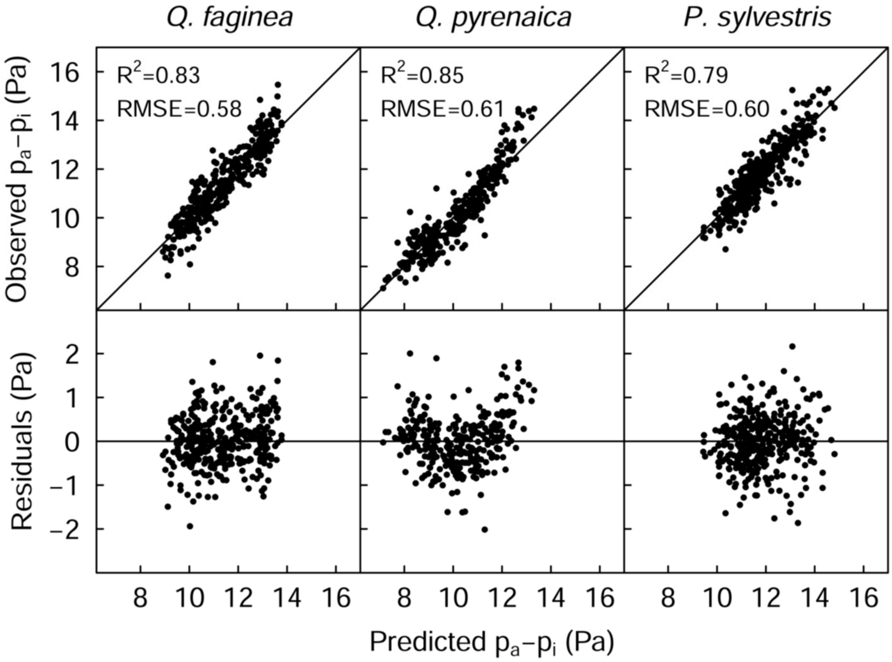
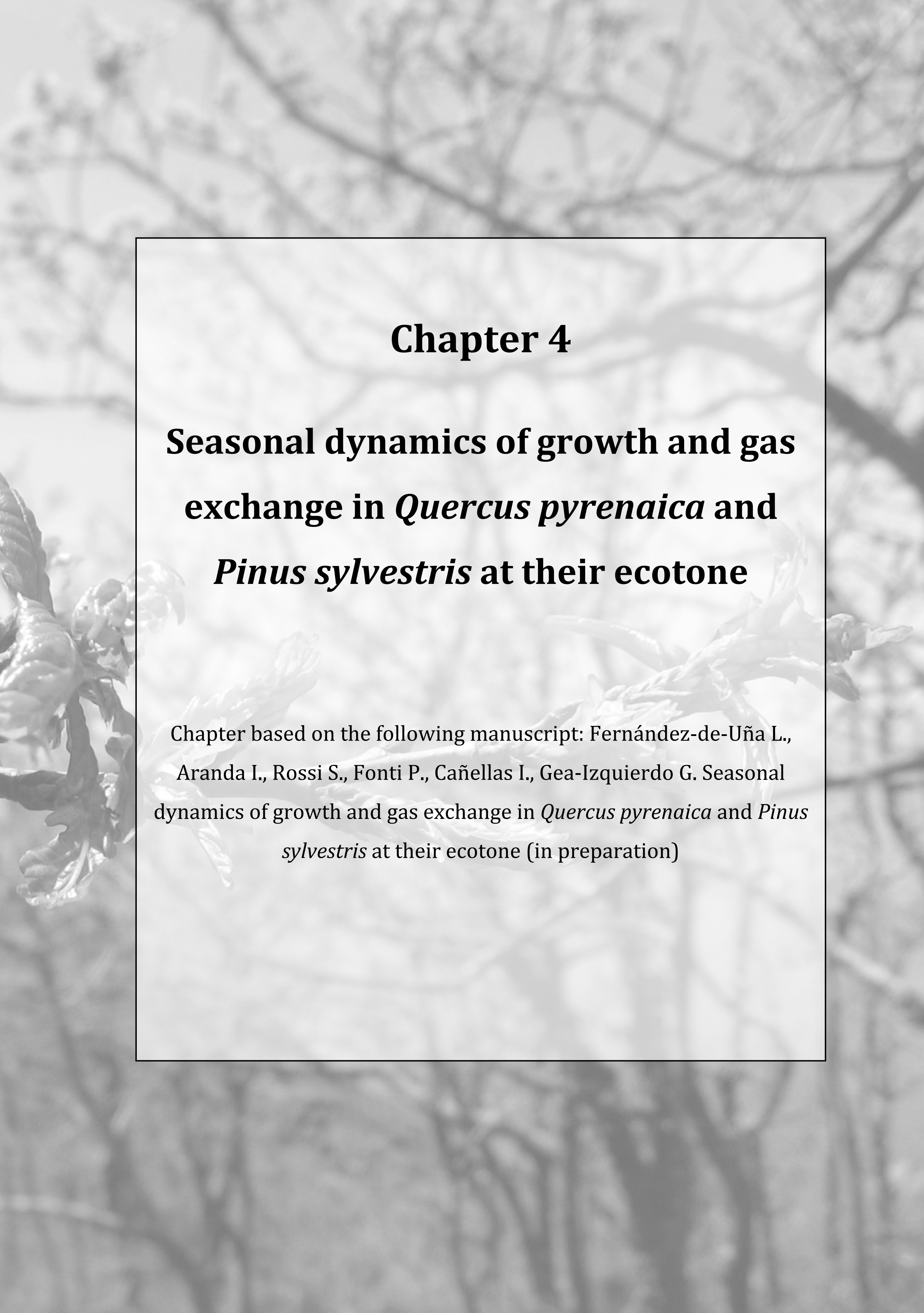


Figure S3.4. Observed vs. Predicted p_a-p_i and Residual plots of the models for *Quercus faginea*, *Quercus pyrenaica* and *Pinus sylvestris*. RMSE: Root-mean-square error



Chapter 4

Seasonal dynamics of growth and gas exchange in *Quercus pyrenaica* and *Pinus sylvestris* at their ecotone

Chapter based on the following manuscript: Fernández-de-Uña L., Aranda I., Rossi S., Fonti P., Cañellas I., Gea-Izquierdo G. Seasonal dynamics of growth and gas exchange in *Quercus pyrenaica* and *Pinus sylvestris* at their ecotone (in preparation)

Resumen

Las especies arbóreas han evolucionado para optimizar el uso de los recursos disponibles, desarrollando diferentes estrategias funcionales que determinan su éxito competitivo y, por tanto, su rango de distribución potencial. Bajo un escenario de clima más cálido, se espera que la especie isohídrica *Pinus sylvestris* L. sea desplazada por especies más tolerantes a la sequía como la anisohídrica *Quercus pyrenaica* Willd. Comprender los factores ambientales que estimulan la fisiología y fenología de los árboles es, por tanto, esencial para evaluar el efecto que las cambiantes condiciones climáticas podrían tener en el rendimiento de estas especies. El objetivo de este estudio es comparar la fenología cambial, la fenología foliar y el intercambio gaseoso de *Q. pyrenaica* y *P. sylvestris* en su límite de distribución altitudinal. Específicamente, evaluamos las variables ambientales que afectan al comienzo y cese de las diferentes fases de fenología cambial y foliar para ambas especies, así como las relaciones entre estas fenofases y las dinámicas estacionales de intercambio gaseoso. *P. sylvestris* presentó una fenología cambial más plástica que *Q. pyrenaica*, presentando una mayor variabilidad inter-anual tanto al principio como al final de la estación de crecimiento. La fenología cambial de *Q. pyrenaica* estaba primordialmente controlada por el fotoperiodo y las temperaturas, mientras que la de *P. sylvestris* estaba principalmente influida por las temperaturas al principio de la estación de crecimiento y el déficit hídrico al final. Además, *Q. pyrenaica* presentó mayor eficiencia fotosintética en el uso del nitrógeno (PNUE, por sus siglas en inglés) y conductancia estomática que *P. sylvestris* a pesar de sus potenciales hídricos foliares más bajos. El mayor control estomático en *P. sylvestris* conllevó una mayor eficiencia intrínseca en el uso del agua (iWUE, por sus siglas en inglés) en esta especie que en *Q. pyrenaica*, indicando una susceptibilidad a la sequía más fuerte. Todo ello sugiere que *P. sylvestris* podría ser más vulnerable al incremento en la

intensidad y recurrencia de sequías predichos bajo escenarios de cambio climático que *Q. pyrenaica*, lo que podría implicar a largo plazo su sustitución por este último, como ya sugieren los modelos de distribución de especies.

Abstract

Tree species have evolved to optimize the use of available resources, developing different functional strategies that determine their competitive success and, therefore, its potential distribution range. Under a warming climate scenario, isohydric *Pinus sylvestris* L. is expected to be displaced by more drought tolerant species such as anyshydric *Quercus pyrenaica* Willd. Understanding the environmental factors that drive tree physiology and phenology is, therefore, essential to assess the effect that changing climatic conditions could have on these species performance. The aim of this study was to compare the cambial phenology, leaf phenology and gas exchange of *Q. pyrenaica* and *P. sylvestris* at their altitudinal distribution limit. Specifically, we assessed the environmental variables that affect the onset and cessation of the different cambial and leaf phenological phases for each species, as well as the relationships among those phenophases and seasonal gas exchange dynamics. *P. sylvestris* had a more plastic cambial phenology than *Q. pyrenaica*, presenting higher inter-year variability both at the beginning and the ending of the growing season. *Q. pyrenaica* cambial phenology was mostly controlled by photoperiod and temperatures, whereas *P. sylvestris* phenology was mainly influenced by temperatures at the beginning of the growing season and water deficit at the end. Additionally, *Q. pyrenaica* had higher photosynthetic nitrogen use efficiency (PNUE) and stomatal conductance than *P. sylvestris* despite its lower leaf water potentials. The tighter stomatal control in *P. sylvestris* led to higher intrinsic water use efficiency (iWUE) in this species than in *Q. pyrenaica*, indicating a stronger susceptibility to drought. Altogether, this research suggests that *P. sylvestris* may be more vulnerable than *Q. pyrenaica* to the increase in intensity and recurrence of droughts predicted under climate change scenarios, which could ultimately entail its replacement by the latter, as already suggested by species distribution models.

4.1 Introduction

Plant species have evolved to optimize the use of available resources, developing different functional strategies to cope with limitations in light, nutrients and water (Limousin *et al.*, 2015). These functional adaptations occur at the whole-tree level, from leaf morphological and xylem structural traits to the timing and duration of physiological processes. At the end, the trade-offs between these functional strategies determine a species competitive success and, therefore, its potential distribution range (Tyree *et al.*, 1994; Vitasse *et al.*, 2014).

One of the major tree functional adaptations is leaf habit. Leaves of evergreen species are able to photosynthesize for a longer span than deciduous species, which entails that they must cope with a wider environmental variability (Warren & Adams, 2004; Baldocchi *et al.*, 2010). Consequently, evergreen species have dense, thick foliage with lower nitrogen contents and higher leaf mass per area, rendering leaves with lower photosynthetic rates than deciduous trees (Reich *et al.*, 1998; Escudero & Mediavilla, 2003; Warren & Adams, 2004). Winter dormancy in deciduous trees prevents freezing damage and reduces leaf respiration losses during periods when potential photosynthetic assimilation is low (Baldocchi *et al.*, 2010; Vitasse *et al.*, 2014). However, this means that deciduous trees must assimilate in a rather short period of time enough carbon to maintain their metabolism, rebuild their canopy and sapwood and replenish their carbohydrate reserves (Gallego *et al.*, 1994). This is particularly important for deciduous ring-porous species, which must renew their main hydraulic system every spring before budburst due to the cavitation of previous-year earlywood vessels (Granier *et al.*, 1994; Delpierre *et al.*, 2016a)

Xylem formation results from a sequence of vascular cambium division and cell differentiation processes. These processes are similar in gymnosperms and angiosperms but result in different xylem anatomical structures, with the conductive system being formed by tracheids in the former and vessels in the latter (Plomion *et al.*, 2001; Delpierre *et al.*, 2016a). As a general rule, gymnosperms tend to have greater hydraulic safety margins than angiosperms, at the expense of lower gas exchange rates (Tyree & Sperry, 1988; Choat *et al.*, 2012). Within each group, further inter-specific differences may arise as a result of the feedback between xylem resistance to cavitation and stomatal conductance (Hacke & Sperry, 2001). Isohydric species, such as *Pinus* spp., generally have higher vulnerability to embolism and, therefore, regulate leaf water potential to prevent xylem cavitation through early stomatal closure (Meinzer *et al.*, 2009; McDowell *et al.*, 2011). On the other hand, anisohydric species, such as *Quercus* spp., tend to be more resistant to xylem cavitation and, thus, maintain relatively open stomata and exert a weaker control on leaf water potential during drought (McDowell *et al.*, 2011; Limousin *et al.*, 2015).

Due to the cyclical intra-annual climatic and photoperiod variability, temperate species adjust their period of maximum activity to optimize the use of available resources while minimizing the exposure to harmful conditions (Cuny *et al.*, 2012; Vitasse *et al.*, 2014; Delpierre *et al.*, 2016a). In Mediterranean mountainous ecosystems, this window of optimal conditions is reduced due to their cold winters and dry summers. An early growth onset may entail a higher risk of frost damage (Vitasse *et al.*, 2014), whereas a late cessation may increase the risk of suffering injuries from summer droughts. Leaf phenology has been extensively monitored, both in terms of leaf habit and biomes. Budburst has been widely recognized to be triggered by temperatures and photoperiod to prevent late spring frost damage (Menzel *et al.*, 2006; Vitasse *et al.*, 2014; Delpierre *et al.*, 2016a). Conversely, little is known regarding the factors triggering xylem formation. Most research on cambial phenology has been conducted in boreal and northern-temperate conifers (Rossi *et al.*, 2006b; Gruber *et al.*, 2010; Eilmann *et al.*, 2011; Cuny *et al.*, 2012), whereas fewer studies have focused on angiosperms (Deslauriers *et al.*, 2009; Michelot *et al.*, 2012b; Pérez-de-Lis *et al.*, 2016). Previous work indicates that temperature and photoperiod are also the main drivers of xylogenesis processes in both groups of species (Rossi *et al.*, 2006b; Delpierre *et al.*, 2016a; Pérez-de-Lis *et al.*, 2016). However, other factors, such as water availability, have been suggested to significantly influence the cessation of xylem formation (Gruber *et al.*, 2010; Eilmann *et al.*, 2011). Likewise, life habit traits such as tree age have been shown to affect the length of the growing season (Rossi *et al.*, 2008b; Li *et al.*, 2013). Nonetheless, as well as studying the environmental factors that drive xylogenesis processes, it is important to understand how these interact with other functional processes such as leaf carbon uptake or tree water use. Research has generally focused on the relationships between cambial and leaf phenological phases (e.g. Cuny *et al.* 2012). Recent work has, however, aimed to further study tree-level processes by unravelling the relationships between cambial and non-structural carbohydrate dynamics (Deslauriers *et al.*, 2009; Oberhuber & Gruber, 2010; Michelot *et al.*, 2012b), whereas fewer have compared wood formation and gas exchange seasonal dynamics (Balducci *et al.*, 2016; Delpierre *et al.*, 2016b).

Pinus sylvestris L. is a widespread Eurosiberian conifer which southernmost, dry limit is found in the mountain ranges of the Iberian Peninsula. Conversely, *Quercus pyrenaica* Willd., a deciduous, ring-porous species, is well adapted to the sub-Mediterranean mountain ranges it inhabits, because of its late budburst, that protects it from early-spring frosts, its high leaf photosynthetic capacity, that maximizes the carbon balance in a short growing season, and a relatively higher drought tolerance compared to other deciduous oaks (Mediavilla & Escudero, 2003; Rodríguez-Calcerrada *et al.*, 2010). Under a warming climate scenario, *P. sylvestris* populations at low elevations are expected to be displaced by more drought tolerant species such as *Quercus* spp. (Ruiz-Labourdette *et al.*, 2012). Understanding the environmental factors that drive tree physiology and phenology is, therefore, essential to assess the effect that changing climatic conditions could have on these species performance. The aim of this study was to compare the cambial phenology, leaf phenology and gas exchange of *Q. pyrenaica* and *P. sylvestris* at their highest and lowest altitudinal distribution limits, respectively. More specifically, we assessed the environmental variables that affect the onset and cessation of the different cambial and leaf phenological phases for each species, as well as the relationships between those phenophases and seasonal gas exchange dynamics. Given the contrasting life strategies of these two species and that the study was performed at the altitudinal distribution limits of *P. sylvestris* and *Q. pyrenaica*, we hypothesize that (i) the two species will differ in the environmental variables triggering phenology, with *P. sylvestris* being more dependent on water availability than *Q. pyrenaica*, efficiency, with the former exhibiting a tighter stomatal control than the latter; (ii) inter-specific differences in stomatal control will translate into contrasting water and nitrogen use efficiencies, and (iii) inter-annual variability in functional traits will be greater in *P. sylvestris* as result of their higher vulnerability to recent episodes of recurrent droughts.

4.2 Materials and Methods

Study site

The study site is located on the northern slope of the Guadarrama mountain range (central Spain, 40º 51’ 35” N, 4º 3’ 52” W, 1350 m.a.s.l.), at the ecotone between low-elevation *Q. pyrenaica*-dominated woodlands and the high-elevation *P. sylvestris* forest. In the area, the two species are distributed in small patches as a result of past harvestings and the species-specific recruitment strategies (Montes & Cañellas, 2007). Two distinct age classes are present, with old *P. sylvestris* trees (sampled trees being 103 ± 5 years old) associated with younger *P. sylvestris* and *Q. pyrenaica* trees (35-47 years old). Mean annual precipitation at the site is 728 mm and mean annual temperature 9.1 ºC during the period 1950-2014, with high inter-annual variability (Figure 4.1).

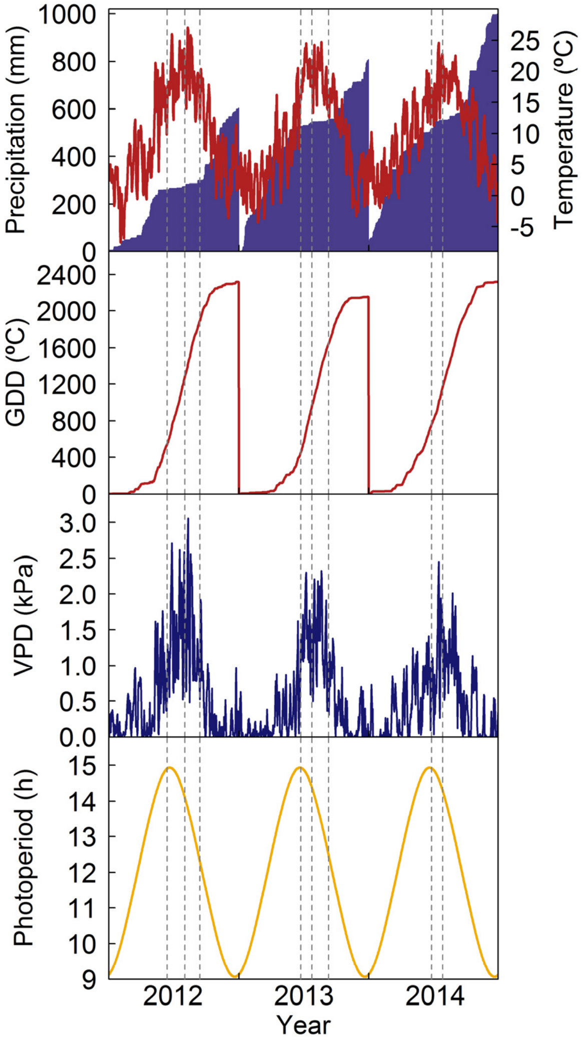


Figure 4.1. Climatic conditions during the study period. GDD: Growing degree days; VPD: vapour pressure deficit. Vertical dashed lines mark the days in which gas exchange measurements were taken.

Intra-annual growth and cambial phenology

Cambial phenology was monitored between April and November during 2012-2014. Micro-cores were extracted with a Trephor (Rossi *et al.*, 2006c) from six trees per species, three per *P. sylvestris* age class, with a frequency of 7-15 days. Different trees were generally selected each study year in order to minimize possible inter-annual bias due to previous year punching injuries. Micro-cores were conserved at 4 ºC in a 70% ethanol solution. *P. sylvestris* 2012 samples were cut in 10-18 µm micro-sections with a sliding micro-tome. This technique was, however, unsuccessful for *Q. pyrenaica*. Therefore, the rest of the samples were dehydrated with increasing concentrations of ethanol and xylene using a tissue processor (Leica TP1020, Leica Biosystems Ltd., Nussloch, Germany), embedded in paraffin and cut in 8-to-12-µm sections with a rotary microtome (Leica RM2135, Leica Biosystems Ltd., Nussloch, Germany). All micro-sections were stained with Safranine O and Astra Blue, dehydrated with increasingly graded ethanol solutions and xylene and mounted with either Canada balsam or a mixture of distyrene, tricresyl phosphate and xylene (DPX).

In order to distinguish the different cell developmental stages (cambium, enlargement, wall thickening and mature cells), microsections were observed under visible and polarized light. For *P. sylvestris*, the number of tracheids in each developmental phase was counted along three radial segments and averaged per sampling date (Figure 4.2). For *Q. pyrenaica*, micro-sections were photographed with an Olympus Colorview camera attached to a microscope (Olympus Vanox AHMT3, Olympus Corporation, Tokio, Japan). For each sampling date, we counted the number of cambium cells and measured the width of earlywood (EW) and latewood (LW) increments at each developmental stage (Figure 4.2) along three radial segments with ImageJ (Schneider *et al.*, 2012). Due to its complex xylem anatomy, the completion of lignification could not be assessed in *Q. pyrenaica*.

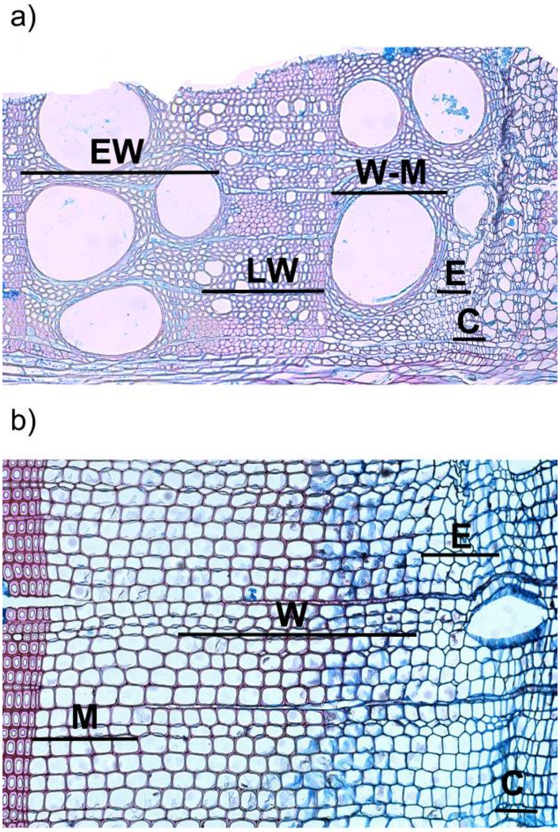


Figure 4.2. Micro-sections of (a) *Quercus pyrenaica* and (b) *Pinus sylvestris* showing the different cambial phenological phases. EW: Earlywood; LW: Latewood; C: Cambial zone; E: Enlargement phase; W: Wall-thickening phase; M: Mature phase.

Leaf phenology

Leaf phenology was recorded with the same frequency than cambial phenology by assessing the dates of bud-burst and full leaf unfolding. In 2012, leaf phenology was recorded at the stand level by species, whereas in 2013 and 2014, it was recorded individually on the trees where the microcores were extracted from.

Physiological measurements

Morning and afternoon net photosynthetic rate (A) and stomatal conductance to water vapour (g_s) and mid-day leaf water potential (Ψ) were recorded in six trees per species in early-summer (ES; June), mid-summer (MS; July-August) and late-summer (LS; September) of 2012-2014; except in September 2012, when Ψ could not be measured, and September 2014, when no measurements could be taken, due to technical problems. Gas exchange measurements were performed on current-year needles and leaves exposed to direct sunlight with an LCpro+ portable photosynthesis system (ADC BioScientific Ltd., Hoddesdon, Herts, UK); except in June 2013 when some measurements on *P. sylvestris* were taken in current- and previous-year needles because current-year needles were not fully developed. Ψ were measured with a Scholander pressure chamber (PMS Instrument Co. 7000, Corvallis, OR, USA). *Q. pyrenaica* leaves collected for gas exchange measurements were scanned and their areas measured. For *P. sylvestris* trees, a subsample of all needles used for gas exchange measurements was scanned to measure needle area. Subsequently, leaves and needles were dried and weighed to obtain their specific leaf area (SLA; cm^2 g^{-1}). SLA was used to calculate the total needle area measured and recalculate A and g_s accordingly. Additionally, instantaneous intrinsic water use efficiency (iWUE=A/g_s) was calculated from leaf gas exchange measurements.

Leaf chemical analysis

Leaves and needles collected during the morning gas exchange measurements were ground to analyse their carbon and nitrogen elemental concentration and stable isotope content ($\delta^{13}C$ and $\delta^{15}N$, respectively). Three-to-4-mg samples were analysed on a PDZ Europa ANCA-GSL elemental analyser connected to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) with an average analytical precision of 0.06‰

and 0.1 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. $\delta^{13}C$ data were transformed to discrimination (Δ) following Farquhar, Ehleringer, & Hubick (1989):

$$\Delta \approx \frac{\delta^{13}C_a - \delta^{13}C}{1 + \delta^{13}C} \qquad [4.1]$$

Source air $\delta^{13}C$ ($\delta^{13}C_a$) data were obtained from NOAA Earth System Research Laboratory (NOAA- ESRL, http://www.esrl.noaa.gov/gmd/dv/data/).

Additionally, foliar carbon and nitrogen concentrations on a leaf basis were used to calculate the C/N ratio, nitrogen concentration per unit of leaf area (N_a), and photosynthetic nitrogen use efficiency (PNUE), i.e. the ratio between net photosynthetic rate and leaf nitrogen content (A/N).

Meteorological data

Air temperature and humidity were measured with a HOBO Pro series temperature/relative humidity data logger (Onset, Cape Cod, Massachusetts, USA). Daily precipitation data and missing temperature and humidity data were obtained from the Spanish Meteorological Agency (AEMET). Raw temperature and humidity data were used to calculate the vapour pressure deficit (VPD), the number of hours a day below 0 ºC, 5 ºC and 7 ºC and the cumulative growing degree days (GDD). Cumulative VPD (CVPD), calculated as the sum of daily VPD before that day, was used as a proxy of the water stress experienced by trees during the growing season. GDD were calculated as the cumulative sum of daily GDD (GDD_{daily}):

$$GDD_{daily} = \frac{T_{\max} + T_{\min}}{2} - T_{base} \qquad [4.2]$$

where T_{max} and T_{min} are daily maximum and minimum temperature, respectively, and T_{base} is set to 5 °C (Gričar *et al.*, 2014). Negative values of GDD_{daily} were set to 0; thus, only positive values of GDD_{daily} were used to calculate GDD since the beginning of the year. According to the literature, trees may remain in dormancy until the spring equinox regardless of temperature (Swidrak *et al.*, 2011); thus, cumulative growing degree days since the spring equinox (GDD_e) were also calculated. Finally, the photoperiod, i.e. daily number of day hours between dawn and sunset, was used to assess the effect of daylight availability on each species xylogenesis.

Data analysis

Logistic functions were used to analyse phenology data and to estimate the day of the year (DOY) at which the onset and cessation of each cambial and foliar developmental phase occurred. Generalized linear mixed models (GLMMs) with a binomial distribution, a logit link function and a random intercept to account for the effect of 'tree' were used to test differences in phenology between species. GLMMs with different fixed factors (DOY, Year, Species and the interaction 'Species × Year') were fitted and assessed using the Akaike Information Criterion (AIC). The fit of a model increases as AIC values decrease. Models with a difference in AIC (ΔAIC) < 2 are equally informative (Burnham & Anderson, 2002) and, therefore, if the ΔAIC between two models was below 2, the most parsimonious model (i.e. with fewer variables) was selected.

Additionally, GLMMs with a binomial distribution and a random intercept associated with tree were also used to assess the effect of climatic factors (GDD, CVPD, temperatures, cumulative precipitation), photoperiod, tree size class (for *P. sylvestris*) and preceding phenological phases on the onset and cessation of cambial and leaf phenology phases. Models with different combinations of these variables were fitted and ranked based on their AIC.

Because data were not normally distributed and measures were repeated within individuals, differences between species in gas exchange and leaf chemical properties were assessed using GLMMs with a Gamma distribution and a log link function, except for g_s, for which an identity link function was used, and Ψ and $\delta^{15}N$, that were tested using linear mixed models (LMMs). Sampling date, species and the interaction 'Species x Date' represented fixed factors. When the model including this interaction had the lowest AIC, differences between categories of fixed factors were assessed using Tukey contrasts. Additionally, we fitted A as a logistic function of g_s and PNUE as a linear function of iWUE for each species and for each period of the summer (early-, mid- and late-summer) to assess whether those relationships differed seasonally and between species.

All analyses were carried out with R version 3.1.1 (R Core Team, 2014). GLMMs were fit with the package "lme4" (Bates *et al.*, 2014). Multiple mean comparisons (Tukey contrasts) were performed with the package "multcomp" (Hothorn *et al.*, 2008).

4.3 Results

Species differences in cambial and leaf phenology

Q. pyrenaica had more cambial cells than *P. sylvestris*, but this difference was observed only during dormancy (Figures 4.3 and 4.4). The onset of xylem formation was significantly different between species, while differences among years were only observed in *P. sylvestris* (Table 4.1 and Figures 4.3 and 4.4). *Q. pyrenaica* xylem growth followed a bimodal pattern, corresponding to the peaks in earlywood and latewood growth (Figure 4.3). The width of enlarging earlywood was maximum in May, on DOY 137 ± 5 in 2012, 135 ± 14 in 2013 and 124 ± 11 in 2014, whereas maximum latewood enlargement occurred between late-June and early-July (Figure 4.3). Maximum enlargement in *P. sylvestris* occurred between the two *Q. pyrenaica* growth peaks, on DOY 147 ± 12 in 2012, 169 ± 12 in 2013 and 148 ± 17 in 2014. *Q. pyrenaica* and *P. sylvestris* xylem formation ceased at similar dates (Table 4.1). However, *P. sylvestris* had a significant year-to-year variability not present in *Q. pyrenaica* (Table 4.1). No xylem growth was observed in autumn (Figures 4.3 and 4.4). *Q. pyrenaica* generally had a longer growing season (119 ± 8 days in 2012, 140 ± 10 days in 2013 and 128 ± 14 days in 2014) than *P. sylvestris* (87 ± 10 days in 2012, 114 ± 19 days in 2013 and 138 ± 17 days in 2014), which however exhibited a higher inter-annual variability.

Table 4.1. Effect of species (Spp) and year on *Quercus pyrenaica* and *Pinus sylvestris* phenology as given by the differences in the Akaike Information Criterion (ΔAIC) of the fitted binomial GLMMs. ΔAIC was calculated as the difference between the AIC of the model being assessed and the AIC of the model with the lowest AIC (i.e. the model with ΔAIC = 0). The best model is marked in bold.

Model	**N par**	**Enlargement onset**		**Enlargement cessation**		**Budburst**	
		LogLik	**ΔAIC**	**LogLik**	**ΔAIC**	**LogLik**	**ΔAIC**
Species effect							
DOY	3	-247.00	42.41	-311.38	32.57	-191.63	157.87
DOY + Spp	4	-231.77	13.95	-310.61	33.04	-171.18	118.98
DOY + Year	5	-245.43	43.28	**-293.09**	**0**	-237.41	253.44
DOY + Spp + Year	6	-226.13	6.69	-292.35	0.54	-168.74	118.10
DOY + Spp + Year + Spp×Year	8	**-220.77**	**0**	-291.35	2.55	**-107.67**	**0**
Year effect on *Quercus pyrenaica*							
DOY	3	**-22.01**	**0**	**-135.19**	**0**	-40.74	46.03
DOY + Year	5	-21.33	2.7	-134.72	3.12	**-15.70**	**0**
Year effect on *Pinus sylvestris*							
DOY	3	-33.59	14.26	-33.74	16.14	-25.25	22.08
DOY + Year	5	**-24.42**	**0**	**-23.63**	**0**	**-12.18**	**0**

Budburst occurred significantly earlier in *Q. pyrenaica* (DOYs 97-133) than in *P. sylvestris* (DOYs 132-147) (Table 4.1 and Figures 4.3 and 4.4). On average, *Q. pyrenaica* and *P. sylvestris* required 34 and 55 days, respectively, to fully unfold leaves. *Q. pyrenaica* showed a secondary leaf sprout event, between DOYs 173 and 189, which coincided with the timing of latewood maximum growth (Figure 4.3).

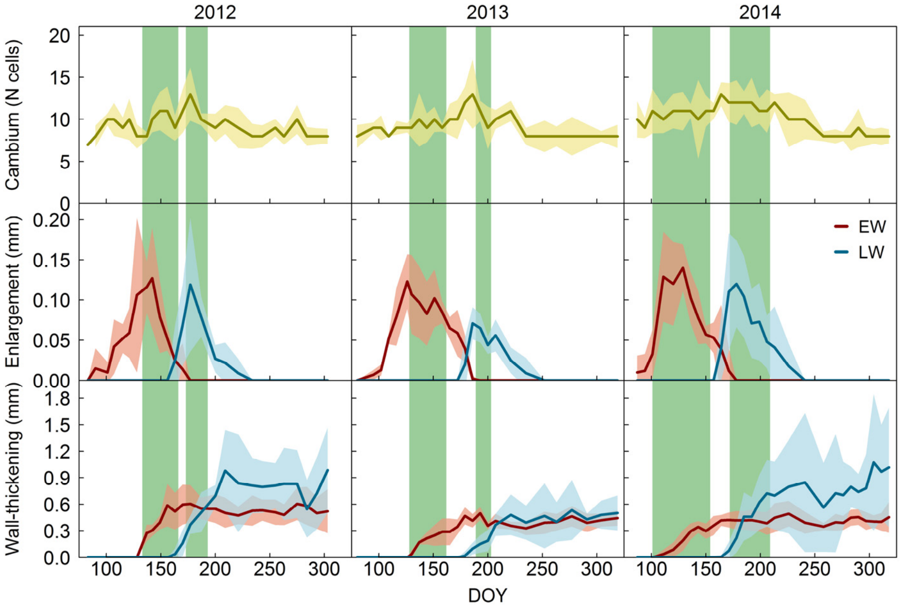


Figure 4.3. *Quercus pyrenaica* cambial and leaf phenology. Light-grey shaded areas represent the standard deviations, whereas dark-grey vertical shaded areas mark the period of leaf development. EW: earlywood; LW: latewood.

Effect of environmental factors on cambial and leaf phenology

The onset of *Q. pyrenaica* xylem growth was mainly influenced by photoperiod (Table 2 and Figure 4.5). Earlywood vessel lignification was triggered by reaching a specific GDD_e. GDD_e also regulated the cessation of earlywood enlargement, which successively affected the beginning of latewood enlargement, which in turn influenced latewood wall-thickening (Table 4.2). The cessation of latewood enlargement was influenced by GDD (Table 4.2 and Figure 4.5). Budburst, which was strongly correlated with earlywood lignification (Pearson correlation coefficient = 0.87; Figure 4.3), was affected by GDD, whereas the second sprout depended on GDD and photoperiod.

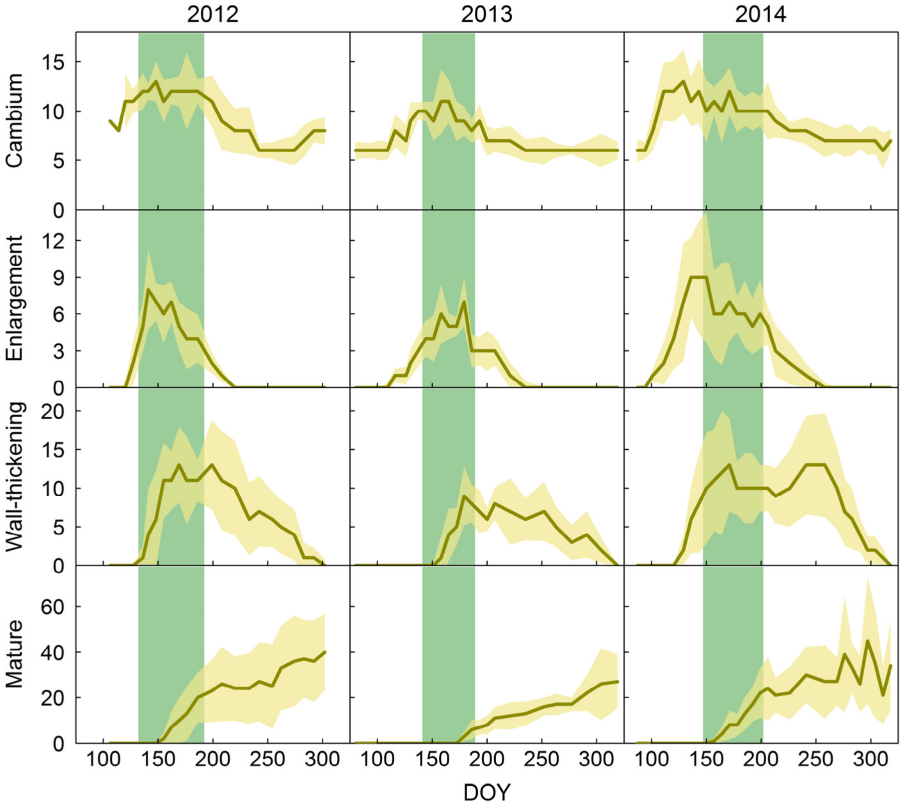


Figure 4.4. *Pinus sylvestris* cambial (number of tracheids at each developmental stage) and leaf phenology. Light-grey shaded areas represent the standard deviations, whereas dark-grey vertical shaded areas mark the period of needle development.

In *P. sylvestris*, the onset of enlargement was influenced by GDD_e and the amount of cold hours below 7ºC (Table 4.3 and Figure 4.6). The onsets of tracheid lignification and maturation were both triggered by GDD_e. The cessation of xylem enlargement was mostly influenced by CVPD, which was a proxy of the seasonal integration of the evaporative demand. Wall thickening cessation was influenced by photoperiod, GDD_{daily}, the accumulated number of cold hours (below 7ºC) since the summer and tree size. Budburst was triggered by GDD_{daily}, photoperiod, the precipitation of the previous month and their interaction (Table 4.3).

Table 4.2. Effect of environmental and endogenous variables on *Quercus pyrenaica* phenology as given by the fitted binomial GLMMs. logLik: log-likelihood; AIC: Akaike Information Criterion; RMSE: Root-mean-square error; EW: Earlywood; LW: Latewood; E: enlargement; W: Wall-thickening; PP: photoperiod; GDD_e: Growing degree days since the spring equinox; GDD: Growing degree days since the beginning of the year; EWE: presence of earlywood enlargement; LWE: presence of latewood enlargement.

Variable	Best model	logLik	AIC
EW E onset	$-306.81 + 24.47 \cdot PP$	-10.57	27.13
EW W onset	$-31.63 + 0.218 \cdot GDD_e$	-18.36	32.52
EW E cessation	$27.29 - 0.054 \cdot GDD_e$	-14.26	34.53
LW E onset	$-2.54 + 0.0268 \cdot GDD_e - 12.95 \cdot EWE$	-12.73	33.46
LW W onset	$-21.69 + 0.0279 \cdot GDD_e + 6.77 \cdot LWE$	-16.67	41.35
LW E cessation	$22.76 - 0.017 \cdot GDD$	-16.85	39.70
Budburst	$-82.73 + 0.548 \cdot GDD$	-0.77	7.55
Second sprout	$-99.43 - 5.86 \cdot PP + 0.271 \cdot GDD$	-3.84	15.68

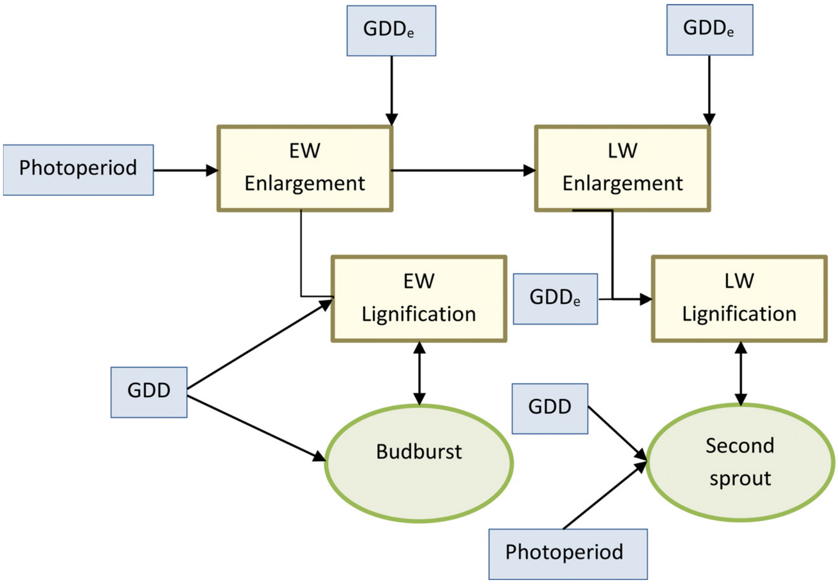


Figure 4.5. Relationships among cambial (boxes) and leaf (circles) phenological phases and environmental variables for *Quercus pyrenaica*. One-directional arrows indicate that an environmental variable or phenological phase had an effect on the phase indicated by the arrow; two-directional arrows indicate correlation between two phases; lines with no arrows join related phases with no significant correlation between them. EW: Earlywood; LW: latewood; GDD: Growing degree days since the beginning of the year; GDD_e: Growing degree days since the spring equinox.

Table 4.3. Effect of environmental and endogenous variables on *Pinus sylvestris* phenology as given by the fitted binomial GLMMs. logLik: log-likelihood; AIC: Akaike Information Criterion; RMSE: Root-mean-square error; E: enlargement; W: Wall-thickening; M: Maturation; GDD_e: growing degree days since March equinox; T7: daily number of hours with temperatures below 7ºC; CVPD: cumulative vapour pressure deficit; PP: photoperiod; GDD_{daily}: daily growing degree days; Ch7: cumulative number of hours with temperatures below 7ºC since summer; SizeS: Size class 'Small'; $Prec_{Prev\_month}$: Precipitation accumulated during the previous month.

Variable	**Best model**	**logLik**	**AIC**
E onset	$-13.13 + 0.177 \cdot GDD_e - 0.224 \cdot T7$	-23.12	54.23
W onset	$-25.50 + 0.104 \cdot GDD_e$	-17.78	41.55
M onset	$-33.30 + 0.084 \cdot GDD_e$	-16.28	38.56
E cessation	$55.56 - 0.439 \cdot CVPD$	-21.56	49.11
W cessation	$-1.15 - 1.34 \cdot PP - 0.735 \cdot GDD_{daily} - 0.097 \cdot Ch7 + 3.59 \cdot SizeS$	-20.19	52.37
Budburst	$-153.91 + 9.99 \cdot PP + 0.613 \cdot GDD_{daily} - 1.48 \cdot Prec_{Prev\ month} + 0.111 \cdot PP \times Prec_{Prev\ month}$	-3.76	19.52

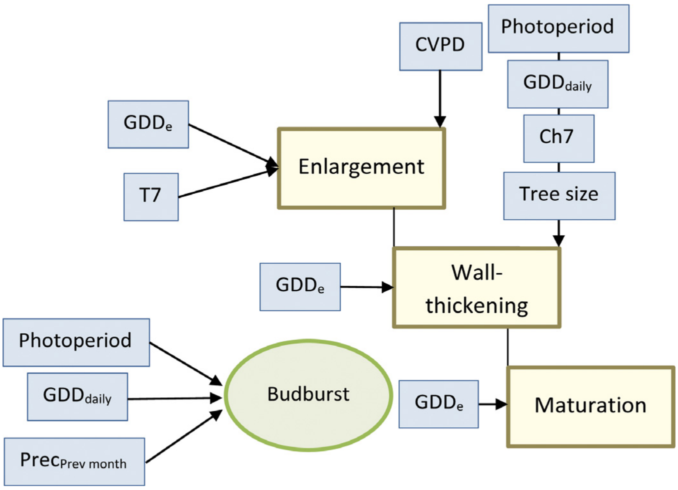


Figure 4.6. Relationships among cambial (boxes) and leaf (circles) phenological phases and environmental variables for *Pinus sylvestris*. One-directional arrows indicate that an environmental variable or phenological phase had an effect on the phase indicated by the arrow; two-directional arrows indicate correlation between two phases; lines with no arrows join related phases with no significant correlation between them. GDD_e: Growing degree days since the spring equinox; T7: daily number of hours with temperatures below 7ºC; CVPD: cumulative vapour pressure deficit; GDD_{daily}: daily growing degree days; Ch7: cumulative number of hours with temperatures below 7ºC since summer; $Prec_{Prev\ month}$: Precipitation accumulated during the previous month.

Gas exchange and leaf chemical properties

Morning net photosynthetic rates were significantly higher in *Q. pyrenaica* than in *P. sylvestris*, particularly during 2013, whereas this difference was reduced in the afternoon, mostly due to a decrease in *Q. pyrenaica* A and g_s (Figure 4.7). For both species, the highest photosynthetic rates tended to occur in mid-summer. *Q. pyrenaica* had significantly lower photosynthetic rates in 2012, the driest year, whereas *P. sylvestris* showed a lower inter-annual variability (Figure 4.7). Generally, stomatal conductance was significantly higher in *Q. pyrenaica* than in *P. sylvestris*. g_s tended to follow the same seasonal dynamics as A in *Q. pyrenaica*, whereas in *P. sylvestris* it dropped more markedly than A at the end of the summer. In general, g_s was significantly lower in 2012, the driest year, for both species. A and g_s dynamics resulted in higher iWUE in *P. sylvestris*, particularly in the morning measurements (Figure 4.7). iWUE significantly increased along the summer in *P. sylvestris*, whereas it was less variable in *Q. pyrenaica*. This divergence occurred also among years, with no significant differences in iWUE among years in *Q. pyrenaica*, whereas it was significantly higher in 2012, the driest year, in *P. sylvestris*. Leaf water potentials decreased along the season and were consistently more negative in *Q. pyrenaica* than in *P. sylvestris*, particularly in mid-summer. Ψ were less negative in *P. sylvestris* in 2013, whereas they were higher in *Q. pyrenaica* in 2014 (Figure 4.7).

SLA and leaf nitrogen content on a leaf mass basis were significantly higher in *Q. pyrenaica* than in *P. sylvestris* (Figure 4.8), which led to similar values of N_a in both species (data not shown). SLA tended to decrease along the growing season, particularly in *P. sylvestris*, whereas the C/N ratio increased in *P. sylvestris* and remain unchanged in *Q. pyrenaica*. Similarly, SLA, C/N and, therefore, N_a, did not vary among years in *Q. pyrenaica*, whereas in P. *sylvestris* SLA was higher and C/N lower in 2013. $\delta^{15}N$ and PNUE were significantly higher in *Q. pyrenaica*, whereas no differences in Δ

were observed between species (Figure 4.8). δ^{15}N and Δ were constant along the summer in both species, whereas PNUE tended to increase in *P. sylvestris* and remained unchanged in *Q. pyrenaica*. No inter-annual change of δ^{15}N was observed among years, whereas PNUE was higher in 2013 for both species. Δ remained unchanged in *P. sylvestris* among years, whereas it was higher in *Q. pyrenaica* in 2014 (Figure 4.8).

The relationship between A and g_s differed between species (Figure 4.9a). The rate of change of A as a function of g_s was relatively constant along the growing season in *Q. pyrenaica*, whereas it was higher for mid-summer in *P. sylvestris*. iWUE decreased with increasing PNUE, except for *P. sylvestris* in the early summer, when iWUE increased with rising PNUE. The slope of the relationship between PNUE and iWUE decreased along the growing season for both *Q. pyrenaica* and *P. sylvestris*. The slopes of this relationship were significantly different ($P<0.02$) between species in early- and mid-summer, whereas they became similar at the end of the summer (Figure 4.9b).

4.4 Discussion

Divergent phenological strategies in Q. pyrenaica *and* P. sylvestris

P. sylvestris had a more plastic cambial phenology than *Q. pyrenaica*, presenting higher inter-year variability both at the beginning and the ending of the growing season. The onset of *Q. pyrenaica* xylem formation was triggered by photoperiod rather than temperatures as found by Pérez-de-Lis et al. (2016). Holding cambial reactivation until a certain day-length is reached prevents the premature onset of growth as a result of mild periods in late winter, which could damage the hydraulic system if frost follows (Swidrak *et al.*, 2011; Vitasse *et al.*, 2014). The rest of *Q. pyrenaica* cambial phenophases, as well as the onset of all *P. sylvestris* xylogenesis phases, were affected by temperatures. This temperature control over cell wall-thickening and maturation would further protect the newly formed xylem when it is already hydraulically active and, thus, vulnerable to freeze-thaw-induced cavitation (Sperry *et al.*, 1994; Hacke & Sperry, 2001). Several studies have found a significant effect of temperatures on the reactivation of cambial activity (Gruber *et al.*, 2010; Swidrak *et al.*, 2011; Delpierre *et al.*, 2016b), which has been further confirmed through stem heating experiments (Kudo *et al.*, 2014). Nonetheless, GDD since the spring equinox was a better predictor than GDD since the beginning of the year, particularly in *P. sylvestris*, indicating that photoperiod exerts some control on the onset of this species xylogenesis too (Swidrak *et al.*, 2011).

In conifers from cold environments, maximum growth rates tend to occur around the summer solstice (Rossi *et al.*, 2006b). However, studies on milder locations have found, concurring with our study, that *P. sylvestris* growth peaks in late-May/early June (Camarero *et al.*, 2010; Gruber *et al.*, 2010; Oberhuber *et al.*, 2011; Cuny *et al.*, 2012; Swidrak *et al.*, 2014). Rossi *et al.* (2006b) hypothesized that the photoperiod constraint on the period of maximum growth may ensure xylem differentiation is completed before winter. However, in warmer environments, trees may have rather adapted to avoid summer drought. Gruber et al. (2010) suggested that, under reduced water availability, an earlier peak in xylem formation would ensure enough carbohydrates could be allotted to bud formation and root growth. In our study, *P. sylvestris* trees finished xylem formation more than a month earlier in 2012, the driest year, than in 2014, the wettest, which indicates a strong control of water availability on this species growing season length, as found in other studies (Gruber *et al.*, 2010; Eilmann *et al.*, 2011). This was confirmed by the model assessing the importance of environmental variables (Table 4.3), where the best predictor of xylogenesis cessation was the cumulative vapour pressure deficit.

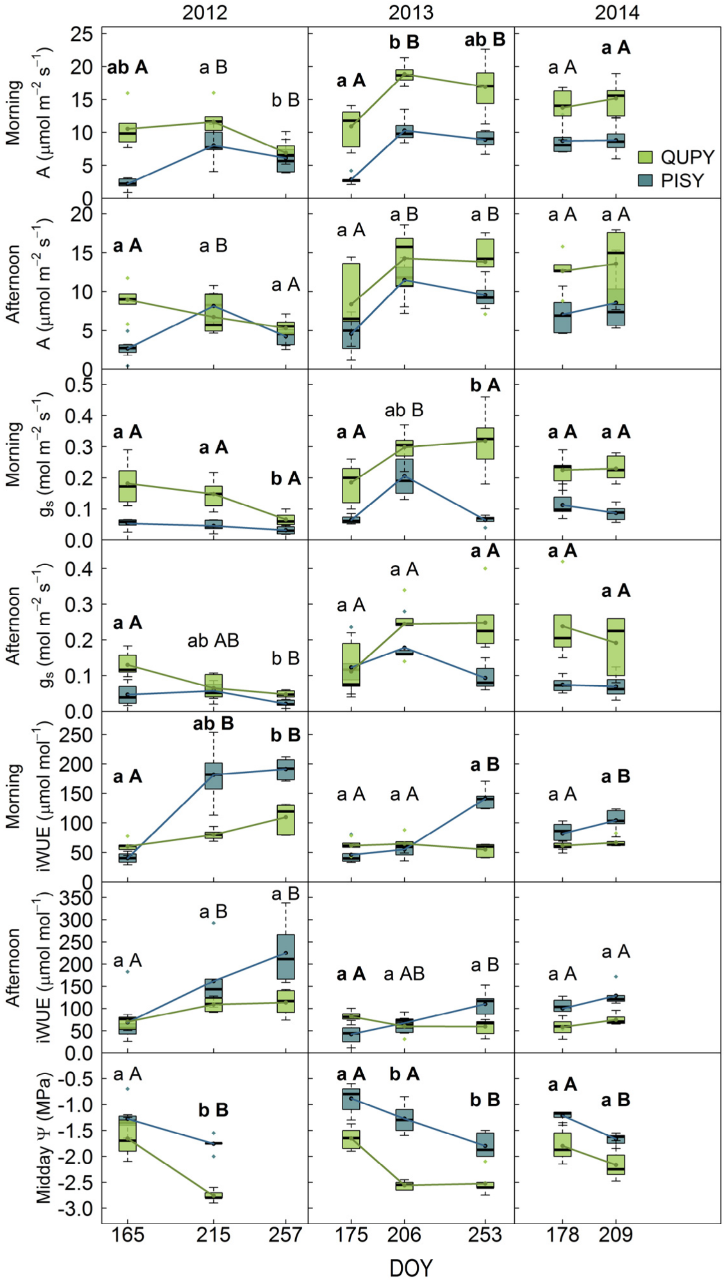


Figure 4.7. Gas exchange differences between species and sampling dates. Lower case letters indicate differences among measurement dates in *Q. pyrenaica* and capital letters indicate differences among measurement dates in *P. sylvestris*. Differences between species are marked in bold. A: photosynthetic rate; g_s: stomatal conductance; iWUE: intrinsic water use efficiency; Ψ: leaf water potential.

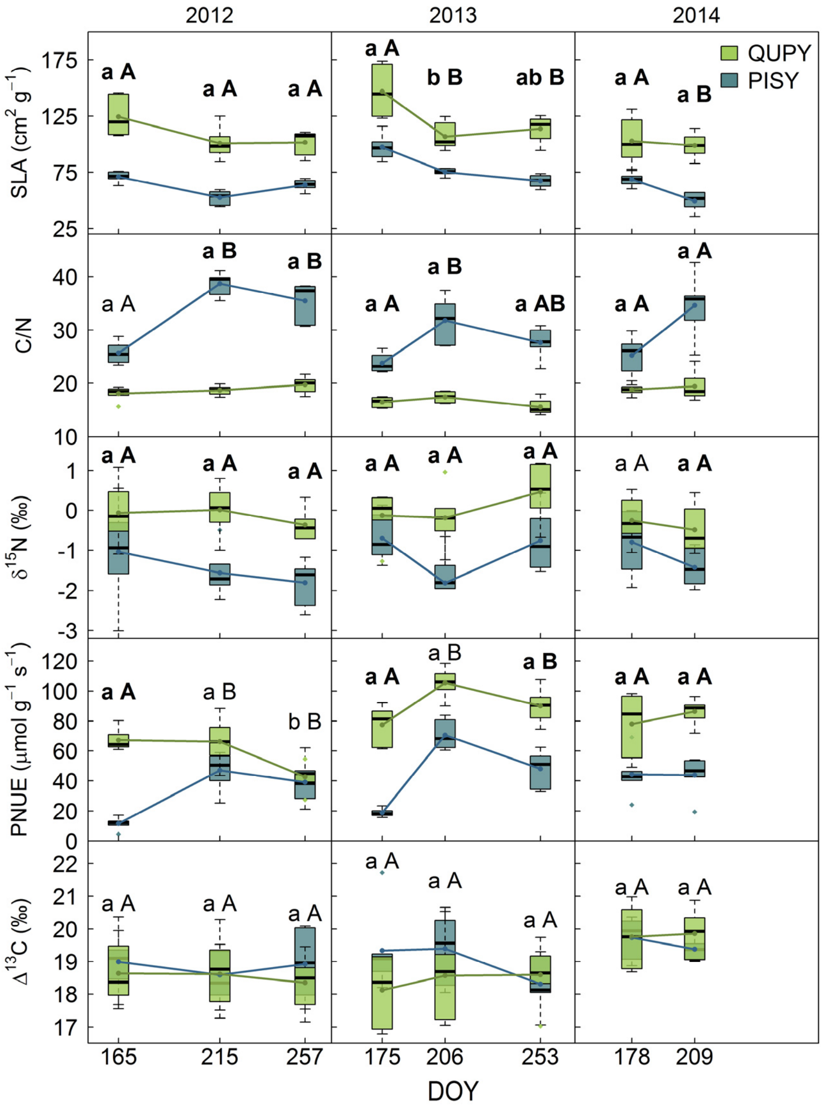


Figure 4.8. Leaf chemical properties differences between species and sampling dates. Lower case letters indicate differences among measurement dates in *Q. pyrenaica* and capital letters indicate differences among measurement dates in *P. sylvestris*. Differences between species are marked in bold. SLA: Specific leaf area; C/N: C/N concentration ratio; $\delta^{15}N$: nitrogen isotope content; PNUE: photosynthetic nitrogen use efficiency; $\Delta^{13}C$: carbon discrimination.

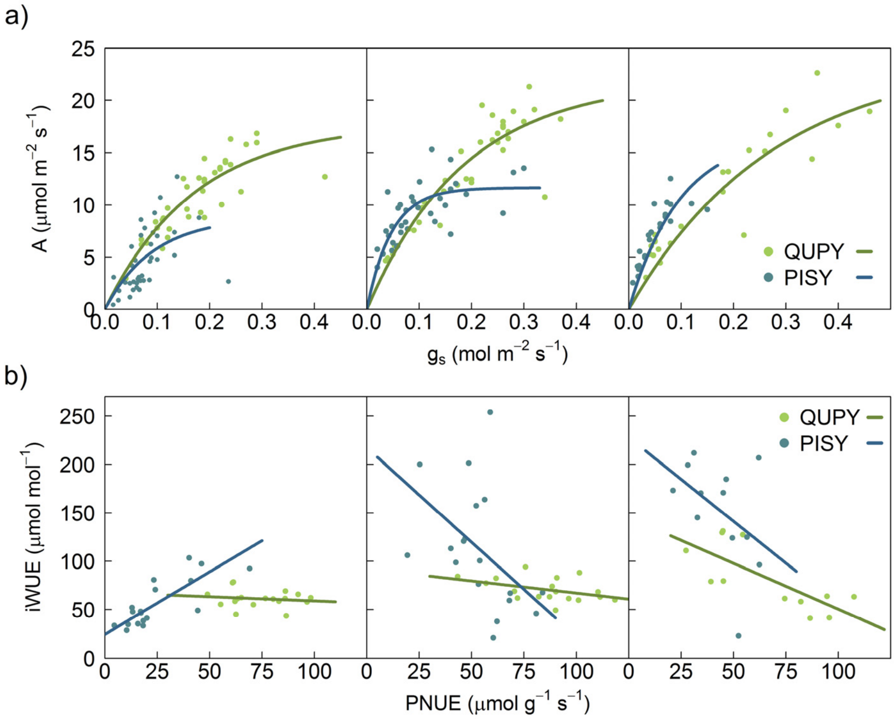


Figure 4.9. Seasonal changes in the relationship between (a) stomatal conductance (g_s) and photosynthetic rate (A) and (b) photosynthetic nitrogen use efficiency (PNUE) and intrinsic water use efficiency (iWUE). *Quercus pyrenaica* relationships: $A_{ES} = 17.753\cdot(1 - e^{-5.827\cdot gs})$; $A_{MS} = 21.91\cdot(1 - e^{-5.398\cdot gs})$; $A_{LS} = 24.172\cdot(1 - e^{-3.638\cdot gs})$; $iWUE_{ES} = 67.53 - 0.09\cdot PNUE$; $iWUE_{MS} = 92.05 - 0.25\cdot PNUE$; $iWUE_{LS} = 145.37 - 0.95\cdot PNUE$; *Pinus sylvestris* relationships: $A_{ES} = 8.812\cdot(1 - e^{-10.908\cdot gs})$; $A_{MS} = 11.6598\cdot(1 - e^{-21.3435\cdot gs})$; $A_{LS} = 16.795\cdot(1 - e^{-10.174\cdot gs})$; $iWUE_{ES} = 24.39 + 1.29\cdot PNUE$; $iWUE_{MS} = 217.39 - 1.96\cdot PNUE$; $iWUE_{LS} = 227.94 - 1.73\cdot PNUE$.

On the other hand, *Q. pyrenaica* latewood maximum growth was concurrent with the summer solstice, which suggests that there exists a photoperiod control to ensure an early cessation of growth and cell differentiation (Rossi *et al.*, 2006b). In fact, unlike for *P. sylvestris*, the cessation of *Q. pyrenaica* xylogenesis was affected by GDD. This strategy would provide this species enough time to replenish carbohydrate reserves to build the following year's earlywood and foliage (Hoch *et al.*, 2003; Michelot *et al.*, 2012b).

As observed in our study, budburst has been widely recognized to be triggered by temperatures and photoperiod in order to avoid frost damage to newly formed leaves (Menzel *et al.*, 2006; Vitasse *et al.*, 2014; Delpierre *et al.*, 2016a). Budburst in *Q. pyrenaica* tended to coincide or shortly followed xylem lignification. Conversely, budburst in *P. sylvestris* was unrelated to cambial phenophases, although it always occurred after cambial activity resumption, concurring with previous studies (Cuny *et al.*, 2012; Michelot *et al.*, 2012b). Due to the cavitation of previous-year earlywood vessels, ring-porous trees must build a functional hydraulic system before leaf unfolding to allow for sufficient water supply to the expanding leaves (Granier *et al.*, 1994; Delpierre *et al.*, 2016a). Therefore, *Q. pyrenaica* relies on a good synchronization among its cambial and leaf phenological phases in order to optimize carbon uptake during the growing season. In *P. sylvestris*, however, old needles provide carbohydrates for the formation of current-year needles and xylem (Michelot *et al.*, 2012b; Delpierre *et al.*, 2016a). This, together to the higher dependence of *Q. pyrenaica* on current year vessels, makes the synchronization between canopy and xylem phenology in *P. sylvestris* less necessary than in *Q. pyrenaica*.

Divergent physiological strategies in Q. pyrenaica *and* P. sylvestris

Leaf gas exchange (A, g_s) and water status from Ψ were within those found in the literature, both for *Q. pyrenaica* (Mediavilla & Escudero, 2003; Hernández-Santana *et al.*, 2008a) and *P. sylvestris* (Irvine *et al.*, 1998; Martínez-Vilalta & Piñol, 2002; Poyatos *et al.*, 2008). A and g_s were higher in the former than in the latter, even in 2012 when *Q. pyrenaica* stomatal conductance dropped as a result of drought. This may be due to the ability of *Q. pyrenaica* to use deeper soil water reserves, as its higher leaf $\delta^{15}N$ might suggest (Resco *et al.*, 2011), therefore avoiding stomatal closure despite the decrease in midday Ψ along the summer due to increasing soil water deficits (Gallego *et al.*, 1994; Mediavilla & Escudero, 2003; Hernández-Santana *et* *al.*, 2008a). Midday Ψ were consistently less negative in *P. sylvestris* as a result of tighter stomatal control (Poyatos *et al.*, 2008), which also resulted in higher iWUE in *P. sylvestris* than in *Q. pyrenaica* as the growing season progressed. These behaviours are consistent with an anisohydric, non-conservative strategy in *Q. pyrenaica* and an isohydric strategy in *P. sylvestris* (Gallego *et al.*, 1994; Poyatos *et al.*, 2008).

These inter-specific ecophysiological differences can be partially explained by the overall divergences between evergreen and deciduous leaf morphologies. SLA was higher and the C/N ratio lower in *Q. pyrenaica* than in *P. sylvestris*, leading to similar N_a values in both species. Given the higher photosynthetic rates in *Q. pyrenaica*, this was translated into a higher PNUE in this species compared to *P. sylvestris*. Evergreen trees generally have lower PNUE than deciduous ones due to a reduced N allocation to photosynthesis, the use of Rubisco for N storage and the higher internal resistance to CO_2 diffusion as a result of leaf morphological adaptations (e.g. increased cell-wall thickness) to optimize leaf toughness (Takashima *et al.*, 2004; Warren & Adams, 2004; Zheng & Shangguan, 2006). Seasonal changes in needle morphology may also explain the increase in PNUE along the summer in *P. sylvestris*, following shifts in the C/N ratio and, to a lesser extent, SLA, as well as changes in *A*. In *Q. pyrenaica*, however, the lack of changes in N concentrations along the summer or among years, probably due to the greater physiological maturity of foliage by the time measurements were taken, indicate that changes in PNUE were only due to fluctuations in *A*. The combined effects of late needle unfolding and tight stomatal control may explain the positive relationship between PNUE and iWUE in *P. sylvestris* in early summer as well as the sharper shifts in iWUE with increasing PNUE in *P. sylvestris* than in *Q. pyrenaica* as water deficit increased. This inverse relationship between iWUE and PNUE has been previously observed as a result of the contrasting effects of stomatal control on these variables (Zheng & Shangguan, 2006; Limousin *et al.*, 2015).

Even though leaf iWUE was significantly higher in *P. sylvestris* than in *Q. pyrenaica*, no significant differences were found between species or between sampling dates in Δ. Instantaneous gas exchange measurements record maximum, light-optimal conditions. Conversely, leaf Δ integrates a longer span of time and, therefore, includes periods of low photosynthetic activity due to, for example, the shading of leaves and needles, or processes of internal carbon discrimination after carbon fixing. This may cause a dilution of the isotopic signal and be partly responsible for the differences between instantaneous and δ^{13}C-derived iWUE.

Relationships among intra-annual growth and gas exchange dynamics

The highest photosynthetic rates were observed in mid-summer, when most of xylem formation was completed. Moreover, photosynthetic carbon uptake remained high in September, particularly in *Q. pyrenaica*, when cambium was already dormant (except in *P. sylvestris* in 2014). Zweifel *et al.* (2006) found that radial growth took only 30-70% of the vegetative period in *P. sylvestris* and *Quercus pubescens*. Therefore, for an important part of the summer, assimilated carbon is allocated to root development and bud formation or accumulated as reserves. Indeed, the allocation of current photosynthates to root growth is known to be maximal in spring, late summer and autumn (Bréda *et al.*, 2006). Likewise, buds are formed from late summer to early autumn (Delpierre *et al.*, 2016a). Moreover, Oberhuber et al. (2011) found accumulation of non-structural carbohydrates when cambial activity had decreased. This period of carbon accumulation is particularly important in *Q. pyrenaica*, which relies on stored carbohydrates to form the following year's earlywood vessels and leaves (Hoch *et al.*, 2003; Michelot *et al.*, 2012b). This is further supported by the fact that in 2014, a wet year, *P.*

sylvestris extended its growing season up to September, whereas *Q. pyrenaica* growth cessation date did not differ from that of other years, indicating that it favoured reserve accumulation.

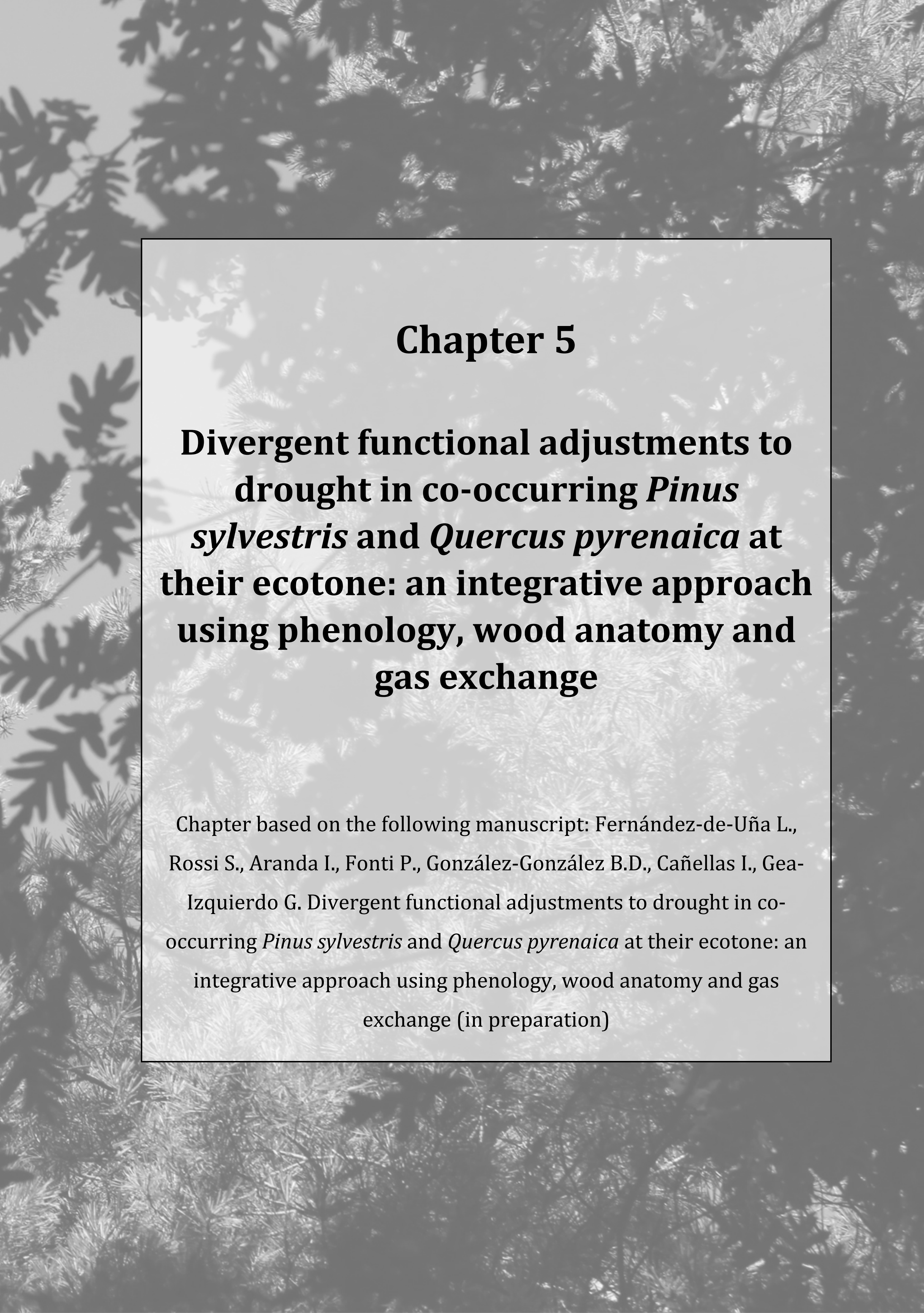
Conversely, both phenology and gas exchange data indicated a significant effect of water availability on *P. sylvestris* physiology. In 2012, drought caused an earlier cessation of growth in *P. sylvestris*, as well as a strong decrease in stomatal conductance. *Q. pyrenaica*, however, maintained relatively open stomata during summer, indicating that for this species water deficits were not strong enough to trigger leaf stomatal closure, probably thanks to its ability to reach deeper soil water reserves (Gallego *et al.*, 1994; Mediavilla & Escudero, 2003).

Conclusions

Our study shows that *Quercus pyrenaica* has adapted its phenology to avoid spring frost damage and maximise carbon uptake during summer to restore carbohydrate reserves. Additionally, this species tended to optimize photosynthetic rates, as indicated by its higher photosynthetic nitrogen use efficiency and higher stomatal conductance than *P. sylvestris* despite its lower water potentials. The tighter stomatal control in *P. sylvestris* led to higher water use efficiency in this species than in *Q. pyrenaica*, indicating a stronger sensitivity to water scarcity. This drought-avoidance strategy by *P. sylvestris* was also evident in cambial phenology. Nonetheless, despite its higher resistance to summer drought, *Q. pyrenaica* may not be able to profit from the increase in spring temperatures if it maintains the photoperiod control over the onset of xylogenesis. Conversely, the tighter stomatal control and the shorter growing season in *P. sylvestris* as a result of dry conditions may portend a higher vulnerability to the increase in intensity and recurrence of droughts predicted under climate change scenarios, as already suggested by species distribution models (Ruiz-Labourdette *et al.*, 2012).

4.5 Acknowledgements

The authors would like to thank the funding from the Spanish Ministry of Economy and Competitiveness for the projects AGL2013-46028R and AGL2010-21153-C02 and the associated PhD scholarship for L.F.U., the Madrid Regional Government for the project S2013/MAE-2760. The authors also thank AEMET for the climate data provided for this work. We would also like to thank A. Bachiller, E. Garriga, A.M. González, A. Parras, M.L. Peña, L. Schneider, and E. Viscasillas for their field and laboratory assistance. Likewise, we appreciate the advice provided by I. García González, G. Pérez de Lis and G. Guada regarding micro-core processing.



Chapter 5

Divergent functional adjustments to drought in co-occurring *Pinus sylvestris* and *Quercus pyrenaica* at their ecotone: an integrative approach using phenology, wood anatomy and gas exchange

Chapter based on the following manuscript: Fernández-de-Uña L., Rossi S., Aranda I., Fonti P., González-González B.D., Cañellas I., Gea-Izquierdo G. Divergent functional adjustments to drought in co-occurring *Pinus sylvestris* and *Quercus pyrenaica* at their ecotone: an integrative approach using phenology, wood anatomy and gas exchange (in preparation)

Resumen

Los escenarios climáticos para la región Mediterránea predicen un aumento en la frecuencia e intensidad de los eventos de sequía. Con el cambio climático, se espera que *Pinus sylvestris* L. reduzca su rango de distribución en la región, siendo sustituido a bajas altitudes por taxones más tolerantes a la sequía como *Quercus pyrenaica* Willd. El objetivo de este estudio era determinar cómo respondían la fenología cambial, la anatomía del xilema y la fenología foliar de estas dos especies a sequía experimental en su ecotono. Se estableció un tratamiento de exclusión de lluvia en un bosque de montaña en el límite de distribución altitudinal entre ambas especies en la zona centro de España. Se recogieron micro-testigos durante 2012-2014 tanto de árboles bajo tratamiento como de árboles control para realizar un seguimiento de su fenología cambial. También se midieron características de la anatomía de la madera (área del lumen, anchura de la pared celular y anchura de la madera temprana y la tardía). Adicionalmente, se midieron en intercambio gaseoso y potenciales hídricos de *P. sylvestris* a lo largo del verano durante el periodo de estudio. Encontramos un cese de la elongación del xilema más temprana bajo el tratamiento de sequía, particularmente en *P. sylvestris*, sin un efecto en la fenología foliar. Esto, junto a una menor asignación de carbono para la formación de traqueidas, en *P. sylvestris*, y madera tardía, en *Q. pyrenaica*, sería consistente con un cambio en la distribución de recursos bajo estrés hídrico del xilema a otros sumideros. La reducción en la madera tardía en *Q. pyrenaica* podría comprometer el rendimiento hidráulico de esta especie, puesto que los vasos de la madera tardía proporcionan transporte de agua cuando los vasos de la madera temprana han cavitado. Además, el mayor control estomático observado en *P. sylvestris* bajo condiciones más secas podría presagiar una limitación en la asimilación de carbono en esta especie. Estos resultados sugieren que tanto *P. sylvestris* como *Q. pyrenaica* serían potencialmente vulnerables al aumento de estrés por sequía predicho. No

obstante, *P. sylvestris* presentó un mayor riesgo de mortalidad inducida por sequía en su límite altitudinal inferior.

Abstract

Climatic scenarios for the Mediterranean region forecast increasing frequency and intensity of drought events. With climate change, *Pinus sylvestris* L. is expected to reduce its distribution range in the region, being displaced at low altitudes by more drought tolerant taxa such as *Quercus pyrenaica* Willd. The objective of this study was to determine how the cambial phenology, xylem anatomy and leaf phenology of these two species responded to experimental drought at their ecotone. A rainfall exclusion experiment was established in a mountain forest stand located at the altitudinal distribution limit of these two species in Central Spain. Micro-cores were sampled during 2012-2014 from both treated and control trees to monitor their cambial phenology. Wood anatomical traits (lumen area, cell-wall thickness and earlywood and latewood widths) were also measured. Additionally, *P. sylvestris* leaf gas exchange and water potential were measured along the summer during the study period. We found an earlier cessation of xylem enlargement under the drought treatment, particularly in *P. sylvestris*, without an effect on leaf phenology. This, together with a lower carbon allocation to the formation of tracheids, in *P. sylvestris*, and latewood, in *Q. pyrenaica*, was consistent with a shift in the allocation of resources under drought stress from xylem to other sinks. The reduction in *Q. pyrenaica* latewood could challenge this species hydraulic performance, as latewood vessels provide water transport once earlywood vessels have cavitated. Moreover, the tighter stomatal control observed in *P. sylvestris* under drier conditions may portend carbon assimilation limitations in this species. These results suggest that both *P. sylvestris* and *Q. pyrenaica* would be potentially vulnerable to the forecasted increasing drought stress, with *P. sylvestris* showing, however, a higher risk of drought-induced mortality at its low altitudinal limit.

5.1 Introduction

The Mediterranean climate is characterized by mild, wet winters and hot, dry summers. An increase in the frequency and severity of drought events is projected under climate change scenarios as a result of rising temperatures without a concurrent increase in precipitation (Giorgi & Lionello, 2008; IPCC, 2013). Consequently, changes in tree growth dynamics and species distribution are expected to occur as a result of climate warming (Hughes, 2000; Ruiz-Labourdette *et al.*, 2012).

Water has a central role in all plant physiological processes and, therefore, changes in its availability will drive shifts from the leaf to the whole-tree level. Drought entails a decrease in soil water availability that is generally related to rainfall scarcity, coupled in many cases to high potential evapotranspiration. These conditions cause a situation of water stress in trees that could translate into a drop of stem and leaf water potentials beyond the boundaries of hydraulic security (McDowell *et al.*, 2008). Hence, limitations to canopy transpiration are induced by stomatal closure to prevent hydraulic failure and excessive water loss (Tyree & Sperry, 1988; Bréda *et al.*, 2006). Consequently, photosynthetic carbon uptake and accumulation of compounds from primary metabolism are reduced, which may eventually lead to a depletion of carbohydrate reserves (Bréda *et al.*, 2006; McDowell *et al.*, 2011). This implies that fewer resources can be allocated to xylem formation (Oberhuber *et al.*, 2011). Likewise, the defensive capacity against other sources of stress can be weakened, predisposing trees to further drought stress, infections or frost damage (Bréda *et al.*, 2006; McDowell *et al.*, 2008, 2011; Eilmann *et al.*, 2010; Gaylord *et al.*, 2013). After a prolonged and severe drought, the depletion of carbohydrate reserves could, ultimately, cause the death of the tree by carbon starvation alone or by its interplay with pathogen attacks or hydraulic failure (Bréda *et al.*, 2006; McDowell & Sevanto, 2010; Sala *et al.*, 2010, 2012; McDowell *et al.*, 2011).

Water availability directly influences cell turgor pressure, affecting in turn cell expansion and, thus, xylem structure and radial growth (Abe *et al.*, 2003). Potential xylem hydraulic conductivity mainly depends on the number of conduits and their diameter, as well as on the features of pit connections between xylem conduits (Tyree *et al.*, 1994; Hacke & Sperry, 2001; Choat *et al.*, 2008). Hence, wide vessels are more effective in water transport, but at the risk of suffering earlier cavitation (Sperry *et al.*, 1994; Tyree *et al.*, 1994). Wood anatomy is, therefore, a key factor determining the trees' resistance to water deficit (Sperry *et al.*, 1994; Fichot *et al.*, 2009). It has been generally assumed that trees under drought tend to form smaller vessels to reduce the risk of cavitation (Sperry *et al.*, 1994). However, recent work has challenged this paradigm (Eilmann *et al.*, 2009, 2011), as vulnerability to cavitation may rely on pit membrane properties rather than conduit diameter (Hacke & Sperry, 2001). Xylem structure and function tend to be optimized to balance safety and water-transport efficiency (Fichot *et al.*, 2009; Zanne *et al.*, 2010), in such way that there could exist a feedback between hydraulic conductance and and stomatal conductance (Hacke & Sperry, 2001). Anisohydric species have high resistance to xylem cavitation and maintain relatively open stomata during drought and, therefore, are considered more drought tolerant (McDowell *et al.*, 2011). On the other hand, isohydric or drought avoiding species are more vulnerable to xylem cavitation, which they prevent through early stomatal closure (McDowell *et al.*, 2011). Regardless of the species-specific strategies to cope with drought, severe dry periods may induce hydraulic damage over time and increase sensitivity to further stress, making trees more vulnerable to decay, which eventually could lead to the death of the tree (Bréda *et al.*, 2006; McDowell *et al.*, 2011; Anderegg *et al.*, 2013).

In this context, rainfall exclusion and irrigation experiments have provided a better understanding of tree performance under drought (Eilmann *et al.*, 2009; Beier *et al.*, 2012; Gaylord *et al.*, 2013; Dickman *et al.*, 2015), as well as they give insight on how tree phenology could be affected by changing climatic conditions. The timing and duration of ecophysiological processes influence tree performance; hence, changes in phenology can significantly modulate crown and xylem development (Rossi *et al.*, 2012; Adams *et al.*, 2015; Pérez-de-Lis *et al.*, 2016). Crown phenology has been widely studied through drought experiments, with delays in phenophases and reductions in seed production occurring as a result of drought (Ogaya & Peñuelas, 2004; Pérez-Ramos *et al.*, 2010; Adams *et al.*, 2015). Cambial phenology has been, however, less considered in drought experiments, with most of the studies being performed in young individuals (Abe *et al.*, 2003; de Luis *et al.*, 2011; Balducci *et al.*, 2013) or in the boreal region (Belien *et al.*, 2012; D'Orangeville *et al.*, 2013), partially neglecting drought-prone areas (Eilmann *et al.*, 2011). Moreover, research has focused on conifer species while deciduous species have been overlooked.

Tree competitive success depends on how they adapt their functional response, xylem structure and phenology to a specific environment, defining a species potential distribution range (Tyree *et al.*, 1994; Fonti *et al.*, 2010; Caffarra & Donnelly, 2011; Vitasse *et al.*, 2014). Changes in tree performance and phenology may, therefore, alter tree-to-tree interactions which, in turn, may lead to shifts in species distribution, particularly at the rear edge of their distribution range. *Pinus sylvestris* L. is a Eurosiberian species which southern distribution limit is found in the mountain ranges of the Iberian Peninsula. As other *Pinus* spp., it is generally considered to follow an isohydric strategy (Poyatos *et al.*, 2008). As a consequence of climate change, this species is expected to reduce its current distribution range, being displaced at low altitudes by more drought-tolerant taxa like sub-Mediterranean *Quercus* spp. (Ruiz-Labourdette *et al.*, 2012). Indeed, previous studies have already found increased mortality in *P. sylvestris* in its lower distribution limit (Galiano *et al.*, 2010; Gea-Izquierdo *et al.*, 2014). The objective of this study is to assess the responses of *P. sylvestris* and *Quercus pyrenaica* Willd. under water stress conditions by analysing cambial and leaf phenology and wood anatomy during a permanent rainfall exclusion experiment located at the altitudinal ecotone between the two species, where *P. sylvestris* is considered to be threatened by increasingly drier conditions (Gea-Izquierdo *et al.*, 2014). We hypothesize that: (i) water deficit will induce a shortening of the xylem growing season; (ii) the effects of drought will be cumulative and more evident as the experiment progresses; and (iii) given its lower drought tolerance, *P. sylvestris* response to the rainfall exclusion treatment will be stronger than that of *Q. pyrenaica*.

5.2 Materials and Methods

Study site and experimental design

The study site is located in Valsaín (Central Spain, 40º 51' 35" N, 4º 3' 52" W) at an altitude of 1350 m.a.s.l., with a 15% slope and north-western exposition. It is found at the transition zone between *P. sylvestris*- and *Q. pyrenaica*-dominated woodlands and, thus, at *P. sylvestris* local xeric altitudinal limit. Both species are distributed in small patches within the stand as a result of their recruitment strategies and the management performed in the area (Montes & Cañellas, 2007), which have produced stands characterized by two distinct age classes (Table 5.1). Mean annual precipitation is 745 mm and the mean annual temperature is 8.8 ºC. In April 2012, three different rainfall exclusion plots of 65-150 m^2 were established based on the structure of the stand: one for *Q. pyrenaica* and two for *P. sylvestris*, one for young trees and another for old trees (Table 5.1). These experimental plots allowed simulating the future intensification of droughts at the study site. The soil at each of plot was covered with waved PVC boards and a 0.5 m ditch was dug and covered with plastic on the upper side of the plot to minimize downwards subsurface water flow. Additionally, soil humidity sensors were set at 25 and 50 cm depth at each exclusion plot and in the control area.

Table 5.1. Characteristics of the studied trees. N trees: number of trees sampled over the study period; DBH: diameter at breast height

	N trees	Age (years)	DBH (cm)	Height (m)
Q. pyrenaica control	18	42 ± 2	16.2 ± 2.5	12.8 ± 1.5
Q. pyrenaica treatment	14	42 ± 1	15.4 ± 2.3	13.1 ± 1.0
P. sylvestris old control	7	103 ± 5	49.1 ± 7.7	23.0 ± 5.1
P. sylvestris old treatment	6	97 ± 7	45.7 ± 8.1	20.6 ± 1.6
P. sylvestris young control	9	41 ± 4	19.4 ± 4.8	14.5 ± 2.2
P. sylvestris young treatment	8	45 ± 2	17.5 ± 5.0	13.6 ± 1.3

Intra-annual growth and cambial phenology

Micro-cores were sampled from six trees per species and treatment, three per age class in the case of *P. sylvestris*, every 7-15 days between April and November during 2012-2014. In order to minimize inter-annual bias due to previous year punching injuries, we generally avoided using the same trees on consecutive years. Samples were extracted with a Trephor (Rossi *et al.*, 2006c) and stored in a 70% ethanol solution at 4 ºC. *P. sylvestris* micro-cores sampled in 2012 were cut in 10-18-µm microsections with a sliding microtome. Since this technique was unsuccessful for *Q. pyrenaica*, the rest of the samples were dehydrated with increasing concentrations of ethanol and xylene and embedded in paraffin. Sections of 8 to 12 µm were cut with a rotary microtome, stained with Safranine O and Astra Blue, dehydrated with increasing concentrations of ethanol and xylene and mounted with either Canada balsam or a mixture of distyrene, tricresyl phosphate and xylene (DPX).

Stained microsections were observed under visible and polarized light to distinguish the different cell developmental stages: cambium, cell enlargement, cell-wall lignification and mature cells. For *P. sylvestris*, the number of tracheids in each phase was counted along three radial segments per date and then averaged. For *Q. pyrenaica*, photographs of the microsections were taken with an Olympus Colorview camera attached to a microscope. The number of cambium cells was counted and the width of earlywood and latewood increments at each cell development phase were measured along three segments with ImageJ (Schneider *et al.*, 2012). Due to the complex anatomy of *Q. pyrenaica*'s xylem, the cessation of the wall-thickening phase could not be assessed on this species. Growth rates were calculated as the ratio between the final number of tracheids or earlywood and latewood widths and the length of the elongation period, which represents a good approximation of the period of cell production.

Cumulative growth and wood anatomy

In November 2014, a micro-core including the period 2011-2014 was taken from every tree sampled to assess the accumulated effect of the treatment on wood anatomy. Anatomical sections were prepared according to the abovementioned protocol and photographed. Cell-wall thickness, lumen area and earlywood and latewood widths were measured along three segments in *P. sylvestris* with ImageJ (Schneider *et al.*, 2012). For *Q. pyrenaica*, earlywood and latewood widths and the area of earlywood vessels with a lumen area > 7500 μm^2 were measured. Earlywood vessel area was additionally measured in all the micro-sections used for the analysis of cambial phenology.

Given the different functionality of earlywood (EW) and latewood (LW), the ratio latewood width/ring width (LW/RW) was calculated for both species to assess whether the treatment had an effect on the proportion of each type of wood. Lumen areas were used to estimate vessel and tracheid radii, which were in turn used to calculate the hydraulic diameter (D_H). D_H, computed as $\Sigma d^5/\Sigma d^4$, where d is conduit diameter, is proportional to the hydraulic conductance given by the Hagen-Pouseuille law and, thus, a proxy of xylem hydraulic conductivity (Sperry *et al.*, 1994).

Leaf phenology

Crown phenology was recorded on the same dates of wood sampling. In 2012, leaf phenology was recorded by speciesat the stand level, whereas in 2013 and 2014 it was recorded individually on the trees used to study cambial phenology. The dates of bud-burst, end of full leaf unfolding and, in *Q. pyrenaica*, the beginning of autumn leaf discolouration, were identified.

Pinus sylvestris *physiological measurements*

Morning and afternoon net photosynthetic rate (A), stomatal conductance to water vapour (g_s) and mid-day leaf water potential (Ψ) were recorded for *P. sylvestris* in early- (June), mid- (July-August) and late- (September) summer between 2012 and 2014; except in 2014, when September measurements could not be taken due to technical problems. The inaccessibility of leaves of *Q. pyrenaica* trees at the rainfall exclusion plot precluded the possibility to carry out gas exchange measurements in this species. Six trees per treatment (three young trees and three old ones) were measured. Gas exchange measurements were performed on current-year needles exposed to direct sunlight with an LCpro+ portable photosynthesis system (ADC BioScientific Ltd., Hoddesdon, Herts, UK). June 2013 was an exception, when some measurements included previous-year needles due to late needle development. Leaf water potential was measured with a Scholander pressure chamber (PMS Instrument Co. 7000, Corvallis, OR, USA). For each tree, a subsample of the measured needles was scanned to measure needle area. Subsequently, they were dried and weighed to obtain their specific leaf area (SLA; cm^2 g^{-1}). SLA was used to recalculate photosynthetic rate (A) and stomatal conductance (g_s) on a leaf area basis.

The needles collected during morning measurements were ground to analyse carbon and nitrogen elemental concentration and stable carbon isotope ratio ($\delta^{13}C$). Samples of 3-4 mg were analysed using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The average $\delta^{13}C$ analytical precision was 0.1‰. $\delta^{13}C$ data were transformed to discrimination (Δ) following Farquhar, Ehleringer, & Hubick (1989):

$$\Delta \approx \frac{\delta^{13}C_a - \delta^{13}C}{1 + \delta^{13}C} \qquad [5.1]$$

Source air $\delta^{13}C$ ($\delta^{13}C_a$) data were obtained from NOAA Earth System Research Laboratory (NOAA- ESRL, http://www.esrl.noaa.gov/gmd/dv/data/).

Needle carbon and nitrogen concentrations on a mass basis were used to calculate needle C/N ratio and photosynthetic nitrogen use efficiency (PNUE), which is the ratio between photosynthetic rate and leaf nitrogen content.

Environmental data

Air temperature and humidity were measured with a HOBO Pro series temperature/relative humidity data logger (Onset, Cape Cod, Massachusetts, USA). Precipitation data and missing temperature and humidity data were obtained from the Spanish Meteorological Agency (AEMET). Raw climatic data were used to calculate the average daily vapour pressure deficit (VPD) and the monthly standardised precipitation-evapotranspiration index (SPEI) (Vicente-Serrano *et al.*, 2010). Soil relative extractable water (REW) was calculated following Granier (1987):

$$REW = \frac{\theta - \theta_m}{\theta_{FC} - \theta_m} \qquad [5.2]$$

where θ is the actual soil water content measured by the humidity sensors, θ_m the minimum soil water content registered and θ_{FC} the soil water content at field capacity.

Data analysis and statistics

In order to analyse cambial and leaf phenology phases, cell count, ring width and leaf phenology data were transformed into presence/absence data. Logistic functions were used to estimate the day of the year (DOY) at which each cambial and foliar developmental phase began and ended. Differences in phenology between treatments were assessed fitting different generalized linear mixed models (GLMM) with a binomial distribution and a random intercept associated with tree. The assessed fixed factors were DOY, Year, Treatment and the interaction 'Treatment × Year'. The Akaike Information Criterion (AIC) was used to assess the information contained in each GLMM fitted. Lower values of AIC indicate a better fit. A model was considered to have a higher explanatory power than another when the difference in AIC (ΔAIC) between models was ≥ 2 (Burnham & Anderson, 2002). If the difference was < 2, the model with fewer variables was selected.

Because data were not normally distributed and measures were repeated within individuals, differences between treatments in total growth, growth rates and wood anatomical features were assessed using GLMMs with a Gamma distribution and an identity link function, except for *Q. pyrenaica* growth rates, for which a log link function was used. We fitted GLMMs with the fixed effects Treatment, Year and the interaction 'Treatment × Year' and a random intercept to account for correlation within trees. If the best model, given by its AIC, included the interaction 'Treatment × Year', we tested the differences in specific years using Tukey contrasts.

For gas exchange and leaf chemical properties, given that within the same sampling date only one measurement was taken per tree, differences between treatments for each sampling date were assessed using Kruskal-Wallis analysis of variance. Regarding differences among measuring dates, A, Ψ, C/N and PNUE were tested using linear mixed models (LMMs), whereas g_s and Δ were tested using GLMM with a Gamma distribution and a log link function due to the non-normality of the data.

All analyses were carried out with R version 3.1.1 (R Core Team, 2014). GLMMs were fit with the packages “lme4” (Bates *et al.*, 2014) and “glmmADMB” (Fournier *et al.*, 2012). LMMs were fit with the package “lme4” (Bates *et al.*, 2014). Multiple mean comparisons (Tukey contrasts) were performed with the package “multcomp” (Hothorn *et al.*, 2008).

5.3 Results

Annual mean temperatures and total precipitation during the study years were 9.7 ºC and 565 mm in 2012, 9.1 ºC and 775 mm in 2013 and 10.1 ºC and 820 mm in 2014. Thus, all three study years were warmer than the long-term average, whereas 2013 had average precipitation, 2012 was drier and 2014 wetter than average. This was translated into generally dry conditions, particularly during the summers of 2012 and 2013, as indicated by the negative monthly SPEI, high VPD – that reached punctual values of 3 kPa in 2012 – and low REW during that period of the year (Figure 5.1). Additionally, although REW greatly fluctuated along the year and during the study period, soil humidity in the top 50 cm was reduced on average 30% at the young-tree *P. sylvestris* plot, 40% at the *Q. pyrenaica* plot and 80% at the old-tree *P. sylvestris* plot, compared to the control (Figure 5.1).

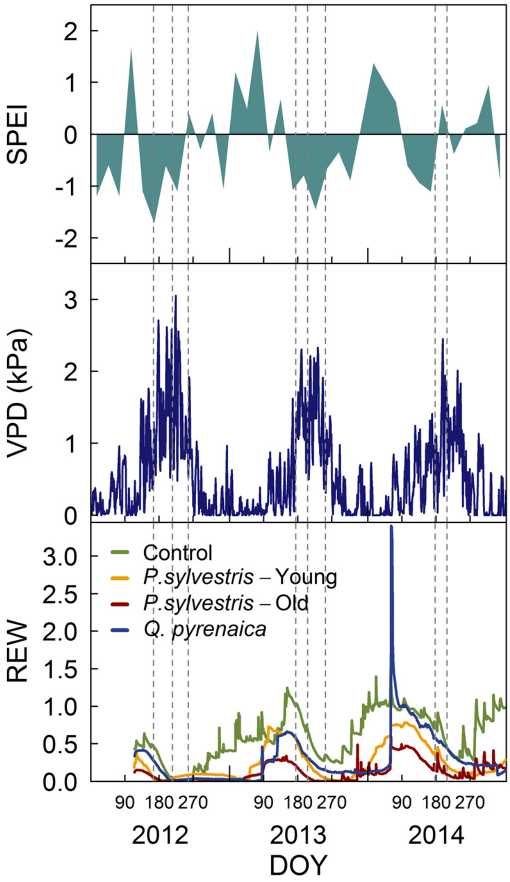


Figure 5.1. Monthly standardised precipitation-evapotranspiration index (SPEI), daily vapour pressure deficit (VPD) and daily relative extractable water (REW) during the study period. REW is given for each of the rainfall exclusion plots and the control area. Vertical dashed lines mark the days in which gas exchange measurements were taken.

Effect of rainfall exclusion on cambial and leaf phenology and intra-annual growth dynamics

In *Pinus sylvestris*, the average number of cambium cells during dormancy was 6 ± 2, whereas active cambium had on average 10 ± 3 cells (Figure 5.2). The number of cambium cells, both active and in dormancy, was significantly lower in trees subjected to rainfall exclusion only in 2013. *P.*

sylvestris xylogenesis started between mid-April and early-May (DOYs 105-122), with cell wall-thickening starting on average two to five weeks later (Figure 5.2). The first cells completed maturation between the end of May and the end of June (DOYs 142-176), depending on the year (Figure 5.2). Xylem enlargement ended between mid-July and early-September (DOYs 203-234), depending on the year, with no reactivation of growth occurring after the summer. Ring maturation was completed in October- early-November (DOYs 283-299). No significant differences between treatments were found in the beginning of the enlargement or wall-thickening phases (Table 5.2), whereas the treatment significantly influenced the end of both phases, with trees subjected to rainfall exclusion finishing growth one to three weeks earlier than control trees (Table 5.2 and Figure 5.2). The strength of the effect depended, however, on the year. The number of enlarging and wall-thickening cells tended to be lower under treatment than in control trees (Figure 5.2).

Table 5.2. Effect of treatment (T) and year (Y) on the onset and cessation of *Pinus sylvestris* phenological phases as given by the differences in the Akaike Information Criterion (ΔAIC) of the fitted GLMMs. ΔAIC was calculated as the difference between the AIC of the model being assessed and the AIC of the model with the lowest AIC (i.e. the model with ΔAIC =0). The best model is marked in bold. E: Enlargement phase; W: Wall-thickening phase; M: Mature cells; ND: Needle development; DOY: Day of the year.

	Phase onset				**Phase cessation**		
Model	**E**	**W**	**M**	**ND**	**E**	**W**	**ND**
DOY	13.93	18.50	34.76	38.60	67.83	16.02	53.04
DOY + Y	**0.00**	**0.00**	**0.00**	**0.00**	4.61	5.88	**0.00**
DOY + T	14.96	20.30	36.73	42.11	64.81	13.47	31.11
DOY + T+ Y	4.64	1.99	2.28	23.34	**0.00**	3.15	19.21
DOY + T + Y + (T × Y)	7.01	1.74	3.39	42.50	2.65	**0.00**	35.91

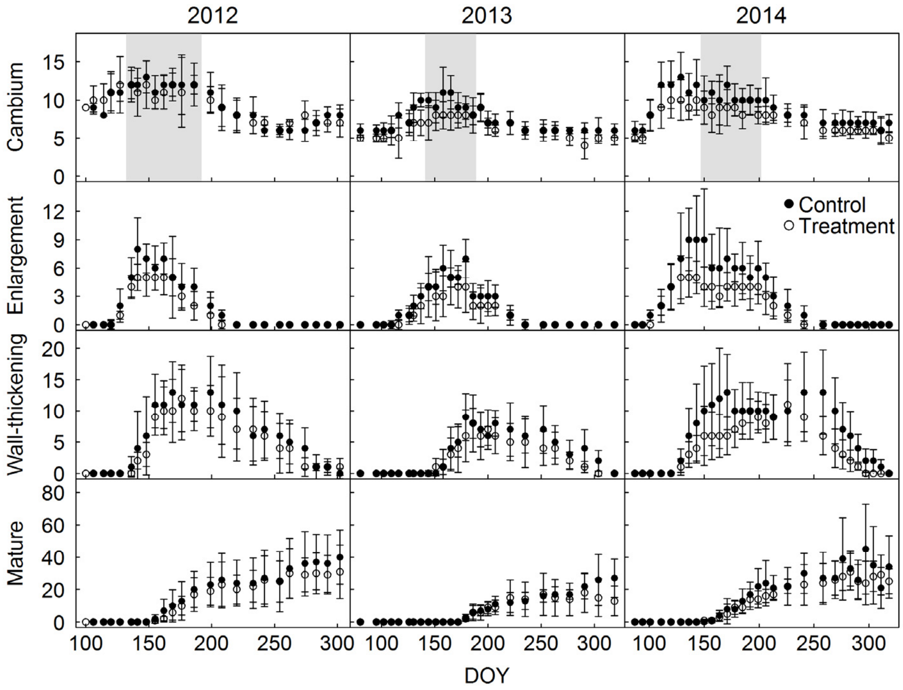


Figure 5.2. *Pinus sylvestris* intra-annual growth dynamics (number of tracheids at each developmental stage) in control and trees subjected to rainfall exclusion. Shaded areas mark the period of needle development.

Regarding *Quercus pyrenaica*, the average number of cambium cells during dormancy was 8 ± 1, whereas active cambium had on average 10 ± 2 cells (Figure 5.3). There were no significant differences between treatments in the number of cambium cells. *Q. pyrenaica* xylem growth presented a bimodal pattern, corresponding to the peaks in earlywood vessel and latewood formation (Figure 5.3). Earlywood growth onset occurred in late-March - early-April (DOYs 91-94). Earlywood vessel lignification started one to eight weeks later (Figure 5.3). Latewood growth started in June - early-July (DOYs 163-182) and latewood lignification began up to two weeks later (Figure 5.3). Latewood enlargement ceased at the end of July-mid-August (DOYs 211-231), with no growth resumption after the summer stop. No significant differences were observed between treatments in *Q. pyrenaica* cambial phenology (Table 5.3 and Figure 5.3), although both earlywood and latewood growth tended to end earlier under rainfall exclusion. Likewise, rainfall exclusion seemed to have an effect on the length of the period between the cessation of earlywood enlargement and the onset of latewood enlargement (longer under rainfall exclusion); however, this was non-significant. There were no significant differences between treatments in the earlywood and latewood widths at each developmental stage, although enlarging earlywood and latewood widths tended to be lower under rainfall exclusion than in control trees, particularly in 2014 (Figure 5.3).

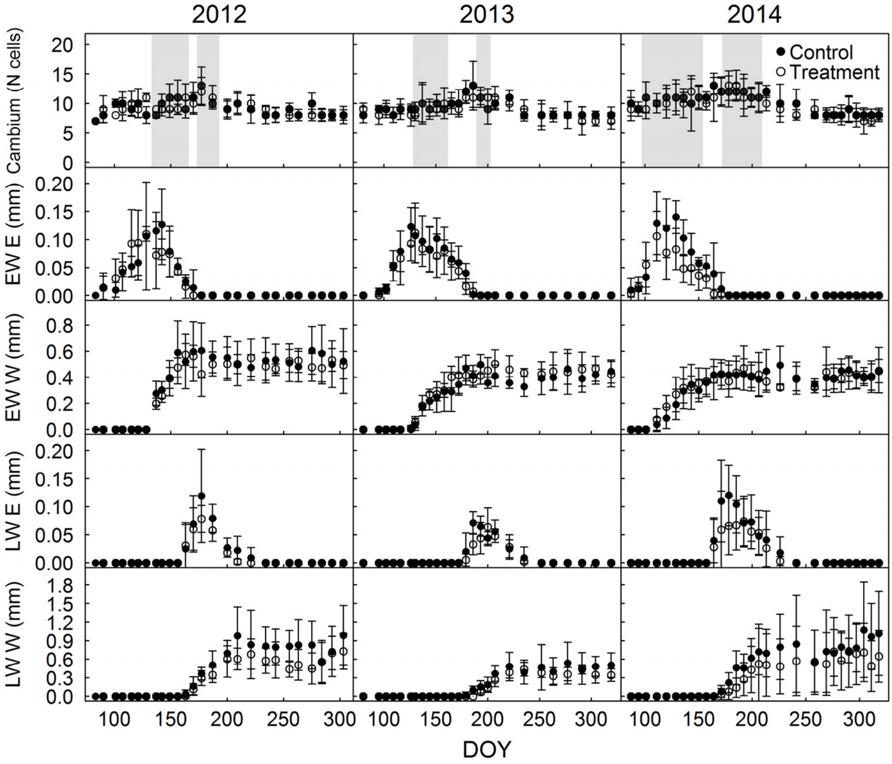


Figure 5.3. *Quercus pyrenaica* intra-annual growth dynamics for control and trees subjected to rainfall exclusion. Shaded areas mark the periods of leaf development. EW E: Enlarging earlywood; EW W: Wall-thickening and mature earlywood; LW E: Enlarging latewood; LW W: Wall-thickening and mature latewood.

Table 5.3. Effect of treatment (T) and year (Y) on the onset and cessation of *Quercus pyrenaica* phenological phases. Differences in the Akaike Information Criterion (ΔAIC) of the fitted GLMMs were calculated as the difference between the AIC of the model being assessed and the AIC of the model with the lowest AIC (i.e. the model with ΔAIC =0). The best model (i.e. the one with fewer number of parameters of those with ΔAIC<2) is marked in bold. EWE: Enlarging earlywood; EWW: Wall-thickening and mature earlywood; LWE: Enlarging latewood; LWW: Wall-thickening and mature latewood; LD1: Spring leaf development; LD2: Summer leaf development; ALD: autumn leaf discolouration; DOY: Day of the year.

	Phase onset							**Phase cessation**			
Model	**EWE**	**EWW**	**LWE**	**LWW**	**LD1**	**LD2**	**ALD**	**EWE**	**LWE**	**LD1**	**LD2**
DOY	**0.00**	83.20	36.12	24.54	203.40	101.75	109.49	48.82	**0.73**	24.47	24.30
DOY + Y	3.42	**0.00**	**0.20**	**0.00**	**0.00**	**1.14**	**1.11**	**0.00**	0.00	**0.00**	**0.00**
DOY + T	1.80	85.19	37.18	21.27	205.34	102.70	111.49	50.54	2.74	25.64	26.89
DOY + T + Y	5.22	1.04	0.00	15.25	2.02	0.00	0.00	0.69	2.01	1.12	8.59
DOY+T +Y+(T×Y)	3.95	4.55	3.10	19.61	6.08	4.08	4.17	3.88	2.89	2.96	19.28

Budburst occurred synchronously within each species; in *P. sylvestris,* between DOYs 132 and 147 (Figure 5.2). In *Q. pyrenaica*, two budburst episodes occurred annually: the main one, between DOYs 97 and 133, and a secondary one, between DOYs 173 and 189 (Figure 5.3). *Q. pyrenaica* leaf discolouration started between DOYs 233 and 279, depending on the year. Leaf phenology was unaffected by treatment in both species (Tables 5.2 and 5.3).

Effect of rainfall exclusion on growth rates, total growth and wood anatomy

No significant differences were found between treatments in *P. sylvestris* growth rates for any of the studied years (Figure 5.4). For both treatments, growth rates were highest in 2012, the driest year. Control trees tended to have more tracheids per ring than trees subjected to rainfall exclusion, even in 2011 before the treatment was applied. This difference was, however, significant in 2012, the driest year (Figures 5.2 and 5.4). None of the other anatomical features assessed (LW/RW ratio, D_H and cell-wall-thickness) showed differences between treatments that did not exist pre-treatment (Figure 5.4). The ratio LW/RW was lowest in 2012, the driest year, and highest in 2014, the wettest. Cell-wall thickness behaved similarly, whereas D_H decreased in 2014, although this difference was not significant for trees under rainfall exclusion.

Despite the lack of differences in growth rates and anatomical features between treatments, one of the old *P. sylvestris* trees subjected to drought died between the 2013 and 2014 growing seasons. During 2013, cambium was mostly inactive and formed at most four tracheids, which in many cases did not lignify (Figure S5.1 in Supporting Information). Cambial inactivity occurred before crown damage was evident. Since the growth of this tree before the treatment was applied did not differ from that of other trees in the stand, its death was likely a direct consequence of the intensification of drought by the rainfall exclusion treatment. There was, however, presence of mistletoe (*Viscum album* L.) on this tree, which might have exacerbated the effect of the treatment.

No significant differences were found between treatments in *Q. pyrenaica* growth rates, neither for EW nor for LW, although LW rates tended to be lower under exclusion (Figure 5.5). Both growth rates were lowest in 2013, although this decrease was mostly non-significant. Ring width and the LW/RW ratio decreased in trees subjected to drought over the study period, becoming the difference between treatments significant in 2014 (Figure 5.5). D_H did not differ between treatments, nor did it significantly fluctuate between years. None of the anatomical features assessed were significantly different between treatments before the rainfall exclusion was applied.

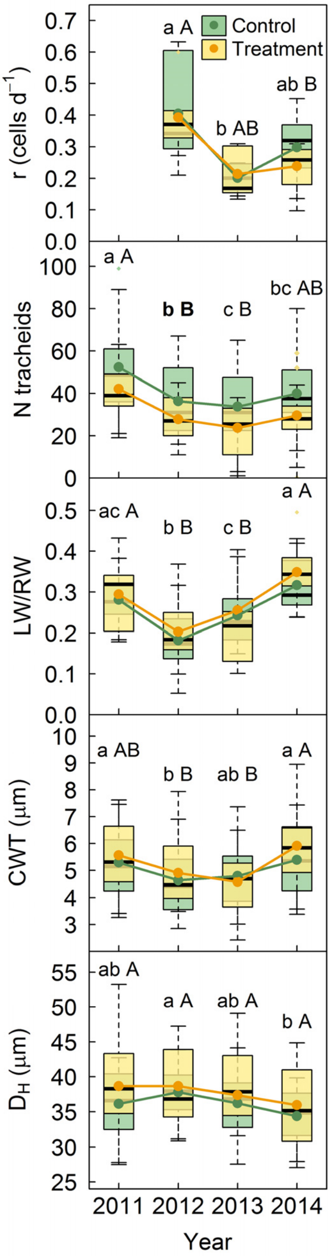


Figure 5.4. Growth rates and anatomical properties for *P. sylvestris* in control trees and trees subjected to rainfall exclusion. Lower case letters indicate differences among years in control trees and capital letters indicate differences among years in treated trees. Bold letters indicate differences between treatments. r: growth rates; N tracheids: number of tracheids; LW/RW: proportion of latewood as a function of ring width; CWT: cell-wall thickness; D$_H$: hydraulic diameter.

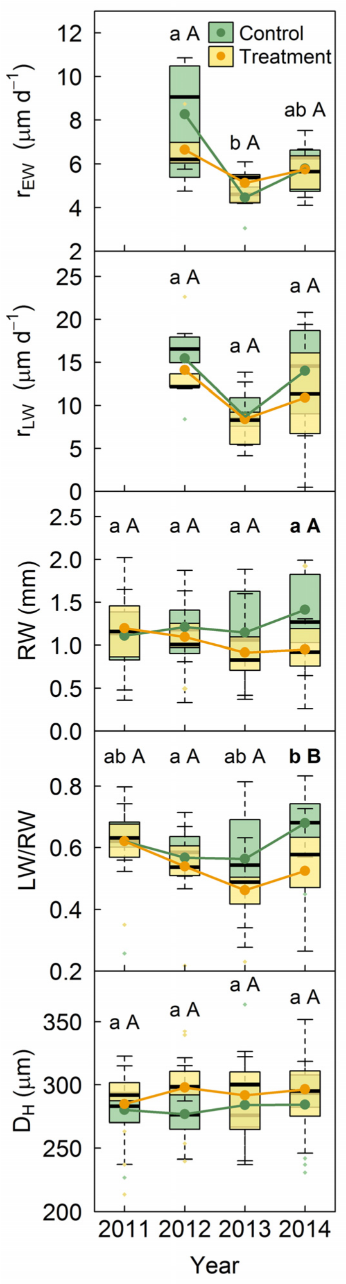


Figure 5.5. *Quercus pyrenaica* growth rates and anatomical properties per treatment. Lower case letters indicate differences among years in control trees and capital letters indicate differences among years in treated trees. Bold letters indicate differences between treatments. r_{EW}: earlywood growth rate; r_{LW}: latewood rate; LW/RW: proportion of latewood as a function of ring width; D_H: hydraulic diameter.

Effect of rainfall exclusion on Pinus sylvestris *gas exchange*

Given that mid-summer measurements (i.e. July-August) were consistently taken over the years and better represent summer conditions, only those measurements are reported here. For the complete measurements see Figures S5.2 and S5.3 in Supporting Information. No significant differences were found between treatments in needle C, N and C/N ratio (Figures 5.6 and S5.2). However, Δ was significantly lower in trees growing under rainfall exclusion than in control trees in 2012 and 2013. No differences in A, g_s or PNUE were observed between treatments, although g_s was marginally lower in trees subjected to rainfall exclusion than in control trees (Figures 5.6 and S5.3). Ψ was significantly more negative in trees subjected to rainfall exclusion than in control ones in 2013. C/N and A did not suffer significant inter-annual variation, although C/N was higher and A marginally lower in 2012, the driest year of the three studied, particularly under rainfall exclusion. PNUE was highest in 2013. Δ significantly increased along the study period for both treatments, although the change was steeper for trees subjected to rainfall exclusion, being lowest in 2012. g_s greatly varied among years, being lowest in 2012, the driest year, and highest in 2013, especially in control trees. Leaf water potentials barely changed among years under the rainfall exclusion treatment but were significantly less negative in control trees in 2013 (Figures 5.6 and S5.3).

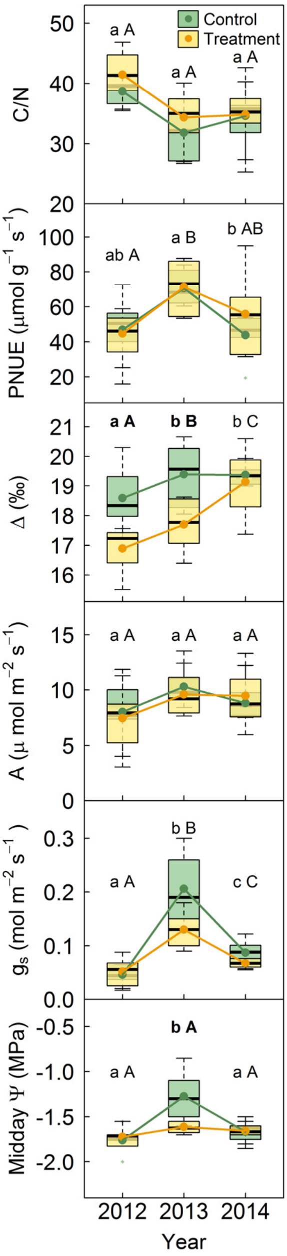


Figure 5.6. Needle chemical properties and gas exchange measurements for mid-summer (July-August) *P. sylvestris* samples per treatment. Lower case letters indicate differences among years in control trees and capital letters indicate differences among years in treated trees. Bold letters indicate differences between treatments. C/N: C/N concentration ratio; PNUE: photosynthetic nitrogen use efficiency; Δ: carbon discrimination; A: morning photosynthetic rate; g_S: morning stomatal conductance; Ψ: leaf water potential.

5.4 Discussion

Drought affects cambial rather than leaf phenology

The growing season tended to end earlier in *Q. pyrenaica* and was significantly shorter in *P. sylvestris* under the rainfall exclusion treatment than in control trees. Conversely, the onset of xylogenesis was not affected by the treatment, indicating that factors other than water availability, such as photoperiod or temperatures (Rossi *et al.*, 2008a; Pérez-de-Lis *et al.*, 2016), triggered the resumption of cambial activity. Thus, as hypothesized, the growing season length was shorter under drought, particularly in *P. sylvestris*, which, therefore, demonstrated a higher vulnerability to drought. Belien *et al.* (2012) and D'Orangeville *et al.* (2013), however, did not observe an effect of drought on cambial phenology in boreal species, neither at the onset nor at the cessation of xylogenesis. Nevertheless, concurring with our results, Eilmann *et al.* (2011) found that trees under drought had shorter growing seasons due to an earlier cessation of growth rather than a later onset. This is consistent with a shift in the allocation of resources under stress, favouring carbon storage, bud formation or fine root growth over secondary growth (Waring, 1987; Wiley *et al.*, 2013).

No differences were found in leaf phenology as a result of the rainfall exclusion treatment. Likewise, Adams *et al.* (2015) found that *Juniperus monosperma* (Engelm.) Sarg. was unaffected by a drought treatment, although they observed a delay in *Pinus edulis* Engelm leaf phenology under the treatment in an already dry year. Similarly, Ogaya & Peñuelas (2004) found a delay in *Arbutus unedo* L. crown phenophases as a result of a drought experiment, but not in *Quercus ilex* L. and *Phillyrea latifolia* L. Therefore, the severity of drought necessary to trigger shifts in leaf phenology seems to be highly species-specific (Peñuelas *et al.*, 2004).

Species-specific plasticity in anatomical traits in response to drought

Contrary to what we hypothesized, no cumulative effect of the treatment was observed in *P. sylvestris* anatomical traits. Control trees tended to have higher number of tracheids than treated trees, even before the rainfall exclusion treatment was applied. This difference was, however, significant in 2012, the driest year studied. Given the lack of differences in growth rates between treatments, this is probably due to tree-intrinsic traits, such as the number of cambial cells, and treatment-derived effects, such as the shorter growing season found in trees subjected to drought. Experiments performed in different conifer species have found an increase in the number of cambial cells and cell production in irrigated compared to non-irrigated trees (Abe *et al.*, 2003; Eilmann *et al.*, 2009; de Luis *et al.*, 2011; Balducci *et al.*, 2013). On the other hand, Belien *et al.* (2012) did not find differences in the number of tracheids formed under drought and D'Orangeville *et al.* (2013) only found differences in tree ring size on the second year of their drought experiment. These variability suggests that the response of cambial production to drought is highly dependent on its severity.

Similarly to Balducci *et al.* (2013), we did not observe differences in *P. sylvestris* wood anatomy between treatments. Nonetheless, hydraulic diameter tended to be higher and cell-wall thickness lower in the driest year, particularly in control trees, indicating that anatomical traits respond to climatic variability. By contrast, Belien *et al.* (2012) and D'Orangeville *et al.* (2013) found that the tracheids of trees under drought had smaller lumen size and thicker cell walls than those of control trees. Eilmann *et al.* (2009, 2011), however, also found greater lumen diameters under drought in *P. sylvestris*. Thus, this species may tend to increase water-transport efficiency under drought, which, given the work by Martínez-Vilalta *et al.* (2009), may not imply an increase in its vulnerability to cavitation.

Conversely, the more-drought tolerant *Q. pyrenaica* did not show differences in earlywood vessel hydraulic diameter between treatments or years, whilst other studies in sub-Mediterranean *Quercus* spp. have found both wider (Corcuera *et al.*, 2004, 2006) and narrower (Eilmann *et al.*, 2009) earlywood vessels under drought. However, consistent with these studies, we found that *Q. pyrenaica* trees subjected to rainfall exclusion suffered an increasing reduction in latewood width along the study period. This hypothesized cumulative effect of the rainfall exclusion treatment might have been more evident in this species than in *P. sylvestris* due to the heavy reliance of ring-porous species on stored carbohydrates to form their annual rings (Michelot *et al.*, 2012b), thus intensifying the experiment's influence as reserves were not fully replenished. Although the treatment did not affect the size of earlywood vessels and, thus, the capacity of the main conductive system remained unaltered, the reduction in latewood could ultimately challenge hydraulic performance in this species as latewood vessels provide water transport for several years once earlywood vessels are not functional due to cavitation (Granier *et al.*, 1994; Umebayashi *et al.*, 2010).

Even though the changes in tracheid size observed may not imply a shift in the amount of carbon allocated to xylem formation (Cuny *et al.*, 2014), *P. sylvestris* formed fewer tracheids in drier years. Hence, this species uses less carbon but builds a more effective, albeit potentially more vulnerable, water transport system under drought. By contrast, *Q. pyrenaica* maintained earlywood vessel size at the expense of reducing latewood and, therefore, invested less carbon in xylem formation but at a greater risk of hydraulic failure, particularly in extremely dry years. Thus, although with different effects on their hydraulic system, both species followed carbon-saving strategies. This is consistent with studies that indicate that trees adjust to drought by firstly limiting secondary growth rather than carbon assimilation (Waring, 1987; McDowell *et al.*, 2008; Oberhuber *et al.*, 2011; Adams *et al.*, 2015; Dickman *et al.*, 2015). This limitation was evident in the old *P. sylvestris* tree that died during the experiment, which barely had any cambial activity prior to its death while the crown was still functioning. The stress suffered by this tree was probably intensified by the presence of mistletoe, which due to its lack of stomatal control induces stomatal closure in *P. sylvestris*, particularly under drought (Galiano *et al.*, 2011; Zweifel *et al.*, 2012). This, together with the inherent stronger hydraulic constraints of large trees, may have impaired the tree's hydraulic performance, causing its death (McDowell *et al.*, 2008; Anderegg *et al.*, 2013; Bennett *et al.*, 2015). Albeit an isolated event within our plots, it is not casual within the study area (Gea-Izquierdo *et al.*, 2014). This could, therefore, portend high vulnerability to drought-induced mortality in *P. sylvestris*, particularly in older trees, as previously observed (Martínez-Vilalta & Piñol, 2002; Vilà-Cabrera *et al.*, 2011).

Tight stomatal control in Pinus sylvestris *determines its response to drought*

The minor reduction observed in leaf N concentrations in trees subjected to drought, probably due to a lower nitrogen uptake, did not affect PNUE, which was unaltered by the rainfall exclusion treatment. PNUE was, however, highest in 2013, suggesting a climatic influence on this variable. PNUE inter-annual changes followed those in g_s and, to a lesser extent, A. This is consistent with studies that show that decreases in g_s reduce PNUE, while increasing intrinsic water use efficiency (iWUE=A/g_s) (Limousin *et al.*, 2015).

Needles of trees under the rainfall exclusion treatment had lower Δ and, thus, higher iWUE, than control trees. The effect of the treatment was, however, less evident in instantaneous gas exchange measurements, which only showed marginal differences between treatments in A and g_s. Due to the non-linearity of the relationship between A and g_s, stomatal closure causes a proportionally greater decrease in transpiration than in photosynthesis (Limousin *et al.*, 2015), which may explain the higher inter-annual variability observed in g_s and Δ, both lowest in the driest year, than in A. Nonetheless, photosynthetic rates are tightly coupled to stomatal control of water use and, therefore, long-term stomatal limitations could significantly alter the tree's carbon reserves (Galiano *et al.*, 2011).

Midday leaf water potentials followed changes in g_s. Midday Ψ observed at our site were above those reported to cause significant xylem cavitation (Martínez-Vilalta & Piñol, 2002; Poyatos *et al.*, 2008) and the absolute minimum values reported for the species (-2.5 MPa; Martínez-Vilalta *et al.* 2009). Hence, thanks to the tight control exerted by stomata to prevent severe damage to the hydraulic system (Irvine *et al.*, 1998; Poyatos *et al.*, 2008), *P. sylvestris* may afford increasing current year tracheid size under dry conditions, as observed in this and other studies (Eilmann *et al.*, 2009, 2011), in order to improve water transport efficiency.

Challenges of rainfall exclusion experiments

Rainfall exclusion and irrigation experiments have been central for improving our understanding of how tree physiology responds to drought (Beier *et al.*, 2012). However, as with any field manipulation, these experiments do not come without shortcomings, particularly when they are performed in mature trees at their natural settings. Our rainfall exclusion treatment was successful at decreasing soil water content. However, the experimental plot for older *P. sylvestris* trees suffered a stronger decrease in soil humidity than the other two, despite their similar design, which may explain the stronger response to drought in those trees, with one of them dying after two years. These differences among plots may be due to plot singularities in terms of canopy interception, micro-topography or species-specific and age-specific hydric requirements. Moreover, trees might have been able to cope with imposed drought by absorbing water from deeper soil layers

(Belien *et al.*, 2012) or from intercepted rainfall (Breshears *et al.*, 2008). Additionally, the field and laboratory intensive requirements characteristic of cambial phenology, wood anatomy and gas exchange studies resulted in small sample sizes, which probably obscured statistically significant effects of the treatment (Metz *et al.*, 2016). Despite these shortcomings, our study provides valuable information on how these traits respond to an intensification of natural droughts.

Conclusions

We demonstrated that drought can affect xylem development in *P. sylvestris* and *Q. pyrenaica*. We observed an earlier cessation of xylem enlargement in our rainfall exclusion experiment, particularly in *P. sylvestris*, without an effect on leaf phenology, together with a lower carbon allocation to tracheid formation in *P. sylvestris* and latewood formation in *Q. pyrenaica*. These findings are consistent with a shift in the allocation of resources under drought stress, favouring carbon storage or bud formation over xylem growth (Waring, 1987; Oberhuber *et al.*, 2011; Wiley *et al.*, 2013; Adams *et al.*, 2015; Dickman *et al.*, 2015). *P. sylvestris* stomatal conductance presented high inter-annual variability and lower values under the treatment in order to maintain leaf water potentials above cavitation-risk levels and maximize photosynthetic uptake and, thus, intrinsic water use efficiency. This suggests that photosynthesis in *P. sylvestris* may also be compromised by increasing drought stress, eventually affecting long-term carbon storage. The outcomes from the experiment indicate that both *P. sylvestris* and *Q. pyrenaica* are potentially vulnerable to the increasing drought stress expected with the ongoing climate change. The death of an old *P. sylvestris* tree under the rainfall exclusion treatment, together with similar events in the study area (Gea-Izquierdo *et al.*, 2014), may, however, forecast a higher risk of drought-induced mortality in this species at its low altitudinal limit.

5.5 Acknowledgements

The authors would like to thank the funding from the Spanish Ministry of Economy and Competitiveness for the projects AGL2013-46028R and AGL2010-21153-C02, and its associated PhD scholarship for L.F.U., as well as the Madrid Regional Government for the project S2013/MAE-2760. We would also like to thank A. Bachiller, E. Garriga, A.M. González, A. Parras, M.L. Peña, L. Schneider, and E. Viscasillas for their field and laboratory assistance. Likewise, we highly appreciate the advice provided by I. García-González, G. Pérez de Lis and G. Guada regarding micro-core processing.

5.6 Supporting information

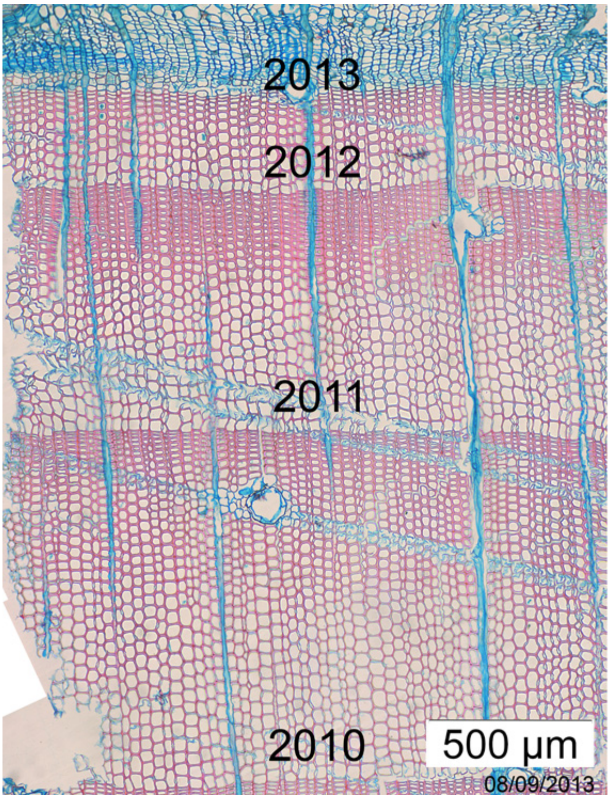


Figure S5.1. Section of the dying *Pinus sylvestris* tree showing inactive cambium and non-lignified cells formed during the 2013 growing season.

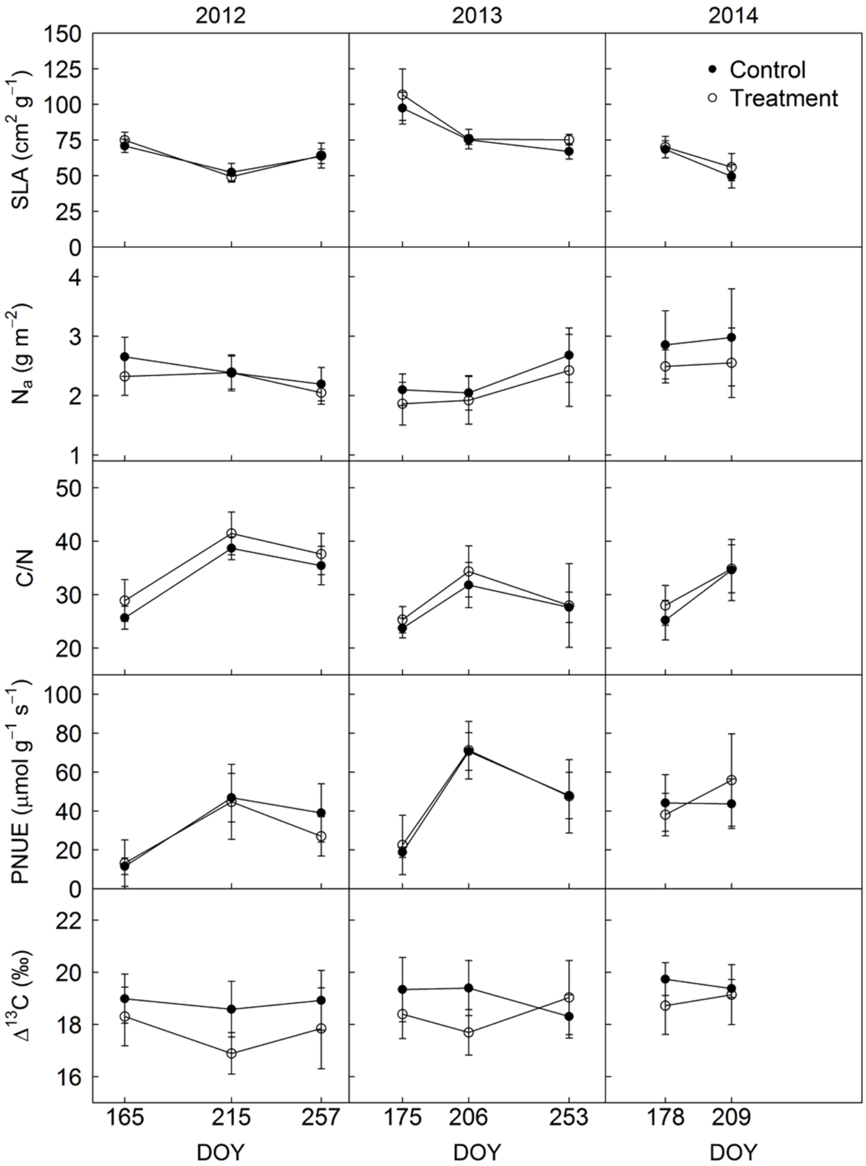


Figure S5.2. *P. sylvestris* needle chemical properties per date. SLA: specific leaf area; N$_a$: nitrogen concentrations per unit of leaf area; C/N: C/N concentration ratio; PNUE: photosynthetic nitrogen use efficiency; Δ^{13}C: carbon discrimination.

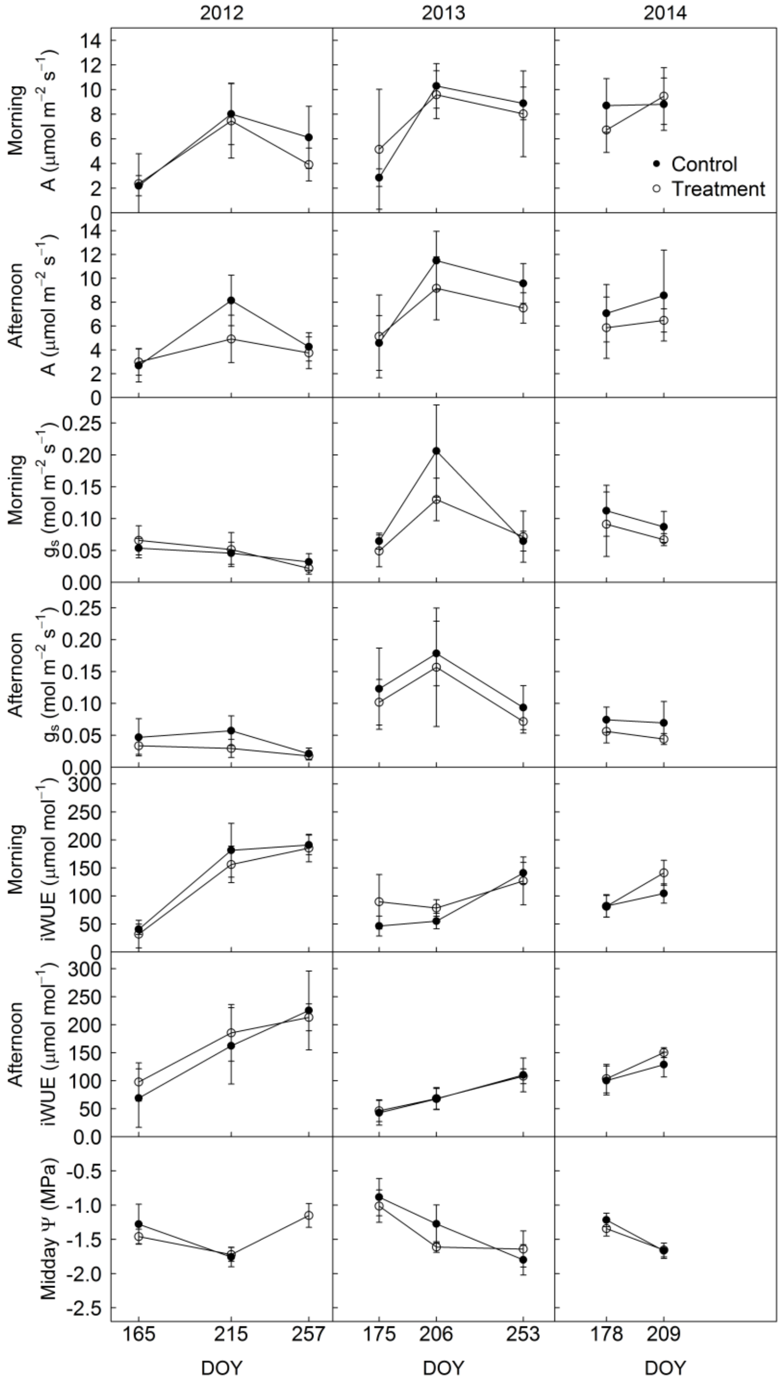
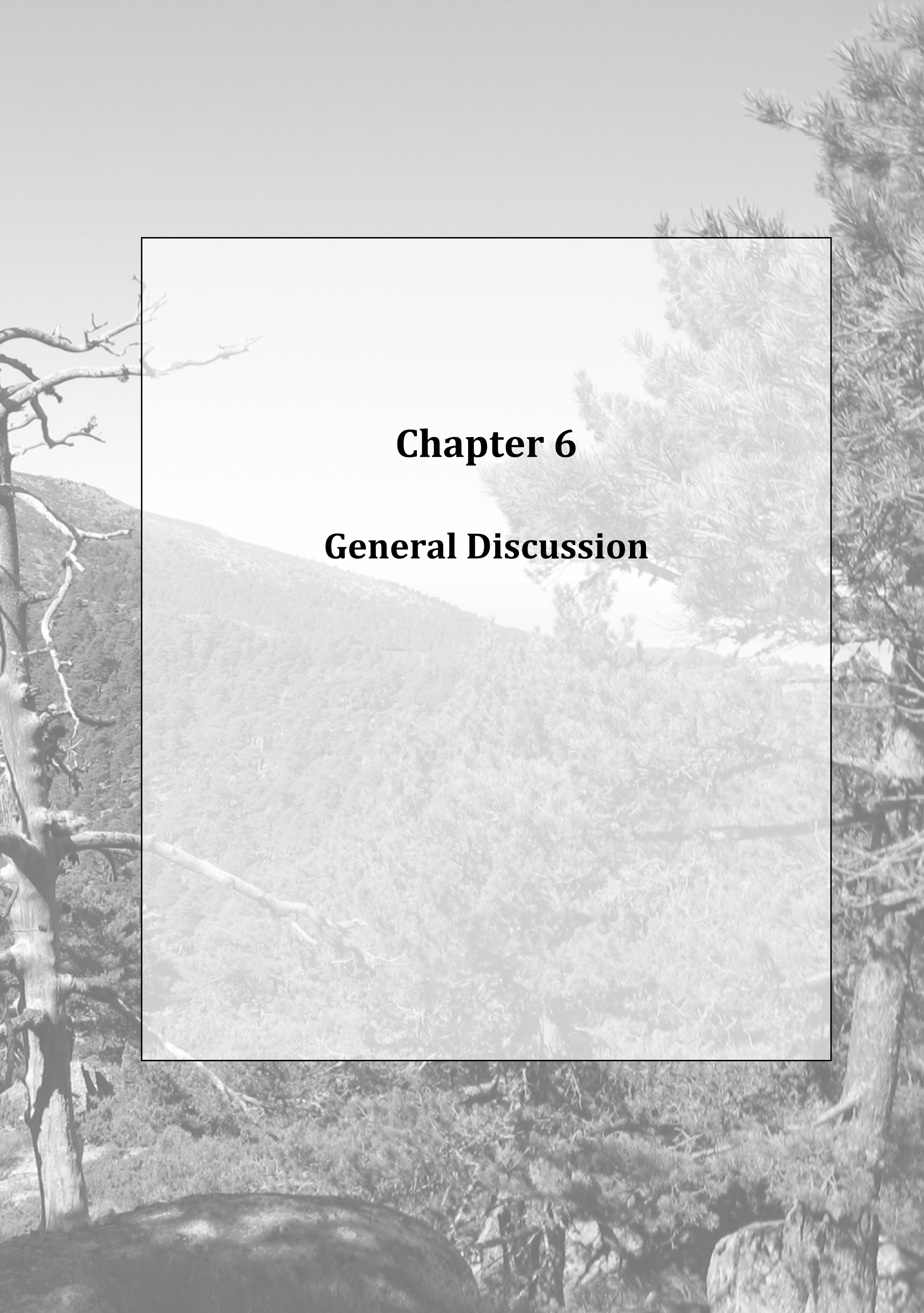


Figure S5.3. *Pinus sylvestris* morning and afternoon gas exchange measurements per date. A: photosynthetic rates; g_s: stomatal conductance; iWUE: intrinsic water use efficiency; Ψ: leaf water potential.



Chapter 6

General Discussion

The ultimate goal of the research carried out throughout this thesis was to ascertain how forest species could respond to global change in order to inform the development of forest management strategies. Specifically, the main objective was to assess the physiological response of *P. sylvestris*, *Q. pyrenaica* and *Q. faginea* to global change processes, particularly climate change, from both a stand- and a tree-level perspective. This objective was fulfilled through the different research studies performed. More concretely, in Chapter 2 the growth projections obtained through the fitted nonlinear models indicated that the less drought-tolerant *P. sylvestris* would be more negatively affected by a warming climate than the sub-Mediterranean *Q. pyrenaica* and *Q. faginea*, even under reduced competition. In Chapter 3 we found that the intrinsic water use efficiency (iWUE) of all three species increased along time due to the combined effect of rising atmospheric CO_2 concentrations, climate and age, whereas competition had no significant effect. Growth was, however, highly influenced by competition and rarely enhanced as a result of increasing iWUE. In Chapter 4 we found that *P. sylvestris* xylogenesis was mostly triggered by temperatures and water deficit, whereas *Q. pyrenaica* cambial phenology was mainly controlled by photoperiod and temperatures. *Q. pyrenaica* had higher photosynthetic performance than *P. sylvestris*, partially due to the tighter stomatal control in the latter, reflecting a stronger susceptibility to drought in the conifer. Concurring with these results, in Chapter 5 we found an earlier cessation of xylem enlargement under the drought treatment, particularly in *P. sylvestris*, as well as lower stomatal conductance in this species in response to enhanced water stress. *Q. pyrenaica*, on the other hand, mostly responded to the rainfall exclusion treatment with a significant reduction in latewood width.

6.1 Climatic drivers of tree physiology: inferences from a multi-scale approach

An evident effect of climatic variability on the analysed tree functional traits, particularly growth, was observed throughout the different research studies of this thesis. Results in Chapter 2 indicated that temperature had a stronger influence on both *Quercus* species growth than precipitation, whereas *P. sylvestris* responded more strongly to the latter. This is, to some extent, consistent with the results found in chapters 4 and 5, with the length of the growing season being more influenced by temperatures in *Q. pyrenaica* and water deficit in *P. sylvestris*.

According to the nonlinear models fitted in Chapter 2, both *Quercus* spp. growth responded more strongly to maximum spring temperatures. *Q. pyrenaica* avoids early-spring frosts through a temperature-regulated phenology in order to prevent freeze-thaw-induced cavitation and frost damage to the leaves (Chapter 4), hence the significance of spring temperatures on the growth of this species. Given their ecological similarities, this strategy may also be followed by *Q. faginea*. In fact, Sanz-Pérez *et al.* (2009) and Albuixech *et al.* (2012) found that most of *Q. faginea* growth occurred in early spring. Conversely, *P. sylvestris* responded to maximum temperatures of the hydrological year (Chapter 2). This evergreen species is able to photosynthesize and thus, accumulate carbon, all year round, even at air and soil temperatures near 0 ºC (Strand *et al.*, 2002), which could explain the significance of temperatures over a wider time span. Nevertheless, low temperatures constrain xylem growth (Rossi *et al.*, 2008), in such way that mild temperatures have a positive effect on growth by inducing an earlier growth onset (Chapter 4) since they positively influence growth-related hormones (Aloni, 2015). However, high temperatures also increase VPD, which induces stomatal closure as well as enhances ABA concentrations, causing the cessation of growth (Aloni, 2015), as observed in chapters 4 and

5 for *P. sylvestris*. This non-linearity was captured in the models fitted in Chapter 2, which temperature functions presented species-specific optima (Figure 2.4).

On the other hand, precipitation showed an asymptotic relationship with growth (Figure 2.4). More concretely, we observed that precipitation of the period May-July was the best climatic predictor for *P. sylvestris*, coinciding with the period of maximum growth rates observed for this species in Figure 4.2 and other studies (Camarero *et al.*, 2010; Gruber *et al.*, 2010; Cuny *et al.*, 2012; Swidrak *et al.*, 2014). Additionally, according to the results in chapters 4 and 5, low precipitation during this period would trigger an earlier cessation of growth and, thus, a reduction in the final ring-width. Indeed, as suggested in Chapter 5 and other studies (Waring, 1987; McDowell *et al.*, 2008; Oberhuber *et al.*, 2011; Adams *et al.*, 2015; Dickman *et al.*, 2015), drought firstly limits xylem growth rather than photosynthesis and carbon storage. In addition, as observed in chapters 4 and 5 and other studies (Irvine *et al.*, 1998; Poyatos *et al.*, 2008), *P. sylvestris* exerts a strong control over stomata under drought, reducing carbon uptake, which could, therefore, limit the amount of carbohydrates available for growth.

Q. pyrenaica growth was, however, more influenced by spring precipitation. Hernández-Santana *et al.* (2008, 2009) found that the soil water reserve before the summer posed a greater limitation on *Q. pyrenaica* gas exchange than summer precipitation itself. Concurring with this, we only observed a significant reduction in this species photosynthetic rates and stomatal conductance in 2012 (Figure 4.7), which suffered the driest spring of the three years studied (Figure 4.1). This suggests that *Q. pyrenaica* is well-adapted to resist summer drought as long as soil water reserves are accumulated in the spring (Gallego *et al.*, 1994; Hernández-Santana *et al.*, 2008a). Moreover, this species mainly responded to the rainfall exclusion experiment through a reduction in latewood width (Chapter 5), as observed by Corcuera *et al.* (2006). Latewood formation begins in June (Chapters 4 and 5), hence being affected by the water accumulated during the spring. This reduction in latewood could, however, challenge this species hydraulic performance during the summer and autumn if soil water reserves are depleted, as latewood vessels provide water transport once earlywood vessels have cavitated (Granier *et al.*, 1994). Tyloses (Figure 6.1) were firstly observed in *Q. pyrenaica* micro-sections around mid-July, and were frequently observed after September, which suggests that latewood vessels play a significant role in this species water transport at the end of the vegetative period.

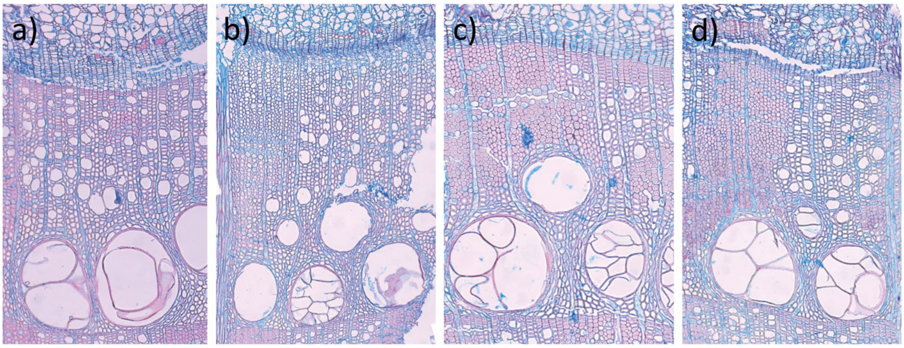


Figure 6.1 Tyloses in previous-year vessels in April (a) and in current-year vessels in July (b), October (c) and November (d)

Similarly to *Q. pyrenaica*, *Q. faginea* latewood width was reduced during dry years, as also found by Corcuera *et al.* (2004). Despite its ecological similarities, however, *Q. faginea* responded to precipitation fallen along the hydrological year. This species was studied at the driest site and, therefore, this result is consistent with those of Pasho *et al.* (2011), who found that species from xeric sites respond more strongly to long-term water deficits (9-12 months), while species from mesic sites rather respond to short-term deficits (1–5 months).

Furthermore, tree-ring width and $\delta^{13}C$ were also significantly correlated with previous-year climatic conditions (Chapter 3). In particular, *Quercus* spp. tree-ring-$\delta^{13}C$-derived iWUE was mostly influenced by previous-year climatic variables. Tree rings are formed from different carbohydrate sources along the year, using stored carbohydrates in the early season and recently produced assimilates as the growing season progresses (Barbaroux & Bréda, 2002; El Zein *et al.*, 2011; Offermann *et al.*, 2011). This carry-over effect from stored carbohydrates, especially between the latewood of one year and the earlywood of the following one (Hoch *et al.*, 2003; Skomarkova *et al.*, 2006; Vaganov *et al.*, 2009). This is particularly relevant for deciduous ring-porous *Quercus* spp., which form the spring vessels before budburst (Chapters 4 and 5) and, therefore, use stored carbohydrates for their formation (Barbaroux & Bréda, 2002; El Zein *et al.*, 2011; Michelot *et al.*, 2012b), supporting the stronger use of carbon reserves for ring formation in both deciduous species than in the conifer. Furthermore, in Chapter 4 we found that maximum photosynthetic rates occurred in mid-summer, when most xylem formation was already completed, indicating that for a significant part of the vegetative period carbon is accumulated or allotted to root and bud formation (Bréda *et al.*, 2006; Delpierre *et al.*, 2016a). Hence, climatic conditions during late summer to early autumn can affect bud formation and, therefore, the following year's leaf cohort development and radial growth (Delpierre *et al.*, 2016a). In fact, drought episodes have been found to strongly influence secondary growth even after several years, either by affecting the recovery of the canopy and its indirect effect on carbon reserves as a result of reduced photosynthetic carbon uptake (Galiano *et al.*, 2011) or by cumulative hydraulic damage (Anderegg *et al.*, 2013, 2015).

Therefore, an important teaching derived from using different methodologies to study climate-growth relationships throughout this thesis, that has already been pointed out in previous studies (e.g. Deslauriers *et al.*, 2003), is that most of the ring width is formed in a climate-dependent, relatively short period of time (Chapters 4 and 5) from, at least partially, carbohydrates accumulated in previous years, particularly for *Quercus* spp. (Chapters 3, 4 and 5). Hence, climate-growth relationships assessed through tree ring proxies must be taken with caution, as they are dependent on multiple factors, including the above-mentioned current and past climatic conditions, which are susceptible to be altered under a changing climate. Moreover, as observed in chapters 2 and 3, growth response to climate can be significantly modulated by other factors, such as tree size and age (Chapters 2, 3 and 4), CO_2 concentrations (Chapter 3) and competition (Chapters 2 and 3).

6.2 Competition impact on the tree functional response to climate

In chapters 2 and 3 we observed that growth was significantly influenced by competition levels, following a negative exponential function. For *Q. faginea* and *P. sylvestris*, the net effect of competition on growth was stronger than that of climatic variables, whereas *Q. pyrenaica* growth was more influenced by temperature than by competition (Table 2.3). This major effect of competition on tree growth is consistent with other studies (Linares *et al.*, 2010; Gómez-Aparicio *et al.*, 2011; Vayreda *et al.*, 2012; Kunstler *et al.*, 2016).

Growth rates were always higher in thinned than in control plots, even in dry years (Figure 3.2). Increased growth rates may be a result of longer growing seasons (Linares *et al.*, 2009a) and higher photosynthetic rates and stomatal conductance under low competition levels (Sala *et al.*, 2005). These changes in gas exchange, however, rarely translate into changes in iWUE, as observed in Chapter 3, but rather into structural changes, particularly increased leaf and sapwood area (McDowell *et al.*, 2006). Regardless of the effect on $\delta^{13}C$, trees under low competition have less negative water potentials (Warren *et al.*, 2001; McDowell *et al.*, 2003, 2006; Sala *et al.*, 2005; Moreno-

Gutiérrez *et al.*, 2012), reducing the risk of drought-induced cavitation. Furthermore, we observed that the growth reduction in *Quercus* spp. under high competition was mostly related to a decrease in or even a lack of latewood (Figure 6.2), as also observed by Corcuera *et al.* (2006), which could compromise the hydraulic performance of trees under high competitive stress. Hence, even though trees may not change their iWUE under low competition levels, the structural changes (e.g. increased sapwood area) promoted by increased resource availability may reduce their vulnerability to drought-induced mortality (Galiano *et al.*, 2010), particularly in stands suffering from densification such as those studied here. Consequently, the sustainability of these stands under climate change scenarios will depend on the synergies between climate forcing and competition intensity, with individuals subject to high competition levels being less likely to survive to enhanced xericity (Bigler *et al.*, 2004; Galiano *et al.*, 2010; Linares *et al.*, 2010; Gómez-Aparicio *et al.*, 2011).

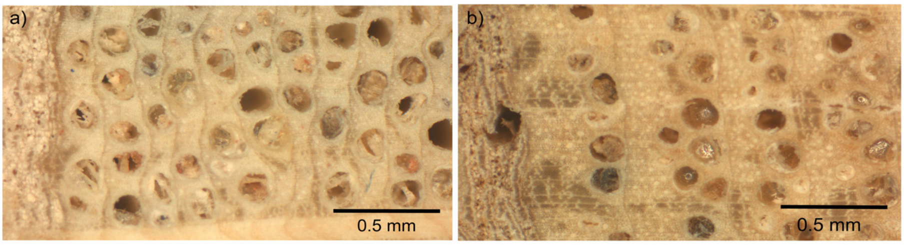


Figure 6.2 *Quercus faginea* xylem in control (a) and heavy thinning (b) plots

6.3 Species-specific projections under climate change scenarios: Management implications

Climate change scenarios predict an increase in temperatures and stable or decreasing precipitation for the Mediterranean region, which will likely enhance evapotranspiration rates, water deficits and, consequently, the frequency and intensity of drought events (Giorgi & Lionello, 2008; IPCC, 2013). In fact, a widespread rise in temperatures and the frequency of drought episodes has already been observed throughout the Mediterranean region, including at our study sites. As a result, we found that trees in control plots had decreasing growth trends since the 1970s in all sites except for *P. sylvestris* at Neila, which had a non-significant increasing trend. Conversely, the negative trend was reversed in thinned plots for *Q. faginea* and *Q. pyrenaica* at Rascafría (Figure 3.2).

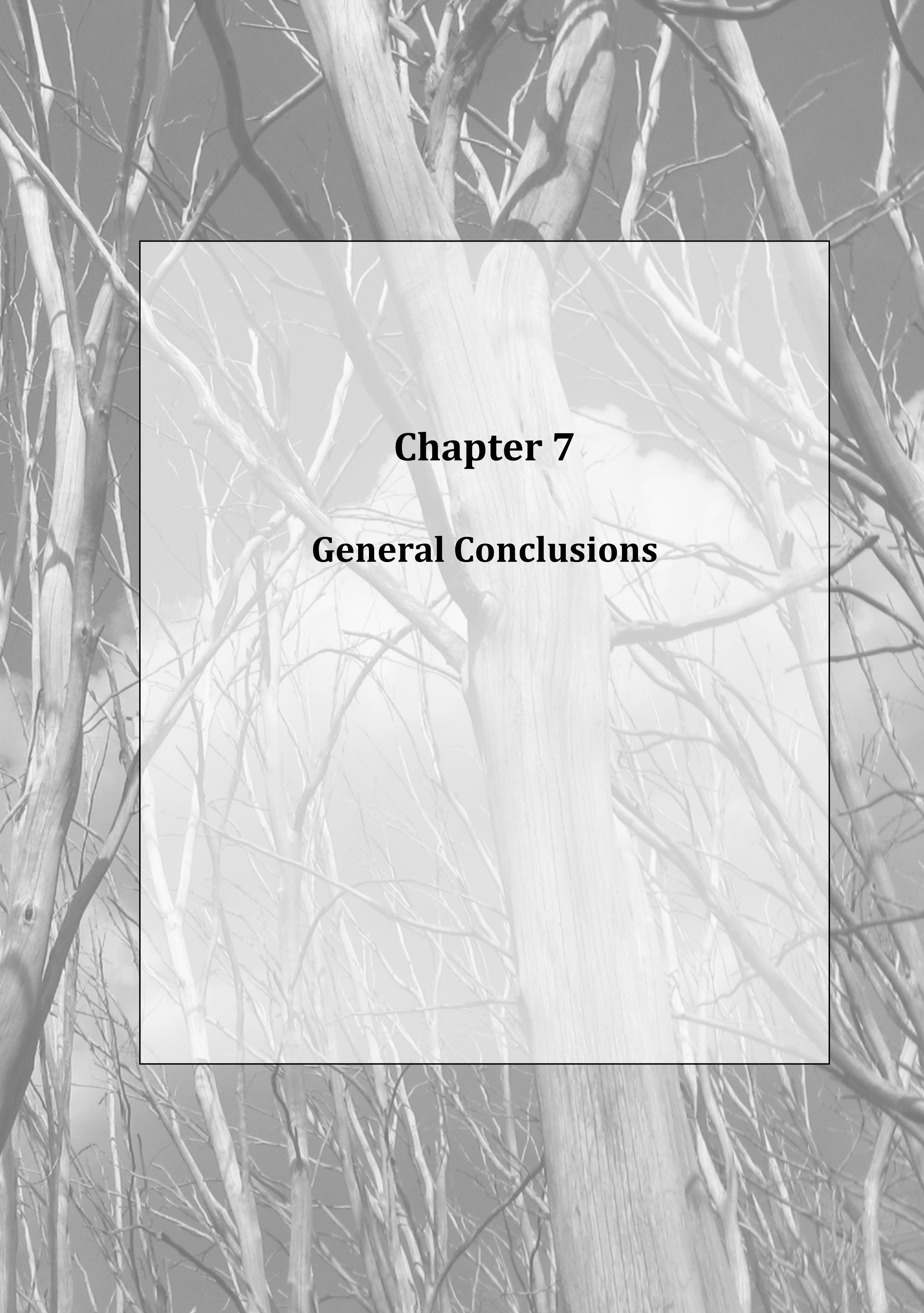
Projections obtained in Chapter 2 predicted a decrease in *P. sylvestris* growth at both study sites regardless of the competition level and despite the increase in iWUE observed in Chapter 3. The stable and decreasing growth trends found in *P. sylvestris* may, therefore, suggest that carbon assimilation has not been enhanced in response to increasing CO_2 concentrations but, rather, the moderate increase observed in iWUE was likely due to reduced stomatal conductance to avoid water loss (Andreu-Hayles *et al.*, 2011; Maseyk *et al.*, 2011; Bader *et al.*, 2013; Voltas *et al.*, 2013). Concurring with this, we found that *P. sylvestris* had strong stomatal control under drought (Chapters 4 and 5; Irvine *et al.* 1998; Poyatos *et al.* 2008). Moreover, even though this species could benefit from an earlier growth onset as a result of warmer springs, this would be likely offset by a concurrent earlier cessation of xylogenesis as a result of increasing summer water deficits, as shown in Chapters 4 and 5. Altogether, evidence gathered throughout this thesis suggests *P. sylvestris* will be highly susceptible to increasingly drier conditions predicted under climate change scenarios, in agreement with other studies (Martínez-Vilalta & Piñol, 2002; Vilà-Cabrera *et al.*, 2011; Hereş *et al.*, 2012; Rigling *et al.*, 2013). Indeed, one of the old trees died during the drought experiment (Chapter 5), seemingly as a result of the drought treatment and probably exacerbated by the presence of mistletoe (*Viscum album* L.) on this tree. *V. album* barely regulates its stomata, which leads to *P. sylvestris* closing its own, reducing carbon uptake without succeeding to avoid water loss, particularly during dry periods (Galiano *et al.*, 2011; Zweifel *et al.*, 2012). Therefore, mistletoe infection, coupled with this species isohydric behaviour, may predispose *P. sylvestris* to die from carbon starvation rather than hydraulic failure under increasingly drier conditions (McDowell *et al.*, 2008; Galiano *et al.*, 2011; Zweifel *et al.*, 2012). High mortality rates have already been observed in this species as a result of increasingly drier conditions, mistletoe infections and higher competition levels (Martínez-Vilalta & Piñol, 2002; Galiano *et al.*, 2010; Vilà-Cabrera *et al.*, 2011), including in the study area where xylogenesis was monitored (Gea-Izquierdo et al. 2014). A reduction of competition, however, may not guarantee the sustainability of these woodlands, as suggested by the decreasing growth trends in Duruelo even under reduced competition (Chapter 3) and by our model projections, which predicted a decline in growth in both *P. sylvestris* sites even under low competition levels (Figures 2.5 and 2.6). Heavier thinning intensities than those applied today could be tested to maintain this species productivity, particularly at drier locations. Nonetheless, the outcomes of thinning treatments should be carefully monitored, as intense logging may have detrimental effects on the growth of the remaining trees (Camarero *et al.*, 2011). Hence, the high vulnerability of *P. sylvestris* to drought observed throughout this thesis, particularly at low elevation sites, may lead, as suggested by species distribution models, to a displacement of this conifer at its low-altitudinal and southernmost distribution limits by sub-Mediterranean species such the studied *Q. pyrenaica* and *Q. faginea* (Sánchez de Dios *et al.*, 2009; Galiano *et al.*, 2010; Ruiz-Labourdette *et al.*, 2012).

Concurring with this, our growth models projected constant or even increasing growth rates for *Q. pyrenaica*, except under the warmest climate scenario (Figures 2.5 and 2.6). This could be partially a result of an earlier phenology, including vessel lignification, leaf flushing and onset of latewood formation as a result of warmer winters and springs and earlier last frosts

(Chapter 4), as reported by Linderholm (2006). Therefore, *Q. pyrenaica* may be able to benefit from the significant iWUE enhancement with increasing CO_2 concentrations observed in Chapter 3 through increased carbon uptake (Saurer *et al.* 2004). Past growth trends were, however, divergent between the two study sites (Figure 3.2). Given this species growth positive correlation with temperatures and following Gea-Izquierdo & Cañellas (2014), the decreasing growth trends observed at Navasfrías may have been likely caused by increasing competition for resources, rather than temperature-induced drought. Supporting this, we predicted an increase in growth under climate change scenarios at the low-competition plots, indicating this species could benefit from the transformation to high forests to cope with increasingly drier conditions (Chapter 2). This species showed high resistance to summer drought, with higher A and g_s than *P. sylvestris* despite the more negative water potentials and even during the driest year (Figure 4.7). Nevertheless, a decrease in growth was projected under the warmest climate change scenario (Chapter 2). As previously observed (Hernández-Santana *et al.*, 2008b, 2009), this species is capable of withstanding summer drought if soil water reserves are accumulated during the spring. If warm temperatures are, however, accompanied by a reduction in spring precipitation or a significant increase in VPD, this species performance may be significantly drought-limited during the summer (Hernández-Santana *et al.*, 2008b, 2009), as observed in Chapter 4. In fact, the cumulative effect of the drought treatment caused a significant reduction in latewood width (Chapter 5), which could compromise the tree’s hydraulic efficiency if drought causes severe damage to earlywood vessels, as previously discussed. Species with high risk of hydraulic damage, such as *Quercus* spp., have been found to have large drought legacy effects and slow growth recovery after drought (Anderegg *et al.*, 2015). Indeed, this species showed an increasing reduction in latewood width as the rainfall exclusion experiment progressed (Figure 5.5), probably due to the heavy reliance of ring-porous species on stored carbohydrates to form their annual rings

(Michelot *et al.*, 2012b). This, coupled with its anisohydric nature (McDowell *et al.*, 2008) and the presence of cavitated earlywood vessels by mid-summer (Figure 6.1), may make *Q. pyrenaica* vulnerable to die from hydraulic failure if a significant reduction in spring precipitation occurs.

Q. faginea suffered a significant decreasing growth trend under control conditions, which was reversed when tree-to-tree competition was reduced (Chapter 3). Likewise, projections estimated a decline in growth rates in control plots, but stable growth rates under reduced competition (Chapter 2). This species suffers substantial growth stagnation as a result of stand densification following the abandonment of traditional management in recent decades (Cañellas *et al.*, 1996). Similarly to *Q. pyrenaica*, this growth reduction was mostly associated with a reduction in latewood width, which also occurred in response to drought (Corcuera *et al.*, 2004; Alla & Camarero, 2012). This long-term decline in or even absence of latewood could, as discussed for *Q. pyrenaica*, significantly constrain the hydraulic capacity of this species under drought, leading to the severely reduced growth rates projected under climate change scenarios and, presumably, extensive dieback. Lowering competition was, however, effective in releasing growth for this species, with slightly increasing growth rates in thinned stands (Chapter 3) and stable growth projections, except under the warmest climate change scenario (Chapter 2). Thus, if competition is reduced, *Q. faginea* may be able to profit from the moderate increase in iWUE observed in Chapter 3 as a result of increasing CO_2 concentrations through a stronger stomatal control without a decrease in photosynthetic assimilation. This is in agreement with this species high stomatal conductance, which allows it to maintain high photosynthetic rates despite reductions in g_s in response to increasing VPD (Peguero-Pina *et al.*, 2015). Nevertheless, even though proactive thinning may enhance these stands' resilience to enhanced drought stress, this practice, which has been rarely applied in sub-Mediterranean *Quercus* spp., must be carefully monitored to ensure the long-term sustainability of these woodlands, as thinning may have a detrimental effect on recruitment (Rey Benayas *et al.*, 2005; Maltez-Mouro *et al.*, 2009). Hence, given the decline in growth rates predicted under the warmest climatic scenario, sub-Mediterranean *Quercus* spp. could be eventually replaced by more drought tolerant species such as *Quercus ilex* or *Pinus halepensis* if increasing temperatures and drought become limiting (Corcuera *et al.*, 2002; Montserrat-Martí *et al.*, 2009; Sánchez de Dios *et al.*, 2009; Ruiz-Labourdette *et al.*, 2012; Peguero-Pina *et al.*, 2015) despite the implementation of adaptive management measures.



Chapter 7

General Conclusions

Conclusiones generales

1. La competencia modula significativamente la respuesta del crecimiento de los árboles al clima. Esta interacción no lineal determinará el impacto de condiciones climáticas cambiantes en el crecimiento de los árboles, de manera que los árboles bajo competencia reducida tendrán mayor capacidad para resistir las condiciones más cálidas predichas bajo escenarios de cambio climático (Capítulo 2).

2. Los árboles responden a una reducción en la competencia mediante cambios estructurales como el incremento en crecimiento radial más que con ajustes en el intercambio gaseoso a nivel de hoja (como por ejemplo, en la eficiencia intrínseca en el uso de agua – iWUE, por sus siglas en inglés) (Capítulo 3).

3. iWUE aumentó a lo largo del tiempo en *Q. faginea, Q. pyrenaica* y *P. sylvestris* debido al efecto combinado del aumento en las concentraciones de CO_2 atmosférico, el clima y la edad de los árboles. Sin embargo, este aumento en iWUE generalmente no se tradujo en un aumento en las tasas de crecimiento debido al aumento simultáneo en el estrés por sequía, lo que impidió una fertilización por CO_2 en el crecimiento (Capítulo 3).

4. El estancamiento en el crecimiento sufrido por *Quercus faginea* bajo condiciones de alta densidad de rodal podría limitar significativamente el crecimiento de esta especie bajo un clima más cálido en el sitio de estudio. Una reducción de la competencia es, no obstante, efectiva para facilitar el crecimiento de esta especie, excepto bajo el escenario de cambio climático más cálido. Por lo tanto, si se reduce la competencia, *Q. faginea* podría beneficiarse del aumento en iWUE mediante una reducción en la conductancia estomática sin comprometer la asimilación fotosintética (Capítulos 2 y 3).

5. *Quercus pyrenaica* podría beneficiarse del aumento en temperaturas e iWUE a través de una mejora en la absorción de carbono y el crecimiento, especialmente si se reduce la competencia, excepto bajo el escenario de cambio climático más cálido (Capítulos 2 y 3).

6. Las fenologías cambial y foliar de *Q. pyrenaica* están sincronizadas para optimizar la absorción de carbono durante la estación de crecimiento. Esta especie podría beneficiarse de los inviernos y primaveras más cálidos predichos bajo escenarios de cambio climático por medio de una fenología más prematura, principalmente de una lignificación de los vasos de primavera, brote de la hoja y comienzo de la formación de madera tardía más tempranos. No obstante, este efecto podría verse minimizado si el fotoperiodo sigue manteniendo un control sobre el comienzo de la xilogénesis (Capítulo 4).

7. *Quercus pyrenaica* tiene una alta resistencia a la sequía estival. Excepto por el año con la primavera más seca, esta especie mantuvo altas tasas fotosintéticas y conductancia estomática durante el verano, a pesar de sus bajos potenciales hídricos foliares de mediodía. No obstante, la reducción significativa en la anchura de la madera tardía bajo sequía podría comprometer la eficiencia hidráulica del árbol si ocurre un daño severo en los vasos de primavera del año en curso. Por lo tanto, esta especie podría ser vulnerable al aumento en condiciones secas, particularmente si éstas se presentan en primavera (Capítulos 4 y 5).

8. Una reducción de la competencia podría no garantizar la sostenibilidad de las masas de *P. sylvestris* bajo escenarios de cambio climático. Se predijo un descenso en el crecimiento de *Pinus sylvestris* independientemente del nivel de competencia y a pesar del aumento en iWUE, el cual fue probablemente debido a una reducción en la conductancia estomática para evitar la pérdida de agua (Capítulos 2 y 3).

9. *Pinus sylvestris* sigue una estrategia de evasión de la sequía, tanto en términos de su intercambio gaseoso foliar como de su fenología cambial. El fuerte control estomático para regular el potencial hídrico foliar y maximizar iWUE indica que la asimilación de carbono podría verse comprometida en esta especie por el incremento en estrés por sequía. La xilogénesis cesó antes como resultado de déficits hídricos en verano más altos, lo que podría neutralizar el potencial beneficio de un comienzo del crecimiento más temprano debido a primaveras más cálidas (Capítulos 4 y 5).

10. La sequía afecta a la formación del xilema tanto en *P. sylvestris* como en *Q. pyrenaica*, lo que podría indicar que ambas especies serán vulnerables al aumento en estrés por sequía como resultado del cambio climático. *P. sylvestris* podría tener, no obstante, un mayor riesgo de mortalidad por sequía que *Q. pyrenaica*. El cese de la elongación del xilema más temprano, junto a una menor asignación de carbono a la formación del xilema en ambas especies, refleja un cambio en la distribución de recursos bajo sequía, favoreciendo la asimilación de carbono y otros sumideros de carbono en vez del crecimiento del xilema (Capítulo 5).

11. En conjunto, la evidencia recogida a lo largo de esta tesis sugiere que *P. sylvestris* será altamente susceptible al aumento en condiciones de sequía predichas bajo escenarios de cambio climático, particularmente en su límite altitudinal inferior. En consecuencia, probablemente podría ser sustituido en el límite de su distribución altitudinal por taxones más tolerantes a la sequía como los *Quercus* spp. submediterráneos estudiados (Capítulos 2, 3, 4 y 5).

General conclusions

1. Competition significantly modulates tree growth response to climate. This nonlinear interaction will determine the impact of changing climatic conditions on growth, with trees under low competition being better able to withstand the warmer conditions predicted under climate change scenarios (Chapter 2).

2. Trees respond to a reduction in competition through structural shifts such as increased radial growth rather than leaf-level gas exchange adjustments (e.g. intrinsic water use efficiency – iWUE) (Chapter 3).

3. iWUE increased along time for *Q. faginea, Q. pyrenaica* and *P. sylvestris* due to the combined effect of rising atmospheric CO_2 concentrations, climate and tree age. This increase in iWUE was, however, generally not translated into an increase in growth rates due to the concurrent increase in drought stress, which hindered CO_2 fertilization on growth (Chapter 3).

4. The growth stagnation suffered by *Quercus faginea* under high stand density conditions could significantly limit this species growth under a warming climate at the study site. Lowering competition is, however, effective in releasing growth in this species, except under the warmest climate change scenario. Thus, if competition is reduced, *Q. faginea* may profit from increasing iWUE through a reduction in stomatal conductance without compromising photosynthetic assimilation (Chapters 2 and 3).

5. *Quercus pyrenaica* may benefit from increasing temperatures and iWUE through enhanced carbon uptake and growth, particularly if competition is reduced, except under the warmest climate change scenario (Chapters 2 and 3).

6. *Q. pyrenaica* cambial and leaf phenology are synchronized to optimize carbon uptake during the growing season. This species could benefit from warmer winters and springs predicted under climate change scenarios through an earlier phenology, namely earlier earlywood vessel lignification, leaf flushing and onset of latewood formation. This effect could be, however, minimized if trees maintain a photoperiod control over the onset of xylogenesis (Chapter 4).

7. *Quercus pyrenaica* has high resistance to summer drought. Except for the year with the driest spring, this species maintained high photosynthetic rates and stomatal conductance during the summer, despite its low midday leaf water potentials. However, the significant reduction in latewood width under drought could compromise the tree's hydraulic efficiency if severe damage occurs to current-year earlywood vessels. Hence, this species may be vulnerable to increasingly drier conditions, particularly if these arise in spring (Chapters 4 and 5).

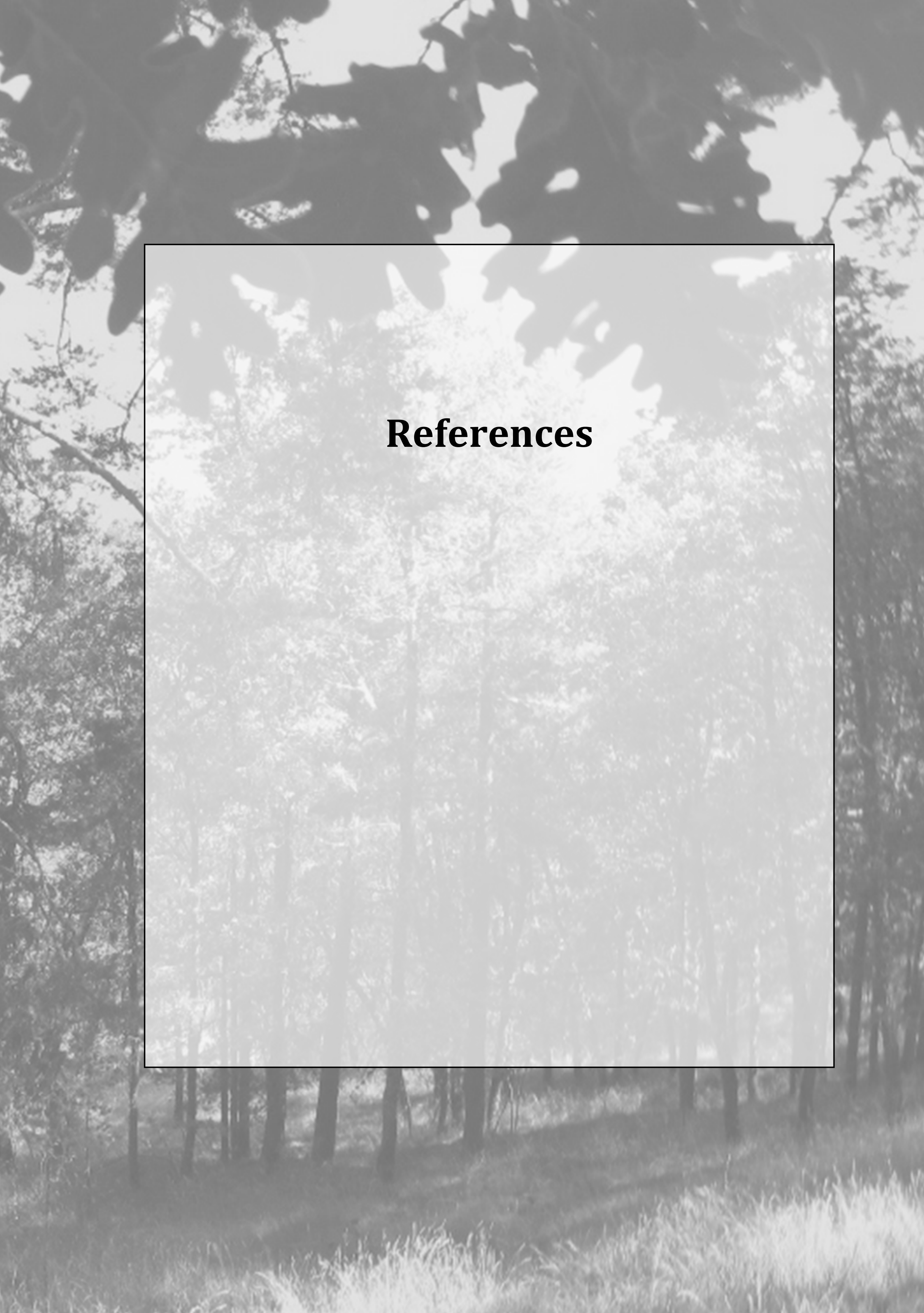
8. A reduction of competition may not guarantee the sustainability of *P. sylvestris* stands under climate change scenarios. A decrease in *Pinus sylvestris* growth was predicted regardless of the competition level and despite the increase in iWUE, which was likely due to reduced stomatal conductance to avoid water loss (Chapters 2 and 3).

9. *Pinus sylvestris* follows a drought-avoidance strategy, both in terms of its leaf gas exchange and cambial phenology. The tight stomatal control to regulate leaf water potential and maximize iWUE indicates that carbon assimilation may be compromised in this species by increasing drought stress. Xylogenesis ceased earlier as a result of higher summer water deficits, which could offset the potential benefit of an earlier growth onset due to warmer springs (Chapters 4 and 5).

10. Drought affects both *P. sylvestris* and *Q. pyrenaica* xylem formation, which could indicate that both species would be vulnerable to increasing drought stress as a result of climate change. *P. sylvestris* may be, however, at a higher risk of drought-induced mortality than *Q. pyrenaica*. The earlier cessation of xylem enlargement, together with a lower carbon allocation to xylem formation in both species, reflects a shift in the allocation of resources under drought, favouring carbon assimilation and carbon sinks other than xylem growth (Chapter 5).

11. Altogether, the evidence gathered throughout this thesis suggests that *P. sylvestris* will be highly susceptible to increasingly drier conditions predicted under climate change scenarios, particularly at its low-elevation limit. Consequently, it could be likely replaced at the edge of its altitudinal distribution by more drought-tolerant taxa such as the studied sub-Mediterranean *Quercus* spp. (Chapters 2, 3, 4 and 5).

References



Abe H, Nakai T, Utsumi Y, Kagawa A (2003) Temporal water deficit and wood formation in *Cryptomeria japonica*. *Tree physiology*, **23**, 859–863.

Ackerly DD, Bazzaz FA (1995) Plant growth and reproduction along CO_2 gradients: non-linear responses and implications for community change. *Global Change Biology*, **1**, 199–207.

Adams HD, Collins AD, Briggs SP et al. (2015) Experimental drought and heat can delay phenological development and reduce foliar and shoot growth in semiarid trees. *Global Change Biology*, **21**, 4210–4220.

Albuixech J, Camarero JJ, Montserrat-Martí G (2012) Dinámica estacional del crecimiento secundario y anatomía del xilema en dos *Quercus* mediterráneos que coexisten Introducción. *Forest Systems*, **21**, 9–22.

Alla AQ, Camarero JJ (2012) Contrasting responses of radial growth and wood anatomy to climate in a Mediterranean ring-porous oak: implications for its future persistence or why the variance matters more than the mean. *European Journal of Forest Research*, **131**, 1537–1550.

Aloni R (2015) Ecophysiological implications of vascular differentiation and plant evolution. *Trees*, **29**, 1–16.

Anderegg WRL, Plavcová L, Anderegg LDL, Hacke UG, Berry JA, Field CB (2013) Drought's legacy: multiyear hydraulic deterioration underlies widespread aspen forest die-off and portends increased future risk. *Global Change Biology*, **19**, 1188–1196.

Anderegg WRL, Schwalm C, Biondi F et al. (2015) Pervasive drought legacies in forest ecosystems and their implications for carbon cycle models. *Science*, **349**, 528–532.

Andreassen K, Solberg S, Tveito OE, Lystad SL (2006) Regional differences in climatic responses of Norway spruce (*Picea abies* L. Karst) growth in Norway. *Forest Ecology and Management*, **222**, 211–221.

Andreu L, Gutiérrez E, Macias M, Ribas M, Bosch O, Camarero JJ (2007) Climate increases regional tree-growth variability in Iberian pine forests. *Global Change Biology*, **13**, 804–815.

Andreu-Hayles L, Planells O, Gutiérrez E, Muntan E, Helle G, Anchukaitis KJ, Schleser GH (2011) Long tree-ring chronologies reveal 20th century increases in water-use efficiency but no enhancement of tree growth at five Iberian pine forests. *Global Change Biology*, **17**, 2095–2112.

Aussenac G, Granier A (1988) Effects of thinning on water stress and growth in Douglas-fir. *Canadian Journal of Forest Research*, **18**, 100–105.

Bader MKF, Siegwolf R, Körner C (2010) Sustained enhancement of photosynthesis in mature deciduous forest trees after 8 years of free air CO_2 enrichment. *Planta*, **232**, 1115–1125.

Bader MKF, Leuzinger S, Keel SG, Siegwolf RTW, Hagedorn F, Schleppi P, Körner C (2013) Central european hardwood trees in a high-CO_2 future: Synthesis of an 8-year forest canopy CO_2 enrichment project. *Journal of Ecology*, **101**, 1509–1519.

Baldocchi DD, Ma S, Rambal S et al. (2010) On the differential advantages of evergreenness and deciduousness in mediterranean oak woodlands: a flux perspective. *Ecological Applications*, **20**, 1583–97.

Balducci L, Deslauriers A, Giovannelli A, Rossi S, Rathgeber CBK (2013) Effects of temperature and water deficit on cambial activity and woody ring features in Picea mariana saplings. *Tree Physiology*, **33**, 1006–17.

Balducci L, Cuny HE, Rathgeber CBK, Deslauriers A, Giovannelli A, Rossi S (2016) Compensatory mechanisms mitigate the effect of warming and drought on wood formation. *Plant, Cell & Environment*, **39**, 1338–1352.

Barbaroux C, Bréda N (2002) Contrasting distribution and seasonal dynamics of carbohydrate reserves in stem wood of adult ring-porous sessile oak and diffuse-porous beech trees. *Tree physiology*, **22**, 1201–1210.

Bates D, Maechler M, Bolker BM, Walker S (2014) lme4: Linear mixed-effects models using Eigen and S4. R package version 1.0-6.

Battipaglia G, Saurer M, Cherubini P, Calfapietra C, McCarthy HR, Norby RJ, Cotrufo MF (2013) Elevated CO_2 increases tree-level intrinsic water use efficiency: insights from carbon and oxygen isotope analyses in tree rings across three forest FACE sites. *New Phytologist*, **197**, 544–54.

Bebber DP, Thomas SC, Cole WG, Balsillie D (2004) Diameter increment in mature eastern white pine *Pinus strobus* L. following partial harvest of old-growth stands in Ontario, Canada. *Trees - Structure and Function*, **18**, 29–34.

Beier C, Beierkuhnlein C, Wohlgemuth T et al. (2012) Precipitation manipulation experiments - challenges and recommendations for the future. *Ecology Letters*, **15**, 899–911.

Belien E, Rossi S, Morin H, Deslauriers A (2012) Xylogenesis in black spruce subjected to rain exclusion in the field. *Canadian Journal of Forest Research*, **1315**, 1306–1315.

Benavides R, Rabasa SG, Granda E et al. (2013) Direct and indirect effects of climate on demography and early growth of *Pinus sylvestris* at the rear edge: changing roles of biotic and abiotic factors. *PloS one*, **8**, e59824.

Bennett AC, McDowell NG, Allen CD, Anderson-Teixeira KJ (2015) Larger trees suffer most during drought in forests worldwide. *Nature Plants*, **1**, 15139.

Bert D, Leavitt SW, Dupouey J-L (1997) Variations of wood $\delta^{13}C$ and water-use efficiency of Abies alba during the last century. *Ecology*, **78**, 1588–1596.

Bickford CP, Hanson DT, McDowell NG (2010) Influence of diurnal variation in mesophyll conductance on modelled ^{13}C discrimination: results from a field study. *Journal of Experimental Botany*, **61**, 3223–33.

Bigler C, Gričar J, Bugmann H, Čufar K (2004) Growth patterns as indicators of impending tree death in silver fir. *Forest Ecology and Management*, **199**, 183–190.

Biondi F, Qeadan F (2008) A theory-driven approach to tree-ring standardization: defining the biological trend from expected basal area increment. *Tree-Ring Research*, **64**, 81–96.

Black BA, Abrams MD (2003) Use of boundary-line growth patterns as a basis for dendroecological release criteria. *Ecological Applications*, **13**, 1733–1749.

Bréda N, Granier A, Aussenac G (1995) Effects of thinning on soil and tree water relations, transpiration and growth in an oak forest (*Quercus petraea* (Matt.) Liebl.). *Tree Physiology*, **15**, 295–306.

Bréda N, Huc R, Granier A, Dreyer E (2006) Temperate forest trees and stands under severe drought : a review of ecophysiological responses, adaptation processes and long-term consequences. *Annals of Forest Science*, **63**, 625–644.

Breshears DD, McDowell NG, Goddard KL, Dayem KE, Martens SN, Meyer CW, Brown KM (2008) Foliar absorption of intercepted rainfall improves woody plant water status most during drought. *Ecology*, **89**, 41–47.

Breshears DD, Myers OB, Meyer CW et al. (2009) Tree die-off in response to global change-type drought: mortality insights from a decade of plant water potential measurements. *Frontiers in Ecology and the Environment*, **7**, 185–189.

Brodribb TJ, Pittermann J, Coomes DA (2012) Elegance versus speed: examining the competition between conifer and angiosperm trees. *International Journal of Plant Sciences*, **173**, 673–694.

Brooks JR, Mitchell AK (2011) Interpreting tree responses to thinning and fertilization using tree-ring stable isotopes. *New Phytologist*, **190**, 770–82.

Brudvig LA, Blunck HM, Asbjornsen H, Mateos-Remigio VS, Wagner SA, Randall JA (2011) Influences of woody encroachment and restoration thinning on overstory savanna oak tree growth rates. *Forest Ecology and Management*, **262**, 1409–1416.

Bunn AG (2008) A dendrochronology program library in R (dplR). *Dendrochronologia*, **26**, 115–124.

Burnham KP, Anderson DR (2002) *Model selection and multimodel inference: a practical information-theoretic approach*, Second edi edn. Springer-Verlag, New York, USA, 488 pp.

Caffarra A, Donnelly A (2011) The ecological significance of phenology in four different tree species: effects of light and temperature on bud burst. *International journal of biometeorology*, **55**, 711–21.

Camarero JJ, Olano JM, Parras A (2010) Plastic bimodal xylogenesis in conifers from continental Mediterranean climates. *New Phytologist*, **185**, 471–80.

Camarero JJ, Bigler C, Linares JC, Gil-Pelegrín E (2011) Synergistic effects of past historical logging and drought on the decline of Pyrenean silver fir forests. *Forest Ecology and Management*, **262**, 759–769.

Canham CD, Papaik MJ, Uriarte M, McWilliams WH, Jenkins JC, Twery MJ (2006) Neighborhood analyses of canopy tree competition along environmental gradients in New England forests. *Ecological Applications*, **16**, 540–54.

Cañellas I, Montero G, Bachiller Á (1996) Transformation of quejigo oak (*Quercus faginea* Lam.) coppice forest into high forest by thinning. *Annali Istituto Sperimentale Selvicoltura*, **27**, 143–147.

Cañellas I, Del Río M, Roig S, Montero G (2004) Growth response to thinning in *Quercus pyrenaica* Willd. coppice stands in Spanish central mountain. *Annals of Forest Science*, **61**, 243–250.

Carnicer J, Coll M, Ninyerola M, Pons X, Sánchez G, Peñuelas J (2011) Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 1474–8.

Cernusak LA, English NB (2015) Beyond tree-ring widths: stable isotopes sharpen the focus on climate responses of temperate forest trees. *Tree Physiology*, **35**, 1–3.

Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought—from genes to the whole plant. *Functional Plant Biology*, **30**, 239–264.

Chen P-Y, Welsh C, Hamann A (2010) Geographic variation in growth response of Douglas-fir to interannual climate variability and projected climate change. *Global Change Biology*, **16**, 3374–3385.

Choat B, Cobb AR, Jansen S (2008) Structure and function of bordered pits: New discoveries and impacts on whole-plant hydraulic function. *New Phytologist*, **177**, 608–626.

Choat B, Jansen S, Brodribb TJ et al. (2012) Global convergence in the vulnerability of forests to drought. *Nature*, **491**, 752–756.

Coates KD, Canham CD, LePage PT (2009) Above- versus below-ground competitive effects and responses of a guild of temperate tree species. *Journal of Ecology*, **97**, 118–130.

Cochard H, Bréda N, Granier A (1996) Whole tree hydraulic conductance and water loss regulation in *Quercus* during drought: evidence for stomatal control of embolism? *Annals of Forest Science*, **53**, 197–206.

Corcuera L, Camarero JJ, Gil-Pelegrín E (2002) Functional groups in *Quercus* species derived from the analysis of pressure–volume curves. *Trees*, **16**, 465–472.

Corcuera L, Camarero JJ, Gil-Pelegrin E (2004) Effects of a severe drought on growth and wood anatomical properties of *Quercus faginea*. *IAWA Journal*, **25**, 185–204.

Corcuera L, Camarero JJ, Sisó S, Gil-Pelegrín E (2006) Radial-growth and wood-anatomical changes in overaged *Quercus pyrenaica* coppice stands: functional responses in a new Mediterranean landscape. *Trees*, **20**, 91–98.

Cuny HE, Rathgeber CBK, Lebourgeois F, Fortin M, Fournier M (2012) Life strategies in intra-annual dynamics of wood formation: example of three conifer species in a temperate forest in north-east France. *Tree Physiology*, **32**, 612–25.

Cuny HE, Rathgeber CBK, Frank D, Fonti P, Fournier M (2014) Kinetics of tracheid development explain conifer tree-ring structure. *New Phytologist*, **203**, 1231–1241.

D'Arrigo R, Wilson R, Liepert B, Cherubini P (2008) On the "Divergence Problem" in Northern Forests: A review of the tree-ring evidence and possible causes. *Global and Planetary Change*, **60**, 289–305.

Do Amaral Franco J (1990) Quercus. In: *Flora Iberica. Vol. II*, Vol. 237 (eds Castroviejo S, Laínz M, López González G, Montserrat P, Muñoz Garmendia F, Paiva J, Villar L), pp. 15–36. Real Jardín Botánico CSIC, Madrid.

D'Orangeville L, Côté B, Houle D, Morin H (2013) The effects of throughfall exclusion on xylogenesis of balsam fir. *Tree Physiology*, **33**, 516–26.

Dawes MA, Hattenschwiler S, Bebi P, Hagedorn F, Handa IT, Körner C, Rixen C (2011) Species-specific tree growth responses to 9 years of CO_2 enrichment at the alpine treeline. *Journal of Ecology*, **99**, 383–394.

Delpierre N, Vitasse Y, Chuine I, Guillemot J, Bazot S, Rutishauser T, Rathgeber CBK (2016a) Temperate and boreal forest tree phenology: from organ-scale processes to terrestrial ecosystem models. *Annals of Forest Science*, **73**, 5–25.

Delpierre N, Berveiller D, Granda E, Dufrêne E (2016b) Wood phenology, not carbon input, controls the interannual variability of wood growth in a temperate oak forest. *New Phytologist*, **210**, 459–470.

De Luis M, Novak K, Raventós J, Gričar J, Prislan P, Čufar K (2011) Cambial activity, wood formation and sapling survival of *Pinus halepensis* exposed to different irrigation regimes. *Forest Ecology and Management*, **262**, 1630–1638.

Deslauriers A, Morin H, Begin Y (2003) Cellular phenology of annual ring formation of *Abies balsamea* in the Quebec boreal forest (Canada). *Canadian Journal of Forest Research*, **33**, 190–200.

Deslauriers A, Giovannelli A, Rossi S, Castro G, Fragnelli G, Traversi L (2009) Intra-annual cambial activity and carbon availability in stem of poplar. *Tree Physiology*, **29**, 1223–35.

De Vries W, Posch M (2011) Modelling the impact of nitrogen deposition, climate change and nutrient limitations on tree carbon sequestration in Europe for the period 1900-2050. *Environmental pollution*, **159**, 2289–2299.

Dickman LT, McDowell NG, Sevanto S, Pangle RE, Pockman WT (2015) Carbohydrate dynamics and mortality in a piñon-juniper woodland under three future precipitation scenarios. *Plant, cell & environment,* **38**, 729–739.

Di Matteo G, De Angelis P, Brugnoli E, Cherubini P, Scarascia-Mugnozza G (2010) Tree-ring Δ^{13}C reveals the impact of past forest management on water-use efficiency in a Mediterranean oak coppice in Tuscany (Italy). *Annals of Forest Science*, **67**, 510.

Dorado Liñán I, Gutiérrez E, Heinrich I, Andreu-Hayles L, Muntán E, Campelo F, Helle G (2011) Age effects and climate response in trees: a multi-proxy tree-ring test in old-growth life stages. *European Journal of Forest Research*, **131**, 933–944.

Dormann CF, Elith J, Bacher S et al. (2013) Collinearity: A review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, **36**, 027–046.

Duursma RA, Kolari P, Perämäki M et al. (2008) Predicting the decline in daily maximum transpiration rate of two pine stands during drought based on constant minimum leaf water potential and plant hydraulic conductance. *Tree Physiology*, **28**, 265–76.

Ehleringer JR (1993) Variation in leaf carbon isotope discrimination in *Encelia farinosa*: implications for growth, competition, and drought survival. *Oecologia*, **95**, 340–346.

Eilmann B, Zweifel R, Buchmann N, Fonti P, Rigling A (2009) Drought-induced adaptation of the xylem in Scots pine and pubescent oak. *Tree Physiology*, **29**, 1011–20.

Eilmann B, Buchmann N, Siegwolf R, Saurer M, Cherubini P, Rigling A (2010) Fast response of Scots pine to improved water availability reflected in tree-ring width and δ13C. *Plant, Cell & Environment*, **33**, 1351–60.

Eilmann B, Zweifel R, Buchmann N, Graf Pannatier E, Rigling A (2011) Drought alters timing, quantity, and quality of wood formation in Scots pine. *Journal of Experimental Botany*, **62**, 2763–2771.

El Zein R, Maillard P, Bréda N, Marchand J, Montpied P, Gérant D (2011) Seasonal changes of C and N non-structural compounds in the stem sapwood of adult sessile oak and beech trees. *Tree Physiology*, **31**, 843–854.

Escudero A, Mediavilla S (2003) Decline in photosynthetic nitrogen use efficiency with leaf age and nitrogen resorption as determinants of leaf life span. *Journal of Ecology*, **91**, 880–889.

Evans MN, Reichert BK, Kaplan A, Anchukaitis KJ, Vaganov EA, Hughes MK, Cane MA (2006) A forward modeling approach to paleoclimatic interpretation of tree-ring data. *Journal of Geophysical Research*, **111**, G03008.

FAO (2015) *Global Forest Resources Assessment 2015*. Rome, 253 pp.

Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology*, **33**, 317–345.

Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology*, **9**, 121–137.

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, **40**, 503–537.

Fernández-de-Uña L, Cañellas I, Gea-Izquierdo G (2015) Stand competition determines how different tree species will cope with a warming climate. *Plos One*, **10**, e0122255.

Fichot R, Laurans F, Monclus R, Moreau A, Pilate G, Brignolas F (2009) Xylem anatomy correlates with gas exchange, water-use efficiency and growth performance under contrasting water regimes: evidence from *Populus deltoides* x *Populus nigra* hybrids. *Tree physiology*, **29**, 1537–49.

Fichtner A, Sturm K, Rickert C, Härdtle W, Schrautzer J (2012) Competition response of European beech *Fagus sylvatica* L. varies with tree size and abiotic stress: minimizing anthropogenic disturbances in forests. *Journal of Applied Ecology*, **49**, 1306–1315.

Fonti P, von Arx G, García-González I, Eilmann B, Sass-Klaassen U, Gärtner H, Eckstein D (2010) Studying global change through investigation of the plastic responses of xylem anatomy in tree rings. *New Phytologist*, **185**, 42–53.

Fournier DA, Skaug HJ, Ancheta J et al. (2012) AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optimization Methods and Software*, **27**, 233–249.

Franco Múgica F, García Antón M, Sainz Ollero H (1998) Vegetation dynamics and human impact in the Sierra de Guadarrama, Central System, Spain. *The Holocene*, **8**, 69–82.

Franks PJ, Adams MA, Amthor JS et al. (2013) Sensitivity of plants to changing atmospheric CO_2 concentration: from the geological past to the next century. *New Phytologist*, **197**, 1077–1094.

Fritts HC (1976) *Tree Rings and Climate*. Academic Press, 510 pp.

Galiano L, Martínez-Vilalta J, Lloret F (2010) Drought-induced multifactor decline of Scots pine in the Pyrenees and potential vegetation change by the expansion of co-occurring oak species. *Ecosystems*, **13**, 978–991.

Galiano L, Martínez-Vilalta J, Lloret F (2011) Carbon reserves and canopy defoliation determine the recovery of Scots pine 4 yr after a drought episode. *New Phytologist*, **190**, 750–9.

Gallego HA, Rico M, Moreno G, Santa Regina I (1994) Leaf water potential and stomatal conductance in *Quercus pyrenaica* Willd. forests: vertical gradients and response to environmental factors. *Tree physiology*, **14**, 1039–1047.

García-Ruiz JM, López-Moreno JI, Vicente-Serrano SM, Lasanta-Martínez T, Beguería S (2011) Mediterranean water resources in a global change scenario. *Earth-Science Reviews*, **105**, 121–139.

Gaylord ML, Kolb TE, Pockman WT et al. (2013) Drought predisposes piñon-juniper woodlands to insect attacks and mortality. *New Phytologist*, **198**, 567–78.

Gea-Izquierdo G, Cañellas I (2009) Analysis of holm oak intraspecific competition using Gamma regression. *Forest Science*, **55**, 310–322.

Gea-Izquierdo G, Cañellas I (2014) Local climate forces instability in long-term productivity of a Mediterranean oak along climatic gradients. *Ecosystems*, **17**, 228–241.

Gea-Izquierdo G, Martín-Benito D, Cherubini P, Cañellas I (2009) Climate-growth variability in *Quercus ilex* L. west Iberian open woodlands of different stand density. *Annals of Forest Science*, **66**, 802–802.

Gea-Izquierdo G, Fernández-de-Uña L, Cañellas I (2013) Growth projections reveal local vulnerability of Mediterranean oaks with rising temperatures. *Forest Ecology and Management*, **305**, 282–293.

Gea-Izquierdo G, Viguera B, Cabrera M, Cañellas I (2014) Drought induced decline could portend widespread pine mortality at the xeric ecotone in managed mediterranean pine-oak woodlands. *Forest Ecology and Management*, **320**, 70–82.

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

Goffe WL, Ferrier GD, Rogers J (1994) Global optimization of statistical functions with simulated annealing. *Journal of Econometrics*, **60**, 65–99.

Gómez-Aparicio L, García-Valdés R, Ruíz-Benito P, Zavala MA (2011) Disentangling the relative importance of climate, size and competition on tree growth in Iberian forests: implications for forest management under global change. *Global Change Biology*, **17**, 2400–2414.

Gómez-Guerrero A, Silva LCR, Barrera-Reyes M et al. (2013) Growth decline and divergent tree ring isotopic composition ($\delta^{13}C$ and $\delta^{18}O$) contradict predictions of CO_2 stimulation in high altitudinal forests. *Global Change Biology*, **19**, 1748–1758.

Grams TEE, Kozovits AR, Häberle KH, Matyssek R, Dawson TE (2007) Combining $\delta^{13}C$ and $\delta^{18}O$ analyses to unravel competition, CO_2 and O_3 effects on the physiological performance of different-aged trees. *Plant, Cell and Environment*, **30**, 1023–1034.

Granda E, Rossatto DR, Camarero JJ, Voltas J, Valladares F (2014) Growth and carbon isotopes of Mediterranean trees reveal contrasting responses to increased carbon dioxide and drought. *Oecologia*, **174**, 307–17.

Granier A (1987) Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements. *Tree physiology*, **3**, 309–320.

Granier A, Anfodillo T, Sabatti M et al. (1994) Axial and radial water flow in the trunks of oak trees: a quantitative and qualitative analysis. *Tree Physiology*, **14**, 1383–1396.

Gričar J, Prislan P, Gryc V, Vavrčík H, de Luis M, Cufar K (2014) Plastic and locally adapted phenology in cambial seasonality and production of xylem and phloem cells in *Picea abies* from temperate environments. *Tree physiology*, **34**, 869–81.

Gruber A, Strobl S, Veit B, Oberhuber W (2010) Impact of drought on the temporal dynamics of wood formation in *Pinus sylvestris*. *Tree Physiology*, **30**, 490–501.

Hacke UG, Sperry JS (2001) Functional and ecological xylem anatomy. *Perspectives in Plant Ecology, Evolution and Systematics*, **4**, 97–115.

Harris I, Jones PD, Osborn TJ, Lister DH (2014) Updated high-resolution grids of monthly climatic observations – the CRU TS3.10 Dataset. *International Journal of Climatology*, **34**, 623–642.

Hereş A-M, Martínez-Vilalta J, Claramunt López B (2012) Growth patterns in relation to drought-induced mortality at two Scots pine (*Pinus sylvestris* L.) sites in NE Iberian Peninsula. *Trees*, **26**, 621–630.

Hernández-Santana V, Martínez-Fernández J, Morán C, Cano A (2008a) Response of *Quercus pyrenaica* (melojo oak) to soil water deficit: a case study in Spain. *European Journal of Forest Research*, **127**, 369–378.

Hernández-Santana V, David TS, Martínez-Fernández J (2008b) Environmental and plant-based controls of water use in a Mediterranean oak stand. *Forest Ecology and Management*, **255**, 3707–3715.

Hernández-Santana V, Martínez-Vilalta J, Martínez-Fernández J, Williams M (2009) Evaluating the effect of drier and warmer conditions on water use by *Quercus pyrenaica*. *Forest Ecology and Management*, **258**, 1719–1730.

Herrera S, Gutierrez JM, Ancell R, Pons MR, Frias MD, Fernandez J (2012) Development and analysis of a 50-year high-resolution daily gridded precipitation dataset over Spain (Spain02). *International Journal of Climatology*, **32**, 74–85.

Hikosaka K, Ishikawa K, Borjigidai A, Muller O, Onoda Y (2006) Temperature acclimation of photosynthesis: mechanisms involved in the changes in temperature dependence of photosynthetic rate. *Journal of Experimental Botany*, **57**, 291–302.

Hoch G, Richter A, Körner C (2003) Non-structural carbon compounds in temperate forest trees. *Plant, Cell & Environment*, **26**, 1067–1081.

Holmes RL (1983) Computer assisted quality control in tree- ring dating and measurement. *Tree-Ring Bulletin*, **43**, 69–78.

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.

Huang J-G, Bergeron Y, Denneler B, Berninger F, Tardif J (2007) Response of forest trees to increased atmospheric CO_2. *Critical Reviews in Plant Sciences*, **26**, 265–283.

Huang J-G, Bergeron Y, Berninger F, Zhai L, Tardif JC, Denneler B (2013) Impact of future climate on radial growth of four major boreal tree species in the Eastern Canadian boreal forest. *PloS one*, **8**, e56758.

Hughes L (2000) Biological consequences of global warming: is the signal already apparent? *Trends in Ecology & Evolution*, **15**, 56–61.

Hultine KR, Marshall JD (2000) Altitude trends in conifer leaf morphology and stable carbon isotope composition. *Oecologia*, **123**, 32–40.

IPCC (2007) *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (ed Solomon, S.; Qin, D.; Manning, M.; Chen, Z.; Marquis, M.; Averyt, K.B.; Tignor, M.; Miller H.). Cambridge University Press,Cambridge, United Kingdom and New York, NY, USA, 996 pp.

IPCC (2013) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM). Cambridge University Press,Cambridge, United Kingdom and New York, New York, USA, 1535 pp.

Irvine J, Perks MP, Magnani F, Grace J (1998) The response of Pinus sylvestris to drought: stomatal control of transpiration and hydraulic conductance. *Tree Physiology*, **18**, 393–402.

Johnson JB, Omland KS (2004) Model selection in ecology and evolution. *Trends in Ecology & Evolution*, **19**, 101–108.

Kalliokoski T, Mäkinen H, Jyske T, Nöjd P, Linder S (2013) Effects of nutrient optimization on intra-annual wood formation in Norway spruce. *Tree Physiology*, **33**, 1145–55.

Kirchner M, Faus-Kessler T, Jakobi G, Leuchner M, Ries L, Scheel H-E, Suppan P (2013) Altitudinal temperature lapse rates in an Alpine valley: trends and the influence of season and weather patterns. *International Journal of Climatology*, **33**, 539–555.

Körner C (2006) Plant CO_2 response: an issue of non definition, time and resource supply. *New Phytologist*, **172**, 393–411.

Kouba Y, Camarero JJ, Alados CL (2012) Roles of land-use and climate change on the establishment and regeneration dynamics of Mediterranean semi-deciduous oak forests. *Forest Ecology and Management*, **274**, 143–150.

Kramer PJ (1962) The role of water in tree growth. In: *Tree growth* (ed Kozlowski TT), pp. 171–182. Ronald Press, New York, USA.

Kudo K, Nabeshima E, Begum S et al. (2014) The effects of localized heating and disbudding on cambial reactivation and formation of earlywood vessels in seedlings of the deciduous ring-porous hardwood, *Quercus serrata*. *Annals of Botany*, **113**, 1021–1027.

Kunstler G, Falster D, Coomes DA et al. (2016) Plant functional traits have globally consistent effects on competition. *Nature*, **529**, 1–15.

Landsberg J, Sands P (2011) *Physiological ecology of forest production: principles, processes and models*. Academic Press/Elsevier, Amsterdam; Boston, 331 pp.

Leavitt SW (2010) Tree-ring C-H-O isotope variability and sampling. *The Science of the Total Environment*, **408**, 5244–53.

Lenoir J, Gégout JC, Marquet PA, de Ruffray P, Brisse H (2008) A significant upward shift in plant species optimum elevation during the 20th century. *Science*, **320**, 1768–1771.

Leonardi S, Gentilesca T, Guerrieri R et al. (2012) Assessing the effects of nitrogen deposition and climate on carbon isotope discrimination and intrinsic water-use efficiency of angiosperm and conifer trees under rising CO_2 conditions. *Global Change Biology*, **18**, 2925–2944.

Lévesque M, Siegwolf R, Saurer M, Eilmann B, Rigling A (2014) Increased water-use efficiency does not lead to enhanced tree growth under xeric and mesic conditions. *New Phytologist*, **203**, 94–109.

Li X, Liang E, Gričar J et al. (2013) Age dependence of xylogenesis and its climatic sensitivity in Smith fir on the south-eastern Tibetan Plateau. *Tree Physiology*, **33**, 48–56.

Limousin JM, Rambal S, Ourcival JM, Rocheteau A, Joffre R, Rodriguez-Cortina R (2009) Long-term transpiration change with rainfall decline in a Mediterranean *Quercus ilex* forest. *Global Change Biology*, **15**, 2163–2175.

Limousin JM, Yepez EA, Mcdowell NG, Pockman WT (2015) Convergence in resource use efficiency across trees with differing hydraulic strategies in response to ecosystem precipitation manipulation. *Functional Ecology*, **29**, 1125–1136.

Linares JC, Camarero JJ, Carreira JA (2009a) Plastic responses of *Abies pinsapo* xylogenesis to drought and competition. *Tree Physiology*, **29**, 1525–36.

Linares JC, Delgado-Huertas A, Camarero JJ, Merino J, Carreira JA (2009b) Competition and drought limit the response of water-use efficiency to rising atmospheric carbon dioxide in the Mediterranean fir *Abies pinsapo*. *Oecologia*, **161**, 611–24.

Linares JC, Camarero JJ, Carreira JA (2010) Competition modulates the adaptation capacity of forests to climatic stress: insights from recent growth decline and death in relict stands of the Mediterranean fir *Abies pinsapo*. *Journal of Ecology*, **98**, 592–603.

Linderholm HW (2006) Growing season changes in the last century. *Agricultural and Forest Meteorology*, **137**, 1–14.

Loehle C (2008) A mathematical analysis of the divergence problem in dendroclimatology. *Climatic Change*, **94**, 233–245.

Lorimer CG, Frelich LE (1989) A methodology for estimating canopy disturbance frequency and intensity in dense temperate forests. *Canadian Journal of Forest Research*, **19**, 651–663.

Maltez-Mouro S, García L V., Freitas H (2009) Influence of forest structure and environmental variables on recruit survival and performance of two Mediterranean tree species (*Quercus faginea* L. and *Q. suber* Lam.). *European Journal of Forest Research*, **128**, 27–36.

Marshall JD, Monserud RA (1996) Homeostatic gas-exchange parameters inferred from $^{13}C/^{12}C$ in tree rings of conifers. *Oecologia*, 13–21.

Martín-Benito D, Del Río M, Heinrich I, Helle G, Cañellas I (2010) Response of climate-growth relationships and water use efficiency to thinning in a *Pinus nigra* afforestation. *Forest Ecology and Management*, **259**, 967–975.

Martín-Benito D, Kint V, del Río M, Muys B, Cañellas I (2011) Growth responses of West-Mediterranean *Pinus nigra* to climate change are modulated by competition and productivity: Past trends and future perspectives. *Forest Ecology and Management*, **262**, 1030–1040.

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

Martínez-Vilalta J, Cochard H, Mencuccini M et al. (2009) Hydraulic adjustment of Scots pine across Europe. *New Phytologist*, **184**, 353–364.

Martínez-Vilalta J, López BC, Loepfe L, Lloret F (2012) Stand- and tree-level determinants of the drought response of Scots pine radial growth. *Oecologia*, **168**, 877–88.

Maseyk K, Hemming D, Angert A, Leavitt SW, Yakir D (2011) Increase in water-use efficiency and underlying processes in pine forests across a precipitation gradient in the dry Mediterranean region over the past 30 years. *Oecologia*, **167**, 573–85.

McCarroll D, Loader NJ (2004) Stable isotopes in tree rings. *Quaternary Science Reviews*, **23**, 771–801.

McDowell NG, Sevanto S (2010) The mechanisms of carbon starvation: how, when, or does it even occur at all? *New Phytologist*, **186**, 264–266.

McDowell N, Brooks JR, Fitzgerald SA, Bond BJ (2003) Carbon isotope discrimination and growth response of old *Pinus ponderosa* trees to stand density reductions. *Plant, Cell & Environment*, **26**, 631–644.

McDowell NG, Licata J, Bond BJ (2005) Environmental sensitivity of gas exchange in different-sized trees. *Oecologia*, **145**, 9–20.

McDowell NG, Adams HD, Bailey JD, Hess M, Kolb TE (2006) Homeostatic maintenance of ponderosa pine gas exchange in response to stand density changes. *Ecological Applications*, **16**, 1164–1182.

McDowell N, Pockman WT, Allen CD et al. (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist*, **178**, 719–39.

McDowell NG, Allen CD, Marshall L (2010) Growth, carbon-isotope discrimination, and drought-associated mortality across a *Pinus ponderosa* elevational transect. *Global Change Biology*, **16**, 399–415.

McDowell NG, Beerling DJ, Breshears DD, Fisher RA, Raffa KF, Stitt M (2011) The interdependence of mechanisms underlying climate-driven vegetation mortality. *Trends in Ecology & Evolution*, **26**, 523–32.

Mediavilla S, Escudero A (2003) Stomatal responses to drought at a Mediterranean site: a comparative study of co-occurring woody species differing in leaf longevity. *Tree Physiology*, **23**, 987–996.

Meinzer FC, Johnson DM, Lachenbruch B, McCulloh KA, Woodruff DR (2009) Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. *Functional Ecology*, **23**, 922–930.

Mencuccini M, Mencuccini M, Grace J, Grace J (1994) Climate influences the leaf/sapwood area ratio in Scots pine. *Tree Physiology*, **15**, 1–10.

Menzel A, Sparks TH, Estrella N et al. (2006) European phenological response to climate change matches the warming pattern. *Global Change Biology*, **12**, 1969–1976.

Metz J, Annighöfer P, Schall P, Zimmermann J, Kahl T, Schulze ED, Ammer C (2016) Site-adapted admixed tree species reduce drought susceptibility of mature European beech. *Global Change Biology*, **22**, 903–920.

Michelot A, Bréda N, Damesin C, Dufrêne E (2012a) Differing growth responses to climatic variations and soil water deficits of *Fagus sylvatica*, *Quercus petraea* and *Pinus sylvestris* in a temperate forest. *Forest Ecology and Management*, **265**, 161–171.

Michelot A, Simard S, Rathgeber C, Dufrêne E, Damesin C (2012b) Comparing the intra-annual wood formation of three European species (*Fagus sylvatica*, *Quercus petraea* and *Pinus sylvestris*) as related to leaf phenology and non-structural carbohydrate dynamics. *Tree Physiology*, **32**, 1033–45.

Millard P, Sommerkorn M, Grelet GA (2007) Environmental change and carbon limitation in trees: A biochemical, ecophysiological and ecosystem appraisal. *New Phytologist*, **175**, 11–28.

Misson L, Tang J, Xu M, McKay M, Goldstein A (2005) Influences of recovery from clear-cut, climate variability, and thinning on the carbon balance of a young ponderosa pine plantation. *Agricultural and Forest Meteorology*, **130**, 207–222.

Misson L, Degueldre D, Collin C, Rodriguez R, Rocheteau A, Ourcival J-M, Rambal S (2011) Phenological responses to extreme droughts in a Mediterranean forest. *Global Change Biology*, **17**, 1036–1048.

Mitchell TD, Jones PD (2005) An improved method of constructing a database of monthly climate observations and associated high-resolution grids. *International Journal of Climatology*, **25**, 693–712.

Monserud RA, Marshall JD (2001) Time-series analysis of $\delta^{13}C$ from tree rings. I. Time trends and autocorrelation. *Tree Physiology*, **21**, 1087–1102.

Montes F, Cañellas I (2007) The spatial relationship between post-crop remaining trees and the establishment of saplings in *Pinus sylvestris* stands in Spain. *Applied Vegetation Science*, **10**, 151–160.

Montserrat-Martí G, Camarero JJ, Palacio S, Pérez-Rontomé C, Milla R, Albuixech J, Maestro M (2009) Summer-drought constrains the phenology and growth of two coexisting Mediterranean oaks with contrasting leaf habit: implications for their persistence and reproduction. *Trees*, **23**, 787–799.

Moreno-Gutiérrez C, Barberá GG, Nicolás E, DE Luis M, Castillo VM, Martínez-Fernández F, Querejeta JI (2011) Leaf δ^{18}O of remaining trees is affected by thinning intensity in a semiarid pine forest. *Plant, Cell & Environment*, **34**, 1009–19.

Moreno-Gutiérrez C, Battipaglia G, Cherubini P, Saurer M, Nicolás E, Contreras S, Querejeta JI (2012) Stand structure modulates the long-term vulnerability of *Pinus halepensis* to climatic drought in a semiarid Mediterranean ecosystem. *Plant, Cell & Environment*, **35**, 1026–39.

Murphy L (2012) likelihood: Methods for maximum likelihood estimation. R package version 1.5.

Ni F, Cavazos T, Hughes MK, Comrie AC, Funkhouser G (2002) Cool-season precipitation in the southwestern USA since AD 1000: comparison of linear and nonlinear techniques for reconstruction. *International Journal of Climatology*, **22**, 1645–1662.

Niinemets Ü (2010) Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: Past stress history, stress interactions, tolerance and acclimation. *Forest Ecology and Management*, **260**, 1623–1639.

Niinemets Ü, Keenan TF, Hallik L (2015) A worldwide analysis of within-canopy variations in leaf structural, chemical and physiological traits across plant functional types. *New Phytologist*, **205**, 973–993.

Nowacki GJ, Abrams MD (1997) Radial-growth averaging criteria for reconstructing disturbance histories from presettlement-origin oaks. *Ecological Monographs*, **67**, 225–249.

Oberhuber W, Gruber A (2010) Climatic influences on intra-annual stem radial increment of *Pinus sylvestris* (L.) exposed to drought. *Trees (Berlin, Germany : West)*, **24**, 887–898.

Oberhuber W, Swidrak I, Pirkebner D, Gruber A (2011) Temporal dynamics of nonstructural carbohydrates and xylem growth in *Pinus sylvestris* exposed to drought. *Canadian Journal of Forest Research*, **41**, 1590–1597.

Offermann C, Ferrio JP, Holst J, Grote R, Siegwolf R, Kayler Z, Gessler A (2011) The long way down--are carbon and oxygen isotope signals in the tree ring uncoupled from canopy physiological processes? *Tree Physiology*, **31**, 1088–102.

Ogaya R, Peñuelas J (2004) Phenological patterns of *Quercus ilex*, *Phillyrea latifolia*, and *Arbutus unedo* growing under a field experimental drought. *Ecoscience*, **11**, 263–270.

Pasho E, Camarero JJ, de Luis M, Vicente-Serrano SM (2011) Impacts of drought at different time scales on forest growth across a wide climatic gradient in north-eastern Spain. *Agricultural and Forest Meteorology*, **151**, 1800–1811.

Peguero-Pina JJ, Sancho-Knapik D, Martín P, Saz MÁ, Gea-Izquierdo G, Cañellas I, Gil-Pelegrín E (2015) Evidence of vulnerability segmentation in a deciduous Mediterranean oak (*Quercus subpyrenaica* E. H. del Villar). *Trees - Structure and Function*, **29**, 1917–1927.

Peñuelas J, Filella I, Zhang X et al. (2004) Complex spatiotemporal phenological shifts as a response to rainfall changes. *New Phytologist*, **161**, 837–846.

Peñuelas J, Hunt JM, Ogaya R, Jump AS (2008) Twentieth century changes of tree-ring $\delta^{13}C$ at the southern range-edge of *Fagus sylvatica*: increasing water-use efficiency does not avoid the growth decline induced by warming at low altitudes. *Global Change Biology*, **14**, 1076–1088.

Peñuelas J, Canadell JG, Ogaya R (2011) Increased water-use efficiency during the 20th century did not translate into enhanced tree growth. *Global Ecology and Biogeography*, **20**, 597–608.

Pérez-de-Lis G, Rossi S, Vázquez-Ruiz RA, Rozas V, García-González I (2016) Do changes in spring phenology affect earlywood vessels? Perspective from the xylogenesis monitoring of two sympatric ring-porous oaks. *New Phytologist*, **209**, 521–530.

Pérez-Ramos IM, Ourcival JM, Limousin JM, Rambal S (2010) Mast seeding under increasing drought: results from a long-term data set and from a rainfall exclusion experiment. *Ecology*, **91**, 3057–68.

Pineda-García F, Paz H, Meinzer FC (2013) Drought resistance in early and late secondary successional species from a tropical dry forest: The interplay between xylem resistance to embolism, sapwood water storage and leaf shedding. *Plant, Cell and Environment*, **36**, 405–418.

Piutti E, Cescatti A (1997) A quantitative analysis of the interactions between climatic response and intraspecific competition in European beech. *Canadian Journal of Forest Research*, **27**, 277–284.

Plomion C, Leprovost G, Stokes A (2001) Wood formation in trees. *Plant physiology*, **127**, 1513–1523.

Powers MD, Pregitzer KS, Palik BJ, Webster CR (2010) Wood $\delta^{13}C$, $\delta^{18}O$ and radial growth responses of residual red pine to variable retention harvesting. *Tree Physiology*, **30**, 326–34.

Poyatos R, Llorens P, Piñol J, Rubio C (2008) Response of Scots pine (*Pinus sylvestris* L .) and pubescent oak (*Quercus pubescens* Willd .) to soil and atmospheric water deficits under Mediterranean mountain climate. *Annals of Forest Science*, **65**, 306.

Pretzsch H, Biber P (2005) A re-evaluation of Reineke's rule and stand density index. *Forest Science*, **51**, 304–320.

Primicia I, Camarero JJ, Imbert JB, Castillo FJ (2013) Effects of thinning and canopy type on growth dynamics of *Pinus sylvestris*: inter-annual variations and intra-annual interactions with microclimate. *European Journal of Forest Research*, **132**, 121–135.

Prus-Glowacki W, Stephan BR (1994) Genetic variation of *Pinus sylvestris* from Spain in relation to other European populations. *Silvae Genetica*, **43**, 7–14.

Prus-Glowacki W, Stephan BR, Bujas E, Alia R, Marciniak A (2003) Genetic differentiation of autochthonous populations of *Pinus sylvestris* (Pinaceae) from the Iberian peninsula. *Plant Systematics and Evolution*, **239**, 55–66.

Quinn Thomas R, Canham CD, Weathers KC, Goodale CL (2009) Increased tree carbon storage in response to nitrogen deposition in the US. *Nature Geoscience*, **3**, 13–17.

R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Reich PB, Ellsworth DS, Walters MB (1998) Leaf structure (specific leaf area) modulates photosynthesis-nitrogen relations: evidence from within and across species and functional groups. *Functional Ecology*, **12**, 948–958.

Resco V, Ferrio JP, Carreira JA et al. (2011) The stable isotope ecology of terrestrial plant succession. *Plant Ecology & Diversity*, **4**, 117–130.

Rey Benayas JM, Navarro J, Espigares T, Nicolau JM, Zavala MA (2005) Effects of artificial shading and weed mowing in reforestation of Mediterranean abandoned cropland with contrasting *Quercus* species. *Forest Ecology and Management*, **212**, 302–314.

Rigling A, Bigler C, Eilmann B et al. (2013) Driving factors of a vegetation shift from Scots pine to pubescent oak in dry Alpine forests. *Global Change Biology*, **19**, 229–240.

Rinntech (2003) TSAP-WIN. Time series analysis and presentation for dendrochronology and related applications. Version 0.53.

Rodríguez-Calcerrada J, Pardos JA, Aranda I (2010) Contrasting responses facing peak drought in seedlings of two co-occurring oak species. *Forestry*, **83**, 369–378.

Rossi S, Deslauriers A, Anfodillo T (2006a) Assessment of cambial activity and xylogenesis by microsampling tree species: an example at the alpine timberline. *IAWA Journal*, **27**, 383–394.

Rossi S, Deslauriers A, Anfodillo T, Morin H, Saracino A, Motta R, Borghetti M (2006b) Conifers in cold environments synchronize maximum growth rate of tree-ring formation with day length. *New Phytologist*, **170**, 301–310.

Rossi S, Anfodillo T, Menardi R (2006c) Trephor: a new tool for sampling microcores from tree stems. *IAWA Journal*, **27**, 89–97.

Rossi S, Deslauriers A, Griçar J et al. (2008a) Critical temperatures for xylogenesis in conifers of cold climates. *Global Ecology and Biogeography*, **17**, 696–707.

Rossi S, Deslauriers A, Anfodillo T, Carrer M (2008b) Age-dependent xylogenesis in timberline conifers. *New Phytologist*, **177**, 199–208.

Rossi S, Morin H, Deslauriers A (2012) Causes and correlations in cambium phenology : towards an integrated framework of xylogenesis. *Journal of Experimental Botany*, **63**, 2117–2126.

Rozas V, García-González I (2012) Too wet for oaks? Inter-tree competition and recent persistent wetness predispose oaks to rainfall-induced dieback in Atlantic rainy forest. *Global and Planetary Change*, **94-95**, 62–71.

Rozas V, De Soto L, Olano JM (2009) Sex-specific, age-dependent sensitivity of tree-ring growth to climate in the dioecious tree *Juniperus thurifera*. *New Phytologist*, **182**, 687–97.

Ruiz-Benito P, Lines ER, Gómez-Aparicio L, Zavala MA, Coomes DA (2013) Patterns and drivers of tree mortality in iberian forests: climatic effects are modified by competition. *PloS one*, **8**, e56843.

Ruiz-Labourdette D, Nogués-Bravo D, Ollero HS, Schmitz MF, Pineda FD (2012) Forest composition in Mediterranean mountains is projected to shift along the entire elevational gradient under climate change. *Journal of Biogeography*, **39**, 162–176.

Sala A, Peters GD, McIntyre LR, Harrington MG (2005) Physiological responses of ponderosa pine in western Montana to thinning, prescribed fire and burning season. *Tree physiology*, **25**, 339–348.

Sala A, Piper F, Hoch G (2010) Physiological mechanisms of drought-induced tree mortality are far from being resolved. *New Phytologist*, **186**, 274–281.

Sala A, Woodruff DR, Meinzer FC (2012) Carbon dynamics in trees: Feast or famine? *Tree Physiology*, **32**, 764–775.

Salomón R, Rodríguez-Calcerrada J, Zafra E et al. (2016) Unearthing the roots of degradation of *Quercus pyrenaica* coppices: A root-to-shoot imbalance caused by historical management? *Forest Ecology and Management*, **363**, 200–211.

Sánchez de Dios R, Benito-Garzón M, Sainz-Ollero H (2009) Present and future extension of the Iberian submediterranean territories as determined from the distribution of marcescent oaks. *Plant Ecology*, **204**, 189–205.

Sanz-Pérez V, Castro-Díez P, Joffre R (2009) Seasonal carbon storage and growth in Mediterranean tree seedlings under different water conditions. *Tree Physiology*, **29**, 1105–1116.

Sarris D, Christodoulakis D, Körner C (2007) Recent decline in precipitation and tree growth in the eastern Mediterranean. *Global Change Biology*, **13**, 1187–1200.

Saurer M, Siegwolf RTW, Schweingruber FH (2004) Carbon isotope discrimination indicates improving water-use efficiency of trees in northern Eurasia over the last 100 years. *Global Change Biology*, **10**, 2109–2120.

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, **9**, 671–675.

Seibt U, Rajabi A, Griffiths H, Berry JA (2008) Carbon isotopes and water use efficiency: sense and sensitivity. *Oecologia*, **155**, 441–54.

Skomarkova M V., Vaganov EA, Mund M, Knohl A, Linke P, Boerner A, Schulze E-D (2006) Inter-annual and seasonal variability of radial growth, wood density and carbon isotope ratios in tree rings of beech (*Fagus sylvatica*) growing in Germany and Italy. *Trees*, **20**, 571–586.

Sohn JA, Kohler M, Gessler A, Bauhus J (2012) Interactions of thinning and stem height on the drought response of radial stem growth and isotopic composition of Norway spruce (*Picea abies*). *Tree Physiology*, **32**, 1199–213.

Sohn JA, Gebhardt T, Ammer C, Bauhus J, Häberle K-H, Matyssek R, Grams TEE (2013) Mitigation of drought by thinning: Short-term and long-term effects on growth and physiological performance of Norway spruce (*Picea abies*). *Forest Ecology and Management*, **308**, 188–197.

Sohn JA, Brooks JR, Bauhus J, Kohler M, Kolb TE, McDowell NG (2014) Unthinned slow-growing ponderosa pine (*Pinus ponderosa*) trees contain muted isotopic signals in tree rings as compared to thinned trees. *Trees*, **28**, 1035–1051.

Sorce C, Giovannelli A, Sebastiani L, Anfodillo T (2013) Hormonal signals involved in the regulation of cambial activity, xylogenesis and vessel patterning in trees. *Plant Cell Reports*, **32**, 885–898.

Sperry JS., Nichols KL., Sullivan JEM, Eastlack SE (1994) Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of northern Utah and interior Alaska. *Ecology*, **75**, 1736–1752.

Strand M, Lundmark T, Söderbergh I, Mellander P-E (2002) Impacts of seasonal air and soil temperatures on photosynthesis in Scots pine trees. *Tree Physiology*, **22**, 839–47.

Swidrak I, Gruber A, Kofler W, Oberhuber W (2011) Effects of environmental conditions on onset of xylem growth in *Pinus sylvestris* under drought. *Tree Physiology*, **31**, 483–93.

Swidrak I, Gruber A, Oberhuber W (2014) Xylem and phloem phenology in co-occurring conifers exposed to drought. *Trees - Structure and Function*, **28**, 1161–1171.

Takashima T, Hikosaka K, Hirose T (2004) Photosynthesis or persistence: Nitrogen allocation in leaves of evergreen and deciduous *Quercus* species. *Plant, Cell and Environment*, **27**, 1047–1054.

Thuiller W, Lavorel S, Aráujo MB, Sykes MT, Prentice IC (2005) Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Sciences*, **102**, 8245–8250.

Tilman D (1983) Some thoughts on resource competition and diversity in plant communities. In: *Mediterranean-Type Ecosystems: The Role of Nutrients* (eds Kruger FJ, Mitchell DT, Jarvis JUM), pp. 322–336. Springer Berlin Heidelberg, Berlin, Heidelberg.

Tolwinski-Ward SE, Evans MN, Hughes MK, Anchukaitis KJ (2010) An efficient forward model of the climate controls on interannual variation in tree-ring width. *Climate Dynamics*, **36**, 2419–2439.

Tyree MT (1997) The Cohesion – Tension theory of sap ascent: current controversies. *Journal of Experimental Botany*, **48**, 1753–1765.

Tyree MT, Sperry JS (1988) Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress?: answers from a model. *Plant physiology*, **88**, 574–580.

Tyree MT, Zimmermann MH (2002) *Xylem structure and the ascent of sap*, 2nd editio edn. Springer, Berlin, Germany, 250 pp.

Tyree MT, Davis SD, Cochard H (1994) Biophysical perspectives of xylem evolution: is there a tradeoff of hydraulic efficiency for vulnerability to dysfunction? *IAWA Journal*, **15**, 335–360.

Uggla C, Magel E, Moritz T, Sundberg B (2001) Function and dynamics of auxin and carbohydrates during earlywood/latewood transition in Scots pine. *Plant physiology*, **125**, 2029–2039.

Umebayashi T, Utsumi Y, Koga S et al. (2010) Xylem water-conducting patterns of 34 broadleaved evergreen trees in southern Japan. *Trees - Structure and Function*, **24**, 571–583.

Vaganov EA, Hughes MK, Shashkin AV (2006) *Growth dynamics of conifer tree rings. Images of past and future environments*, Vol. 183. Springer, Berlin Heidelberg, 354 pp.

Vaganov EA, Schulze ED, Skomarkova M V, Knohl A, Brand WA, Roscher C (2009) Intra-annual variability of anatomical structure and $\delta^{13}C$ values within tree rings of spruce and pine in alpine, temperate and boreal Europe. *Oecologia*, **161**, 729–745.

Valbuena-Carabaña M, González-Martínez SC, Gil L (2008) Coppice forests and genetic diversity: A case study in *Quercus pyrenaica* Willd. from Central Spain. *Forest Ecology and Management*, **254**, 225–232.

Valbuena-Carabaña M, López de Heredia U, Fuentes-Utrilla P, González-Doncel I, Gil L (2010) Historical and recent changes in the Spanish forests: A socio-economic process. *Review of Palaeobotany and Palynology*, **162**, 492–506.

Vayreda J, Martinez-Vilalta J, Gracia M, Retana J (2012) Recent climate changes interact with stand structure and management to determine changes in tree carbon stocks in Spanish forests. *Global Change Biology*, **18**, 1028–1041.

Venables WN, Ripley BD (2002) *Modern Applied Statistics with S*, 4th edn. Springer, New York, USA, 498 pp.

Vicente-Serrano SM, Beguería S, López-Moreno JI (2010) A multiscalar drought index sensitive to global warming: The standardized precipitation evapotranspiration index. *Journal of Climate*, **23**, 1696–1718.

Vieira J, Campelo F, Rossi S, Carvalho A, Freitas H, Nabais C (2015) Adjustment capacity of maritime pine cambial activity in drought-prone environments. *Plos One*, **10**, e0126223.

Vilà-Cabrera A, Martínez-Vilalta J, Vayreda J, Retana J (2011) Structural and climatic determinants of demographic rates of Scots pine forests across the Iberian Peninsula. *Ecological Applications*, **21**, 1162–72.

Vitasse Y, Lenz A, Körner C (2014) The interaction between freezing tolerance and phenology in temperate deciduous trees. *Frontiers in plant science*, **5**, 541.

Voelker SL, Muzika R-M, Guyette RP, Stambaugh MC (2006) Historical CO_2 enhancement declines with age in *Quercus* and *Pinus*. *Ecological Monographs*, **76**, 549–564.

Voltas J, Camarero JJ, Carulla D, Aguilera M, Ortiz A, Ferrio JP (2013) A retrospective, dual-isotope approach reveals individual predispositions to winter-drought induced tree dieback in the southernmost distribution limit of Scots pine. *Plant, Cell & Environment*, **36**, 1435–48.

Wang J, Ives NE, Lechowicz MJ (1992) The relation of foliar phenology to xylem embolism in trees. *Functional Ecology*, **6**, 469–475.

Waring RH (1987) Characteristics of treespredisposed to die: stress causes distinctive changes in photosynthate allocation. *BioScience*, **37**, 569–574.

Warren CR, Adams MA (2004) Evergreen trees do not maximize instantaneous photosynthesis. *Trends in plant science*, **9**, 270–4.

Warren C, McGrath J, Adams M (2001) Water availability and carbon isotope discrimination in conifers. *Oecologia*, **127**, 476–486.

Webb WL, Newton M, Starr D (1974) Carbon dioxide exchange of *Alnus rubra*. A mathematical model. *Oecologia*, **17**, 281–291.

Weigelt A, Jolliffe P (2003) Indices of plant competition. *Journal of Ecology*, **91**, 707–720.

Whitehead D, Edwards WRN, Jarvis PG (1984) Conducting sapwood area, foliage area, and permeability in mature trees of *Picea sitchensis* and *Pinus contorta*. *Canadian Journal of Forest Research*, **14**, 940–947.

Wiley E, Huepenbecker S, Casper BB, Helliker BR (2013) The effects of defoliation on carbon allocation: Can carbon limitation reduce growth in favour of storage? *Tree Physiology*, **33**, 1216–1228.

Williams AP, Allen CD, Macalady AK et al. (2013) Temperature as a potent driver of regional forest drought stress and tree mortality. *Nature Climate Change*, **3**, 292–297.

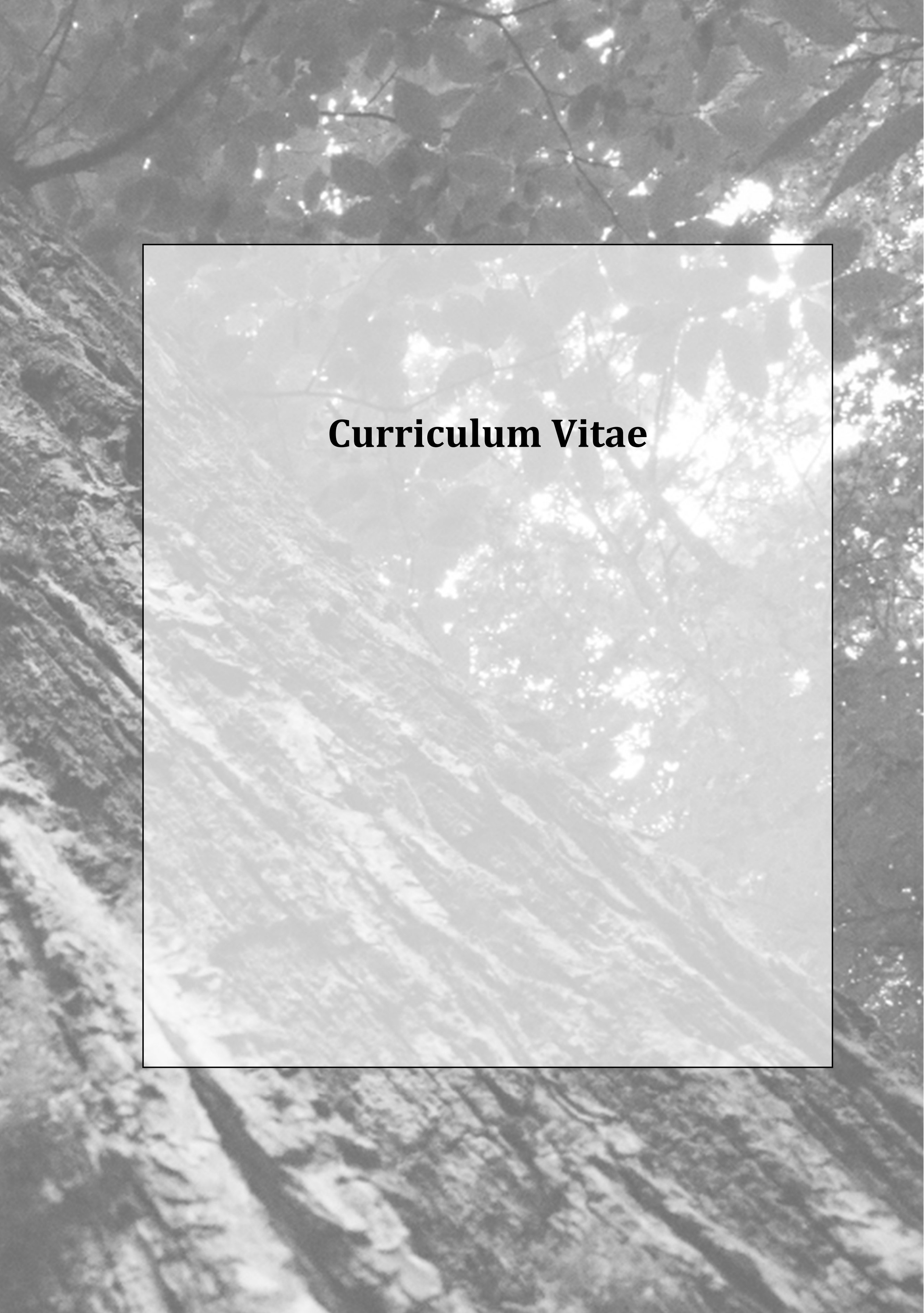
Zanne AE, Westoby M, Falster DS, Ackerly DD, Loarie SR, Arnold SEJ, Coomes DA (2010) Angiosperm wood structure: Global patterns in vessel anatomy and their relation to wood density and potential conductivity. *American Journal of Botany*, **97**, 207–215.

Zheng S, Shangguan Z-P (2006) Relationship between $\delta^{13}C$ and photosynthetic parameters and their responses to leaf nitrogen content in six broadleaf tree species. *Photosynthetica*, **44**, 109–115.

Zuur A, Ieno E, Walker N, Saveliev A, Smith G (2009) *Mixed effects models and extensions in ecology with R* (eds Gail M, Krickeberg K, Samet JM, Tsiatis A, W.Wong).

Zweifel R, Zimmermann L, Zeugin F, Newbery DM (2006) Intra-annual radial growth and water relations of trees: Implications towards a growth mechanism. *Journal of Experimental Botany*, **57**, 1445–1459.

Zweifel R, Bangerter S, Rigling A, Sterck FJ (2012) Pine and mistletoes: how to live with a leak in the water flow and storage system? *Journal of Experimental Botany*, **63**, 2565–78



Curriculum Vitae

Publications

Fernández de Uña L., McDowell N.G., Cañellas I., Gea-Izquierdo G. (2016) Disentangling the effect of competition, CO_2 and climate on intrinsic water-use efficiency and tree growth. *Journal of Ecology*, 104: 678–690

Fernández de Uña L., Cañellas I., Gea-Izquierdo G. (2015) Growth of Mediterranean species under a warming climate will be determined by the interaction between climate and stand competition. *PLoS ONE*, 10(3): e0122255

Fernández de Uña L., Fonti P., Aranda I., Cañellas I., Gea-Izquierdo G. (2013) Impact of drought on the intra-annual growth of *Pinus sylvestris* L. *The Open Forest Science Journal*, 6 (Suppl 1: M3): 43-45

Gea-Izquierdo G., **Fernández de Uña L.**, Cañellas I. (2013) Growth projections reveal local vulnerability of Mediterranean oaks with rising temperatures. *Forest Ecology and Management*, 305: 282-293

Jovellar Lacambra L.C., **Fernández de Uña L.**, Mezquita Santos M., Bolaños López de Lerma F. and Escudero San Emeterio V. (2013) Structural characterization and analysis of the regeneration of woodlands dominated by *Juniperus oxycedrus* L. in west-central Spain. *Plant Ecology*, 214:61–73

Congress communications

Fernández de Uña L., Rossi S., Aranda I., Fonti P., Montes F., Cañellas I., Gea-Izquierdo G. Intra-annual growth, gas exchange and foliar phenology response to drought in *Quercus pyrenaica* and *Pinus sylvestris*. 2015 ESA Annual Meeting, August 9-14 2015, Baltimore, USA. Oral communication

Fernández de Uña L., Rossi S., Aranda I., Fonti P., Montes F., Cañellas I., Gea-Izquierdo G. Intra-annual growth, gas exchange and leaf phenology response to drought in *Pinus sylvestris* and *Quercus pyrenaica* at their altitudinal distribution limit. Tree Rings in Archaeology, Climatology and Ecology (TRACE) 2015, May 20-23 2015, Seville, Spain. Poster communication

Fernández de Uña L., McDowell N.G., Cañellas I., Gea-Izquierdo G. Disentangling the effects of competition, climate and CO_2 concentrations on tree growth and water use efficiency. EuroDendro Conference 2014, September 8-11 2014, Lugo, Spain. Oral communication

Fernández de Uña L., Cañellas I., Gea-Izquierdo G. The interaction between climate and competition determines future growth of different Mediterranean species under a changing climate. 9th International Conference on Dendrochronology, January 13-17 2014, Melbourne, Australia. Oral communication

Gea Izquierdo G., **Fernández de Uña L.**, Cañellas I. Non-linear climate-growth models suggest vulnerability of Mediterranean oaks with rising temperatures. 9th International Conference on Dendrochronology, January 13-17 2014, Melbourne, Australia. Oral communication

Fernández de Uña L., Cañellas I., Bachiller A., Viscasillas E., Gea-Izquierdo G. Efecto de la selvicultura en la respuesta al cambio climático de masas de *Quercus* submediterráneos. 6º Congreso Forestal Español, June 10-14 2013, Vitoria, Spain. Oral communication

Fernández de Uña L., Fonti P., Aranda I., Cañellas I., Gea-Izquierdo G. Impact of increasing drought on the intra-annual growth of *Pinus sylvestris* L. International Symposium on Wood Structure in Plant Biology and Ecology, April 17-20 2013, Naples, Italy. Oral communication

Fernández de Uña L., Cañellas I., Gea-Izquierdo G. Influence of stand competition on the growth response to climate change in Mediterranean tree species. Tree Rings in Archaeology, Climatology and Ecology (TRACE), May 9-12 2012, Potsdam, Germany. Oral communication

Short-term research visits

April – July 2015 (2 months) Centre de Recherche et d'Enseignement de Géosciences de l'Environnement (CEREGE), Aix-en-Provence, France. Supervisors: Guillermo Gea Izquierdo and Joël Guiot

September – December 2014 (3 months) Université du Québec à Chicoutimi, Canada. Supervisor: Sergio Rossi

September – December 2013 (3 months) Earth and Environmental Sciences Division, Los Alamos National Laboratory, Los Alamos, NM, USA. Supervisor: Nathan G. McDowell

August – November 2012 (3 months) Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Birmensdorf, Switzerland. Supervisor: Patrick Fonti

Grants and fellowships

September 2011- August 2015 Spanish Ministry of Economy and Competitiveness Personnel Research Training Programme fellowship

September 2007 - December 2008 Foundation of Canadian Studies and La Caixa Foundation scholarship to pursue postgraduate studies in Canadian Universities

December 2006 - June 2007 Spanish Ministry of Education and Science Undergraduate research fellowship

Participation in research projects

633464-DIABOLO "Distributed, Integrated And Harmonised Forest Information For Bioeconomy Outlooks". 2015-2019. Project coordinator: Tuula Packalen (Natural Resource Institute Finland)

S2013/MAE-2760-BOSSANOVA "Bosques Sanos y Variados: gestión sostenible en sistemas forestales de la CM en el contexto del cambio global". 2014-2017. Project coordinator: Luis Gil Sánchez (UPM)

AGL2013-46028R "La gestión forestal frente a los cambios en la dinámica de los ecosistemas forestales: un enfoque multiescala". 2014-2017. Project coordinator: Fernando Montes Pita (INIA)

AGL2010-21153-C02-01 "Mitigación y adaptación al cambio global en ecosistemas forestales en zonas de transición: efecto de la selvicultura en la regeneración, estructura y procesos funcionales. S1". 2011-2014. Project coordinator: Isabel Cañellas Rey de Viñas (INIA)

Research experience

March 2016 – Present Research technician, INIA-CIFOR, Spain

September 2015 – January 2016 Research technician, INIA-CIFOR, Spain

September 2011 – August 2015 PhD researcher (FPI fellow), INIA-CIFOR, Spain

May - August 2010 Field assistant, Faculty of Forestry, University of Toronto, Canada at the Smithsonian Tropical Research Institute facilities on Barro Colorado Island, Panama

November 2009 - February 2010 Research assistant, Faculty of Forestry, University of Toronto, Canada

February - May 2009 Research assistant, Faculty of Forestry, University of Toronto, Canada

May - August 2008 Intern, INRA-Bordeaux-Aquitaine, France

December 2006 - June 2007 Undergraduate research fellow, Department of Botany, University of Salamanca, Spain

Teaching experience

30 hours Bachelor's Degree in Environmental Sciences, University of Alcalá

Other professional experience

March - July 2011 Outreach campaigner, Environmental Defence Canada, Toronto, Canada

June - October 2009 Urban forestry field assistant, Toronto and Region Conservation Authority, Toronto, Canada

July - September 2006 Intern, Nature Restoration Office, Provincial Department of Environment of Salamanca, Spain

Additional training

May 2014 COST Training School “Modelling drought stress responses” (3 day-course), Ghent University, Belgium

January 2012 “Spatial Analysis and Geostatistics: Forest Research Applications” (12-hour course), Technical University of Madrid, Spain

November 2007 “Learning ArcGIS Desktop (for ArcGIS 9.0-9.1)” (24-hour online course), Environmental Systems Research Institute Inc.

July 2006 “Geomatic Applications in Remote Sensing” (30-hour course), University of Salamanca, Spain